BIOTRANSFORMATION OF DRUGS

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• Phase I reactions
• Phase II reactions
• The effect of biotransformation on the biological activity of drugs: How the biological (pharmacological and toxic) activity of the metabolite compares to that of the parent compound?

PART B: Alterations in biotransformation
• Biological alterations
  - Decreased biotransformation capacity in neonates
  - Genetic alterations in biotransformation
    Mutation or multiplication of genes coding for drug metabolizing enzymes – possible consequences

• Chemical alterations
  - Induction of CYP – possible consequences
  - Inhibition of CYP – possible consequences
INTRODUCTION

ELIMINATION MECHANISMS

Physical mechanism: EXCRETION

Chemical mechanism: BIOTRANSFORMATION

CONTRIBUTION OF EXCRETION AND BIOTRANSFORMATION TO ELIMINATION OF DRUGS - examples

<table>
<thead>
<tr>
<th>Drugs NOT biotransformed, excreted unchanged</th>
<th>Drugs excreted both unchanged and as metabolites</th>
<th>Drugs fully biotransformed, excreted only as metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzylpenicillin, Aminoglycosides, Metformin, Tubocurarine, Amantadine</td>
<td>Salicylates, Acetaminophen, Phenobarbital</td>
<td>TCADs, Phenothiazines, Chloramphenicol</td>
</tr>
</tbody>
</table>

The sites of biotransformation:

- Predominantly, the liver
  The liver contributes to both the presystemic and the systemic elimination of many drugs.

- Often other tissues, as well.
  e.g., in intestinal mucosa cells → presystemic elimination of several drugs in renal tubular cells, etc

- The colon, by bacteria - e.g., azo reduction, hydrolytic reactions
## BIOTRANSFORMATION: classification

<table>
<thead>
<tr>
<th>Phase I reactions</th>
<th>Phase II reactions (conjugations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Oxidation</td>
<td>1. Glucuronidation</td>
</tr>
<tr>
<td>2. Reduction</td>
<td>2. Sulfation</td>
</tr>
<tr>
<td>3. Hydrolysis</td>
<td>3. Conjugation with glycine (Gly)</td>
</tr>
<tr>
<td></td>
<td>4. Conjugation with glutathione (GSH)</td>
</tr>
<tr>
<td></td>
<td>5. Acetylation</td>
</tr>
<tr>
<td></td>
<td>6. Methylation</td>
</tr>
</tbody>
</table>

### The chemical role of Phase I and Phase II biotransformations:

- **Phase I:** A functional group is added to the molecule or explored in the molecule at which conjugation can take place.
- **Phase II:** An organic acid (or acetyl or methyl group) is conjugated to the molecule at a preexisting functional group or at a functional group acquired in Phase I biotransformation.

### General examples

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Conjugates</th>
</tr>
</thead>
<tbody>
<tr>
<td>RH → oxidation R-O</td>
<td>R-O-glucuronic acid</td>
</tr>
<tr>
<td></td>
<td>R-O-sulfonic acid</td>
</tr>
<tr>
<td>R-HC=O → reduction R-CH₂-O</td>
<td>R-CH₂-O-glucuronic acid</td>
</tr>
<tr>
<td></td>
<td>R-CH₂-O-sulfonic acid</td>
</tr>
<tr>
<td>R₁-O-(C=O)-R₂ → hydrolysis R₁</td>
<td>R-O-glucuronic acid</td>
</tr>
<tr>
<td></td>
<td>R-O-sulfonic acid</td>
</tr>
</tbody>
</table>

### The pharmacological role of Phase I and Phase II biotransformations:

**How the fate and effect of metabolites compare to the fate and effect of the parent drug?**

<table>
<thead>
<tr>
<th>Phase I metabolites</th>
<th>Phase II metabolites (conjugates)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water-solubility:</strong></td>
<td><strong>Water-solubility:</strong> for 1-4: [\bigarrow\bigarrow\bigarrow\bigarrow] (rapid excr.) for 5-6: [\downarrow] (slower excretion)</td>
</tr>
<tr>
<td><strong>Biological activity:</strong> in general [\downarrow] but often [\uparrow]</td>
<td><strong>Biological activity:</strong> almost always [\downarrow] very seldom [\uparrow]</td>
</tr>
</tbody>
</table>
Part A. BIOTRANSFORMATION REACTIONS

1. PHASE I BIOTRANSFORMATIONS

1.1. OXIDATION

1.1.1. Microsomal oxidation

1.1.1.1. CYP-catalyzed reactions

1.1.1.1.1. Oxigenation

1.1.1.1.1.1. Insertion of oxygen produces a stable oxigenated metabolite

1.1.1.1.1.1. C-hydroxylation: aliphatic hydroxylation, aromatic hydroxylation

1.1.1.1.1.2. N-hydroxylation

1.1.1.1.1.3. Epoxidation

1.1.1.1.1.2. Insertion of oxygen produces an unstable oxigenated metabolite which undergoes spontaneous cleavage into two molecules

1.1.1.1.2.1. Oxidative dealkylation (N- and O-dealkylation)

1.1.1.1.2.2. Oxidative deamination

1.1.1.1.2.3. Oxidative dehalogenation

1.1.1.1.2.4. Oxidative desulfuration

1.1.1.1.2. Dehydrogenation

1.1.1.1.3. Reduction (reductive dehalogenation)

1.1.1.2. FMO-catalyzed oxidations

1.1.2. Non-microsomal oxidation

1.1.2.1. MAO-catalyzed oxidations

1.1.2.2. Oxidations catalyzed by molydenum-containing oxidases

1.1.2.2.1. Xanthine oxidase-catalyzed oxidations

1.1.2.2.2. Aldehyde oxidase-catalyzed oxidations

1.1.2.3. Alcohol dehydrogenase and aldehyde dehydrogenase-catalyzed oxidations

1.2. REDUCTION

1.2.1. Azo-reduction

1.2.2. Nitro-reduction

1.2.3. Carbonyl-, aldehyde- and aldose-reduction (by aldo-keto reductases; AKR)

1.3. HYDROLYSIS

1.3.1. Hydrolysis by carboxylesterases

1.3.2. Hydrolysis by alkaline phosphatase

1.3.3. Hydrolysis by paraoxonases

1.3.4. Hydrolysis by epoxide hydrolase (illustrated under epoxidation)

1.3.5. Hydrolysis by microbial hydrolases in the colon

2. PHASE II BIOTRANSFORMATIONS (CONJUGATIONS)

2.1. GLUCURONIDATION

2.2. SULFATION

2.3. CONJUGATION WITH GLYCINE

2.4. CONJUGATION WITH GLUTATHIONE (Glu-Cys-Gly)

2.5. ACETYLATION

2.6. METHYLATION
PHASE I BIOTRANSFORMATIONS

OXIDATION - summary

<table>
<thead>
<tr>
<th>MICROSONAL catalyzed by:</th>
<th>NON-MICROSOMAL catalyzed by:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Cytochrome P-450 (CYP)</td>
<td>• Monoamino-oxidases (MAO) - mitochondrial</td>
</tr>
<tr>
<td>• Microsomal flavine-containing monooxigenase (FMO)</td>
<td>• Mo-containing oxidases (cytosolic): Xanthine oxidase (XO) and Aldehyde oxidase (AOX)</td>
</tr>
<tr>
<td></td>
<td>• Alcohol- and aldehyde dehydrogenases (ADH, ALDH) - cytosolic</td>
</tr>
</tbody>
</table>

OXIDATIONS CATALYZED BY CYTOCHROME P-450 (CYP)

CYP is a heme-containing protein embedded in the membranes of the smooth endoplasmic reticulum (SER), the fragments of which in a tissue homogenate are sedimented after ultracentrifugation (at 100,000g) in the microsomal fraction.

Origin of the name cytochrome P-450:
• Cytochrome: it is a coloured intracellular protein
• P: it is pink
• 450: its absorption spectrum has a maximum at 450 nm

CYP constitutes a superfamily of enzymes that are classified into families (numbered) and subfamilies (marked with capital letters), the latter of which contain the individual enzymes (numbered), e.g., CYP1A2, CYP2C9, CYP2D6, CYP3A4.
**CYTOCHROME P-450 (CYP)-CATALYZED REACTIONS – Mechanisms**

CYP is an extremely versatile enzyme as it can catalyze numerous types of reaction, out of which three types are presented below.

**Oxygenation** involves insertion of an O atom (from O\(_2\)) into a C-H bond, forming a hydroxylated metabolite, or into a C=C double bond, forming an epoxide. A hydroxylated metabolite may be stable, or unstable. From an unstable hydroxylated metabolite a group may break off spontaneously: an alkyl group, ammonia, a halogen atom, or sulfur atom; such reactions are called oxidative dealkylation, oxidative deamination, oxidative dehalogenation, and oxidative desulfuration, respectively.

CYP can also catalyze **dehydrogenation**, i.e. removal of 2 H atoms from a drug molecule (this is how the reactive hepatotoxic paracetamol metabolite is formed).

Surprisingly, CYP may also catalyze **reduction**, by transferring only 1 electron to a compound (e.g. in a reductive dehalogenation reaction) or as many as 6 electrons to a nitro group, thus converting it into an amino group (nitro reduction). As catalyzed by CYP, these reductions are also shown here.

<table>
<thead>
<tr>
<th><strong>I. OXYGENATION</strong> - the most typical CYP-catalyzed reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General scheme:</strong> RH + O(_2) + (NADPH + H(^+)) (\rightarrow) R-OH + HOH + NADP(^+)</td>
</tr>
<tr>
<td><strong>Note:</strong> 1 atom of O(_2) is inserted into the drug, whereas the 2nd O atom is reduced to HOH by NADPH-CYP red.</td>
</tr>
<tr>
<td><strong>Catalytic cycle</strong> (simplified):</td>
</tr>
<tr>
<td><img src="image" alt="Catalytic cycle diagram" /></td>
</tr>
</tbody>
</table>

**II. DEHYDROGENATION** e.g. acetaminophen (=paracetamol), nifedipine, ethanol

**III. REDUCTION** e.g. nitro reduction of clonazepam; reductive dehalogenation of CCl\(_4\)
CYP-CATALYZED REACTIONS – Overview

OXYGENATION (insertion of one O atom) - two variations:

1. INSERTION OF O PRODUCES A STABLE METABOLITE:
   a. Hydroxylation
      • C-hydroxylation (insertion of O into a C-H bond to form a hydroxyl group):
        - Aliphatic hydroxylation: tolbutamide (-), terfenadine (+), cyclophosphamide (+)
        - Aromatic hydroxylation: warfarin (-), phenytoin (-), propranolol (-)
      • N-hydroxylation (insertion of O into an N-H bond): dapsone (†)
   b. Epoxidation (insertion of O into a C=O bond to form an epoxide): carbamazepine (-/o)

2. INSERTION OF O PRODUCES AN UNSTABLE METABOLITE WHICH SPONTANEOUSLY BRAKES INTO TWO MOLECULES:
   a. Oxidative dealkylation (→ dealkylated metabolite + an aldehyde):
      • N-dealkylation: Diazepam → nordiazepam (+),
        Carisoprodol → meprobamate (+),
        Amitriptyline → nortriptyline (+) → desmethyl-nortriptyline (-)
        Lidocaine → monoethylglycylxylidine (+) → glycylxylidine (-)
        Caffeine → paraxanthine (-), or theophylline, or theobromine
      • O-dealkylation: Codeine → morphine (+)
        Dextromethorphan → dextrorphan (=D form of levorphanol) (+)
        Phenacetin → acetaminophen (=paracetamol) (+)
   b. Oxidative deamination (→ O-containing metabolite + NH₃):
        Amphetamine → phenylacetone (-)
   c. Oxidative dehalogenation (→ O-containing metabolite + HBr):
        Halothane → trifluoroacetyl chloride (†)
   d. Oxidative desulfuration (→ O-containing metabolite + S):
        Thiopental → pentobarbital (+),
        Parathion → paraoxon (+); thio-TEPA → TEPA (+)

DEHYDROGENATION (removal of 2 H atoms):
• Acetaminophen → N-acetylbenzoquinone imine (†)
• Nifedipine (a dihydropyridine parent compound) → "pyridine" metabolite (-)

REDUCTION (reductive dehalogenation, nitro reduction):
• CCl₄ → CCl₃ (+) + Cl⁻; Halothane → debrominated halothane free radical (†) + Br⁻
• For reduction of nitro group of clonazepam (or nitrazepam) to amino group by CYP, see under nitro reduction (6-electron reduction)

+ = active metabolite; – = inactive metabolite; † = toxic metabolite
CYP-CATALYZED REACTIONS:
1. INSERTION OF O PRODUCES A STABLE METABOLITE

HYDROXYLATION = insertion of O into a C-H or N-H bond

a) C-HYDROXYLATION: aliphatic

Tolbutamide (antidiabetic)

\[
\text{HO} - \text{CH}_2 - \text{SO} - \text{NH} - \text{C} - \text{NH} \rightarrow \text{HO} - \text{CH}_2 - \text{SO} - \text{NH} - \text{C} - \text{NH}
\]

Hydroxymethyl-tolbutamide (inactive)

Ibuprofen (NSAID)

\[
\text{HOOC} - \text{CH} - \text{CH}_2 - \text{NH}_2 \rightarrow \text{HOOC} - \text{CH} - \text{CH}_2 - \text{OH}
\]

2-hydroxy-ibuprofen

CONJUGATES

3-hydroxy-ibuprofen

b) C-HYDROXYLATION: aromatic

Phenytoin (antiepileptic, antiarrhythmic)

\[
\text{HO} - \text{CH} - \text{NH} - \text{C} - \text{NH} \rightarrow \text{HO} - \text{CH} - \text{NH} - \text{C} - \text{NH}
\]

4'-Hydroxyphenytoin (inactive)

Warfarin (anticoagulant)

\[
\text{HO} - \text{CH}_2 - \text{C} - \text{CH}_3 \rightarrow \text{HO} - \text{CH}_2 - \text{C} - \text{CH}_3
\]

7-hydroxy-warfarin (inactive)

c) N-HYDROXYLATION

Dapsone (antileprotic)

\[
\text{H}_2 \text{N} - \text{SO} - \text{NH}_2 \rightarrow \text{H}_2 \text{N} - \text{SO} - \text{NH}_2
\]

Dapsone-hydroxylamine (hemotoxic and allergenic)
**EPOXIDATION** = insertion of O into a C=C double bond

**Carbamazepine**
(antiepileptic)

**Carbamazepine**
(less active metabolite)

**benzo(a)pyrene**

**benzo(a)pyrene-7,8-oxide**

**benzo(a)pyrene-7,8-dihydro-diol**

**benzo(a)pyrene-7,8-dihydro-diol-9,10-epoxide**

"ultimate carcinogen"

**NOTE:**
Leucotriene A4 (LTA4) is also an epoxide!
LTA4 is converted into either LTB4 (a diol) by *epoxide hydrolase*, or into LTC4 (a GSH conjugate) by *glutathione S-transferase*. 
CYP-CATALYZED REACTIONS:
2. INSERTION OF O PRODUCES AN UNSTABLE METABOLITE WHICH UNDERGOES SPONTANEOUS CLEAVAGE INTO TWO MOLECULES

First, the drug becomes hydroxylated at the C atom of the alkyl group that is linked to the N (or the O) atom. This hydroxylated metabolite is unstable. It breaks spontaneously into two molecules: the dealkylated metabolite (e.g., an amine or alcohol/phenol), and an aldehyde (e.g., formaldehyde after demethylation, acetaldehyde after deethylation, etc.).

N-dealkylation

**General scheme:**

![Diagram showing N-dealkylation](image_url)

**Examples:**

![Diagram showing examples](image_url)

**Other examples:**

- lidocaine → monoethylglycylxylidine
- imipramine → desmethylimipramine
- erythromycin → desmethylerthyromycin

Theophylline may undergo:
- N-demethylation at N1 and N3 by CYP
- C-hydroxylation at C8 by CYP
- N-methylation at N7 by MT

In neonates more caffeine is formed from theophylline than in adults because the CYP levels in the neonatal liver are low, however, the methyltransferase (MT) levels are relatively high.
O-dealkylation

**General scheme:**

\[ R-O-CH_3 \rightarrow [R-O-CH_2] \rightarrow R-OH \rightarrow R-OH + HCHO \]

NOTE: After hydroxylation at the O-bound C atom of the alkyl group, the alkyl group breaks off (as an aldehyde) from the O atom, producing a dealkylated *hydroxylated metabolite* of the drug molecule.

**Example:**

\[
\begin{align*}
\text{CH}_3-O & \quad \text{(antitussive drug)} \\
\text{Dextromethorphan} & \\
\text{N-CH}_3 & \\
\text{CYP2D6} & \quad HCHO \\
\text{Dextrorphan} & \quad \text{(active metabolite)} \\
\text{N-CH}_3 &
\end{align*}
\]

*Other examples:*

- codeine $\rightarrow$ morphine
- phenacetine $\rightarrow$ paracetamol
**Oxidative deamination**

\[
\begin{align*}
&\text{Amphetamine} \\
&\text{(centrally acting sympathomimetic)}
\end{align*}
\]

\[
\begin{align*}
&\text{CYP} \\
&\text{OH} \\
&\text{NH}_2 \\
&\text{CYP} \\
&\text{Phenylacetone} \\
&\text{(inactive)}
\end{align*}
\]

**Oxidative dehalogenation**

\[
\begin{align*}
&\text{Halothane} \\
&\text{(inhalation anesthetic)}
\end{align*}
\]

\[
\begin{align*}
&\text{Trifluoro-acetyl-chloride} \\
&\text{binds covalently to hepatic proteins,} \\
&\text{thus forming a neoantigene and} \\
&\text{causing "halothane hepatitis"}
\end{align*}
\]

**Oxidative desulfuration**

\[
\begin{align*}
&\text{Parathion} \\
&\text{(an organophosphate insecticide)}
\end{align*}
\]

\[
\begin{align*}
&\text{Paraoxon} \\
&\text{(active insecticide,} \\
&\text{an irreversible inhibitor of} \\
&\text{acethylcholinesterase)}
\end{align*}
\]

\[
\begin{align*}
&\text{Thiopental} \\
&\text{(an i.v. anesthetic)}
\end{align*}
\]

\[
\begin{align*}
&\text{Pentobarbital} \\
&\text{(active as hypnotic)}
\end{align*}
\]

The arrows point to the bond into which an O atom is inserted, which then promotes cleavage of the molecule to release NH\textsubscript{3}, HBr, or sulfur.
CYP-CATALYZED REACTIONS: 3. DEHYDROGENATION

General scheme:

6 \[(\text{FeO})^{3+} \quad \text{RH}_2\] \rightarrow \text{Fe}^{3+} 7 \[\text{HOH}\]

NOTE: While an oxygenation produces 1 molecule hydroxylated metabolite plus 1 molecule HOH, dehydrogenation produces 2 molecules HOH.

Examples:

- Acetaminophen 
  Analgetic and antipyretic drug

- N-Acetyl-p-benzoquinoneimine (NAPBQI) 
  Hepatotoxic

- Nifedipine 
  A dihydropyridine Ca^{++}-channel blocker

"Pyridine metabolite" 
  Inactive

Other example: Dehydrogenation of nicotine to nicotine $\Delta^{1(5)}$ iminium ion (see under aldehyde oxidase)

CYP-CATALYZED REACTIONS: 4. REDUCTION

In CYP-catalyzed reduction, the second electron is not transferred to the CYP-bound O$_2$, but to the CYP-bound substrate, e.g. carbon tetrachloride or clonazepam (see also under nitro reduction). With carbon tetrachloride, its reduced intermedier then undergoes a homolytic cleavage to form a free radical and a chloride anion. The reactive trichloromethyl free radical formed in the liver, can initiate lipid peroxidation in the liver cell membranes and can thus induce hepatic necrosis.

General scheme:

4 \[\text{Fe}^{2+} \quad \text{O}_2 \quad \text{RH}\] \rightarrow \text{Fe}^{2+} 5 \[\text{O}_2 \quad \text{[RH]}^{-}\]

Examples:

1. Reductive dehalogenation of carbon tetrachloride (1-electron reduction):

2. Nitro-reduction of clonazepam to 7-amino-clonazepam (6-electron reduction):

NOTE for those interested: Homolytic cleavage occurs when a covalent bond (formed by two electrons shown as :) is cleaved in a way so that one electron remains with one of the breakage products, whereas the other electron will belong to the other breakage product. Thus, homolytic cleavage yields products with an unpaired electron (called free radicals), which are reactive.
**OXIDATIONS CATALYZED BY MICROsomAL FLAVIN-CONTAINING MONooXYGENASE (FMO)**

**FMO-CATALYZED OXIDATIONS** – Overview

Take place on *heteroatoms* in drugs:

**Oxidation of $S$ atom:** Cimetidine $\rightarrow$ cimetidine-S-oxide

**Oxidation of tert. $N$ atom:** Nicotine $\rightarrow$ nicotine-1’-N-oxide

Trimethylamine $\rightarrow$ trimethylamine N-oxide

*(Deficiency: Fish-odor syndrome)*

---

**OXIDATIONS CATALYZED BY FMO - Examples:**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Reaction</th>
<th>Product</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cimetidine (an H2-receptor antagonist)</td>
<td>$\text{FMO}$</td>
<td>Cimetidine S-oxide (inactive)</td>
<td></td>
</tr>
<tr>
<td>Nicotine</td>
<td>$\text{FMO}$</td>
<td>Nicotine-1’-N-oxide (inactive)</td>
<td></td>
</tr>
<tr>
<td>Trimethylamine</td>
<td>$\text{FMO3}$</td>
<td>Trimethylamine N-oxide</td>
<td>(water-soluble metabolite excreted in urine)</td>
</tr>
</tbody>
</table>

*Trimethylamine* is an endogenous volatile compound; it accumulates in FMO3 deficiency, causing *fish odor syndrome*. 
### OXIDATIONS CATALYZED BY NON-MICROSOMAL ENZYMES

#### NON-MICROSOMAL OXIDATIONS – Overview

The oxygen incorporated into the substrate is derived from HOH rather than O<sub>2</sub>.

#### OXIDATION CATALYZED BY MAO

MAO catalyzes oxidative deamination of amines.

*Examples:*
- Dopamine → 3,4-dihydroxy-phenylacetaldehyde
- Norepinephrine → 3,4-dihydroxy-mandelic aldehyde (-)
- N-deisopropyl-propranolol → the respective aldehyde (-)
- MPTP → MPP⁺(†) – kills the DAergic neurons, causing Parkinson’s disease

#### OXIDATIONS CATALYZED BY MOLYBDENUM-CONTAINING OXIDASES:

- **XANTHINE OXIDASE-CATALYZED OXIDATION:**
  *Examples:*
  - Hypoxanthine → xanthine → uric acid
  - Allopurinol → alloxanthine (compet. inhibition of uric acid formation)
  - 6-Mercaptopurine → 6-thiouric acid (-)

- **ALDEHYDE OXIDASE-CATALYZED OXIDATION:**
  *Examples:*
  - Nicotine Δ¹(⁵) iminium ion → cotinine
  - Benzaldehyde → benzoic acid
  - 3-Methoxy 4-hydroxy-mandelic aldehyde → VMA

#### OXIDATIONS CATALYZED BY ALCOHOL- AND ALDEHYDE DEHYDROGENASES:

*Examples:*
- Methanol → formaldehyde → formic acid (†) – causes blindness
- Ethanol → acetaldehyde (†) → acetic acid
- Ethylene glycol →
  - glycolic acid (†) → glyoxylic acid → oxalic acid (†) – cause renal injury
- Chloral (= trichloroacetaldehyde) → trichloroethanol (+) – ADH in the reverse reaction (using NADH) can reduce chloral; ethanol facilitates this by increasing NADH supply.
MAO-CATALYZED OXIDATIVE DEAMINATION OF AMINES INTO ALDEHYDES

General scheme:

Dopamine

1. R-CH₂NH₂ + FAD > R-CH=NH + FADH₂
   Dehydrogenation
2. R-CH=NH + H₂O > R-CH(OH)-NH₂
   Hydration
3. R-CH(OH)-NH₂ > R-CH=O + NH₃
   Deamination
4. FADH₂ + O₂ > FAD + H₂O₂
   FAD

FOUR STEPS - the byproducts are ammonia and HOOH!

Example:

Propranolol

N-Desisopropylpropranolol

4-Hydroxypropranolol

Oxidation by ALDH
Reduction by AR

Oxidation by AOX
Reduction by AR

Carboxylic acid
Glycol
OXIDATIONS CATALYZED BY Mo-CONTAINING OXIDASES, XANTHINE OXIDASE (XO) and ALDEHYDE OXIDASE (AOX)

**Mechanism of oxygenation by XO and AOX** – two steps:

NOTE: The oxygen atom incorporated into the substrate is derived from water rather than O₂.
1. A water molecule is added into the parent compound, forming an intermediary metabolite.
2. Thereafter, 2 H atoms are removed from the intermediary metabolite by molecular oxygen (O₂), thus forming hydrogen peroxide (HOOH) and the oxygenated metabolite, which carries now a keto-group or a carboxylic acid group. These 2 steps are shown only in the reactions presented for AOX. For simplicity, they are not shown in the reactions presented for XO.

**Oxygenations catalyzed by XO and/or AOX:**

1. **Oxygenation of a C atom double-bonded to a N atom in a heterocyclic ring,** such as:
   - in purines, e.g. hypoxanthine and xanthine (preferred by XO),
   - in purine analogues, e.g. 6-mercaptopurine and 6-thioxanthine (preferred by XO),
   - in zaleplone (preferred by AOX)
   - in the dehydrogenated nicotine metabolite, nicotine-\(\Delta^{1(5)}\)-iminium ion (mainly by AOX)

2. **Oxygenation of aromatic (but not aliphatic) aldehydes,** such as benzaldehyde (product of benzyl alcohol oxidation by ADH – see gasping syndrome).

### XANTHINE OXIDASE-CATALYZED OXIDATIONS

![Xanthine Oxidase-Catalyzed Oxidations Diagram](image)

Administration of the XO inhibitor allopurinol to patients treated with 6-mercaptopurine (6MP) would decrease the elimination of 6-mercaptopurine and in turn could result in 6MP-induced toxicity, i.e. bone marrow depression. **Therefore, allopurinol is contraindicated for the 6-mercaptopurine-treated patient even if hyperuricemia developed.** Patients have died because this contraindication was disregarded by ignorant physicians!

**NOTE:** 6MP can also be eliminated by methylation at the SH group by thiopurine methyltransferase (TPMT, see later). TPMT deficiency increases the toxicity of 6MP, just like cotreatment with allopurinol does.
**ALDEHYDE OXIDASE-CATALYZED OXIDATIONS**

**Zaleplon**
short-acting hypnotic

NOTE: Zaleplon is also N-deethylated by CYP3A.

**Nicotine**
nicotine-Δ¹(5')-iminium ion

**Benzaldehyde**
formed from benzylic alcohol,
(see gasping syndrome)

**NOTE:**
Cotinine is the main metabolite of nicotine and as such it may be used as a biomarker of nicotine exposure (i.e. smoking). Cotinine is conveniently analyzed from the saliva of the smoker.
ALCOHOL DEHYDROGENASE (ADH)- AND ALDEHYDE DEHYDROGENASE (ALDH)-CATALYZED OXIDATIONS

**Ethanol**

\[ \text{CH}_3\text{CH}_2\text{OH} \xrightarrow{\text{ADH}} \text{CH}_3\text{C}==\text{H} \xrightarrow{\text{ALDH}} \text{CH}_3\text{CO}_2\text{H} \]

Acetaldehyde

\[ \text{ADH} \]

Acetic acid

**Methanol**

\[ \text{CH}_3\text{OH} \xrightarrow{\text{ADH}} \text{H}==\text{C}==\text{H} \xrightarrow{\text{ALDH}} \text{H}==\text{C}==\text{O} \]

Formaldehyde

\[ \text{ALDH} \]

Formic acid

**Ethylene glycol**

\[ \text{CH}_2\text{OH} \xrightarrow{\text{ADH}} \text{H}==\text{C}==\text{O} \xrightarrow{\text{ALDH}} \text{COOH} \]

Glycolaldehyde

\[ \text{ALDH} \]

Glycolic acid

**Acidic metabolites cause acidosis and renal injury**

3,4-Dihydroxy-phenylacetaldehyde (DOPAL) formed by MAO in dopaminergic neurons

\[ \text{ADH} \]

3,4-Dihydroxy-phenylacetic acid (DOPAC)

Vanillyl mandelic aldehyde formed by COMT and MAO from epinephrine and NE

Vanillyl mandelic acid (VMA)

Fomepizole (=4-methylpyrazole; ANTIZOL®) is a potent competitive inhibitor of ADH and is the most reliable antidote in poisonings with methanol or ethylene glycol. Fomepizole is also used to alleviate the acetaldehyde syndrome caused by ethanol consumption in disulfiram-treated patients.

2. As shown in Part 6 (Appendix-2), disulfiram is biotransformed into an electrophilic metabolite which covalently binds to and irreversibly inhibits aldehyde dehydrogenase. For this reason, a disulfiram-treated individual should not consume alcohol for at least a week after discontinuation of disulfiram administration.

3. Genetic and gender variations in the biotransformation of ethanol are presented in Part 6, Appendix-3.

4. Elimination of the potentially toxic DOPAL by ALDH from nigrostriatal dopaminergic neurons is a protective mechanism. Inhibition of ALDH by some pesticides (e.g. the fungicide benomyl) has been speculated to be a mechanism of pesticide-induced Parkinson disease.

5. VMA is the final product of the biotransformation of epinephrine (E) and norepinephrine (NE) via the COMT – MAO – ALDH pathway. Thus, VMA is the biomarker of E and NE production. Urinary excretion of large amounts of VMA is a diagnostic sign for pheochromocytoma.
## REDUCTION – Overview

### AZO-REDUCTION \((R_1-N=N-R_2 \rightarrow R_1-NH_2 + R_2-NH_2)\)

catalyzed by *microbial enzymes* in the colon

- Prontosyl → sulfanilamide + triaminobenzene
- Sulfasalazine (salicylazosulfapyridine)
  → 5-aminosalicylic acid + sulfapyridine

### NITRO-REDUCTION \((R-NO_2 \rightarrow R-NH_2)\):

catalyzed by *CYP*

- Clonazepam → 7-amino-clonazepam
- Chloramphenicol → an arylamine metabolite (†)

### CARBONYL-, ALDEHYDE-, and ALDOSE-REDUCTION \((R-C=O \rightarrow R-C-OH)\):

catalyzed by cytosolic enzymes of the *aldo-keto reductase (AKR)* superfamily, using NADPH

- Haloperidol → reduced haloperidol
- Oxcarbazepin → 10-hydroxy-carbazepin (+)
- Doxorubicin → doxorubicinol (†)
- 3,4-Dihydroxyphenylacetaldehyde (DOPAL; produced from DA by MAO)
  → 3,4-dihydroxyphenylethanol (DOPET)
**REDUCTION**

**AZO-REDUCTION** \[R-N=N-R' \rightarrow R-NH_2 + R'-NH_2\]

\[
\begin{align*}
\text{Prontosil} & \quad (\text{Gerhard Domagk, Nobel Prize, 1939}) \\
& \quad \text{tiraminobenzene} \\
\text{sulfanilamide} & \quad (\text{antibacterial, the prototype of sulfonamides})
\end{align*}
\]

\[
\begin{align*}
\text{Sulfasalazine} (\text{Salicylazosulfapyridine}) & \quad (\text{to treat ulcerative colitis}) \\
\text{5-aminosalicylic acid} & \quad (\text{antiinflammatory}) \\
\text{sulfapyridine}
\end{align*}
\]

**NITRO-REDUCTION** \[(R-\text{NO}_2) \rightarrow R-\text{NO} \rightarrow R-\text{NHOH} \rightarrow R-\text{NH}_2\]

**Clonazepam** (anxiolytic and antiepileptic effects)

\[
\begin{align*}
& \quad \text{Clonazepam} \\
& \quad \text{Haloperidol} (\text{antipsychotic}) \\
& \quad \text{Oxcarbazepine} (\text{antiepileptic, inactive prodrug}) \\
& \quad \text{Doxorubicin} (\text{antitumor drug})
\end{align*}
\]

\[
\begin{align*}
& \quad \text{Haloperidol} (\text{inactive}) \\
& \quad \text{7-amino-clonazepam} (\text{inactive}) \\
& \quad \text{10-hydroxycarbazepine} (\text{active}) \\
& \quad \text{doxorubicinol} (\text{cardiotoxic})
\end{align*}
\]

\[
\begin{align*}
\text{AKR} & \quad \text{aldo-keto reductase}
\end{align*}
\]
HYDROLYSIS – Overview

BY CARBOXYLESTERASES (CES, microsomal enzymes) and/or BUTYRYLCHOLINESTERASES (BChE, soluble enzymes, also in plasma)

- HYDROLYSIS OF CARBOXYLIC ACID ESTERS: rapid
  Examples:
  - Drugs: Succinylcholine, mivacurium, procaine, tetracaine, cocaine, esmolol, meperidine, remifentanil, acetylsalicylic acid, clopidogrel, oseltamivir – the active drugs are converted into inactive (or less active) metabolites
  - Prodrugs: Fibrate esters, e.g. fenofibrate (-) → fenofibric acid (+); ACE-inhibitor esters, e.g. enalapril (-) → enalaprilat (+) Antipsychotic esters, e.g. pipothiazine palmitate (-) → pipothiazine (+) Mycophenolate mofetil (-) → mycophenolic acid (+) – by CES Bambuterol → terbutaline (+); Also: heroin (+) → morphine (+)

- HYDROLYSIS OF CARBOXYLIC ACID AMIDES: slow
  Example: Procainamide

BY ALKALINE PHOSPHATASES: hydrolyzes phosphoric acid monoesters
Examples (i.v. injectable prodrugs): Fosphenytoin (-) → phenytoin (+) Fospropofol (-) → propofol (+) Clindamycin phosphate (-) → clindamycin (+)

BY PARAOXONASES (Lactonases)
- HYDROLYSIS OF PHOSPHORIC ACID TRI-ESTERS
  Examples: Paraoxon and other organophosphate insecticides
- HYDROLYSIS OF LACTONES
  Examples: Statins, e.g. lovastatin, or simvastatin (lactone) → hydroxy-acid (+), Spironolactone → hydroxy-acid (-)

BY EPOXIDE HYDROLASE: hydrolyzes the epoxide into dihydrodiols
Examples: Carbamazepine-epoxide (little-reactive epoxide, active metabolite) Benzpyrene-epoxide (DNA-reactive epoxide, mutagenic and carcinogenic)
NOTE: Leucotriene A₄ (LTA₄) is also an epoxide! It is converted into either LTB₄ (a diol) by epoxide hydrolase, or into LTC₄ (a GSH conjugate) by glutathione S-transferase.

BY PANCREATIC LIPASE IN THE SMALL INTESTINE
Example: castor oil (ricinoleic acid-containing triglyceride) → ricinoleic acid (+)

BY MICROBIAL HYDROLASES IN THE COLON
Examples (laxative prodrugs): Sennoside A, bisacodyl, sodium picosulfate

(-) = inactive metabolite, (+) = active metabolite, ASA = acetylsalicylic acid

NOTE: BChE is protective against organic phosphate ester insecticides and “nerve agents” whose oxone form phosphorylates and irreversibly inactivates not only acetylcholinesterase (causing the toxicity) but also BChE, which has no role in the toxic action. Thus BChE represents a “silent binding site” for OP insecticides and chemical warfare agents by preventing their binding to the target of toxic action, i.e. the neuronal acetylcholinesterase.
Some drugs (e.g., enalapril) containing carboxyl groups are esterified with an alcohol to make them more lipophilic for better absorption by diffusion from the GI tract. After absorption, such a drug is readily hydrolyzed by carboxylesterase and the original organic acid (e.g., enalaprilate from enalapril) is released. Enalapril is an inactive drug (i.e., prodrug), whereas enalaprilate is the active ACE inhibitor metabolite. Similarly, the lipophilic heroin readily diffuses into brain, where its hydrolysis produces morphine.
NOTE: Fosphenytoin, fospropofol and clindamycin phosphate are phosphate ester prodrugs that were synthesized in order to convert the poorly water-soluble phenytoin, propofol and clindamycin, respectively, into water-soluble, i.v.-injectable forms.

As phenytoin does not contain a hydroxyl group at which to esterify it with phosphoric acid, and the hydroxyl group of propofol is sterically hindered from phosphoric acid by the neighboring isopropyl groups, first a $-\text{CH}_2\text{-OH}$ group had to be linked to these molecules. This linker group breaks off spontaneously as formaldehyde after the AP-catalyzed hydrolysis.
**HYDROLYSIS BY PARAOXONASES (PON)**

**Hydrolysis of phosphoric acid tri-esters:**

\[
\begin{align*}
\text{Paraoxon} & \quad \xrightarrow{\text{PON}} \quad \text{active metabolite of parathion, an OP insecticide, irreversible acetylcholinesterase inhibitor} \\
\end{align*}
\]

**Hydrolysis of lactone rings:**

\[
\begin{align*}
\text{Lovastatin} & \quad \xrightarrow{\text{PON}} \quad \text{active lovastatin} \\
\text{Spironolactone} & \quad \xrightarrow{\text{PON}} \quad \text{inactive spironolacton} \\
\end{align*}
\]

(HMG-CoA reductase inhibitor, cholesterol lowering drug)  
(aldosterone antagonist, K-sparing diuretic)

**Notes:**

- PON is also called aromatic esterase 1 or serum aryl-dialkylphosphatase 1
- PON1 is synthesized in the liver and transported along with HDL in the plasma. It functions as an antioxidant; it is an anti-atherosclerotic component of HDL.
Hydrolysis produces stimulatory laxatives in the colon

**Sennoside A**
- **antraquinone glycoside** (prodrug)
- Hydrolysis: 2 HOH → 2 glucose
  - **active antraquinone aglycone**

**Bisacodyl**
- **Diacetic acid ester of diphenol-methane laxative (prodrug)**
- Hydrolysis: 2 HOH → 2 acetate
  - **Active diphenol-methane laxative metabolite**

**Sodium picosulfate**
- **Disulfonic acid ester of diphenol-methane laxative (prodrug)**
- Hydrolysis: 2 HOH → 2 sulfate
  - **Active diphenol-methane laxative metabolite**
### Phase II Biotransformations (Conjugations)

#### Glucuronidation

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cosubstrate</th>
<th>Substrates:</th>
<th>OH-containing compounds:</th>
<th>COOH-containing compounds:</th>
<th>N-containing drugs:</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-glucuronosyl transferases</td>
<td>UDP-glucuronic acid</td>
<td>drugs, endogenous compounds</td>
<td>morphine, paracetamol, chloramphenicol, oxazepam, phenol, propofol, diethylstilbestrol, estradiol, thyroxine</td>
<td>valproic acid, diclofenac, furosemide, telmisartan, bilirubin</td>
<td>lamotrigine</td>
</tr>
<tr>
<td>(in the endoplasmic reticulum = microsomes)</td>
<td></td>
<td></td>
<td>6-OH phenytoin, etc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N-OH-dapsone</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Sulfation

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cosubstrate</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfotransferases</td>
<td>PAPS</td>
<td>OH-containing compounds: pravastatin + see above</td>
</tr>
<tr>
<td>(in cytosol)</td>
<td></td>
<td>OH-containing metabolites: see above</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N-oxide-containing compounds: minoxidil</td>
</tr>
</tbody>
</table>

#### Conjugation with Glycine

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cosubstrate</th>
<th>Substrates:</th>
<th>COOH-containing compounds:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyl-CoA synthetase + Glycine N-acyltransf.</td>
<td>ATP + CoA-SH + Glycine</td>
<td>salicylic acid, benzoic acid, nicotinic acid</td>
<td></td>
</tr>
<tr>
<td>(in mitochondrial matrix)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Conjugation with Glutathione (Glu-Cys-Gly)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cosubstrate</th>
<th>Substrates:</th>
<th>Electrophilic C-containing compounds:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione S-transf.</td>
<td>Glutathione (glu-cys-gly)</td>
<td>ethacrinic acid, epoxides (e.g. LTA&lt;sub&gt;4&lt;/sub&gt;)</td>
<td></td>
</tr>
<tr>
<td>(in cytosol)</td>
<td></td>
<td></td>
<td>Electrophilic C-containing metabolites:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N-acetyl-p-benzoquinone imine, acrolein</td>
</tr>
</tbody>
</table>

#### Acetylation

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cosubstrate</th>
<th>Substrates:</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetyltransferases</td>
<td>Acetyl-CoA</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;-containing compounds:</td>
</tr>
<tr>
<td>(in cytosol)</td>
<td></td>
<td>NAT1 substrates: p-aminobenzoic acid, sulfamethoxazol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NAT2 substrates: isoniazid, procainamide, dapsone, hydralazine, sulfamethazine</td>
</tr>
</tbody>
</table>

#### Methylation

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cosubstrate</th>
<th>Substrates:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyltransferases</td>
<td>S-adenosylmethionine</td>
<td>OH-containing compounds: L-Dopa (COMT)</td>
</tr>
<tr>
<td>(in cytosol)</td>
<td></td>
<td>N-containing compounds: histamine, nicotine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SH-cont. compounds: 6-mercaptopurine (TPMT)</td>
</tr>
</tbody>
</table>
**GLUCURONIDATION**

**Enzymes:** UDP-glucuronosyltransferases (UDP-GT, UGT)

**Cosubstrate:**
UDP-glucuronic acid (UDP-GA)

**Examples:**
- **Ether glucuronide formation** (from compounds with a hydroxyl group)

  ![Ether glucuronide formation](image)

  *Chloramphenicol*  
  (antibiotic)

  ![Chloramphenicol glucuronide](image)

  *Chloramphenicol glucuronide*  
  (rapidly excreted in urine)

  Others: Endogenous compounds: Thyroxine, Estradiol
  - Drugs: Acetaminophen, Diethylstilbestrol, Ezetimibe, Morphine, Oxazepam, Phenolphthalein
  - Hydroxylated metabolites of drugs

- **Ester glucuronide formation** (from compounds with a carboxylic group)

  ![Ester glucuronide formation](image)

  *Valproic acid*  
  (antiepileptic drug)

  ![Valproic acid glucuronide](image)

  *Valproic acid glucuronide*  
  (rapidly excreted in urine)

  Others: - Bilirubin (forms mono- and diglucuronides)
  - Drugs: NSAID (e.g., diclofenac), furosemide, telmisartan
  - Acidic metabolites (e.g., fenofibric acid) produced from fibric acid esters by ester hydrolysis

- **N-glucuronide formation**

  ![N-glucuronide formation](image)

  *Lamotrigine*  
  (antiepileptic drug)

  ![Lamotrigine N2-glucuronide](image)

  *Lamotrigine N2-glucuronide*  
  (rapidly excreted in urine)

Other: Carbamazepine
**SULFATION**

**Enzymes:** Sulfotransferases (SULT)

**Cosubstrate:** 3'-Phosphoadenosine-5'-phosphosulfate (PAPS)

![Chemical structure of PAPS](image)

**Examples:**

\[
\text{NH} - \text{C} - \text{CH}_3 
\]

\[
\text{SULT} \quad \text{PAPS} 
\]

\[
\text{NH} - \text{C} - \text{CH}_3 
\]

Acetaminophen = paracetamol  
(analgetic and antipyretic)  

acetaminophen-sulfate = paracetamol-sulfate  
(rapidly excreted in urine)

Others: catecholamines (e.g., dopamine)  
minoxidyl (see later)  
hydroxylated metabolites of drugs
CONJUGATION WITH GLYCINE

Enzymes: (1) acyl-CoA synthetase (ACS)  
(2) acyl-CoA: glycine N-acyltransferase (GNT)  
Both enzymes are located in the mitochondrial matrix.

Cosubstrates: (1) ATP, (2) Coenzyme A (CoA-SH), (3) Glycine (Gly)

Examples:

Salicylic acid  
\[ \text{Salicyl-CoA} \]  
\[ \text{Salicyl-glycine = salicyluric acid} \]  
(rapidly excreted in urine)

Acetylsalicylic acid  
(aspirin)  
\[ \text{acetlylation} \]  
and inactivation of COX

Benzoic acid  
\[ \text{Benzoyl-CoA} \]  
\[ \text{Benzoyl-glycine} \]  
(hippuric acid)

Note: Benzoyl-glycine was the first identified metabolite of a xenobiotic. In 1830, it was found in the urine of horses given benzoic acid. Then it was named hippuric acid (hippos means horse in Greek).
CONJUGATION WITH GLUTATHIONE

**Enzymes:** Glutathione S-transferases (GST)

**Cosubstrate:** Glutathione (GSH)

Notice that the SH group in GSH is electron-rich (i.e., nucleophilic), therefore it is reactive with electron-deficient (i.e., electrophilic) atoms, thus forming a glutathione conjugate.

As drugs and chemicals with electrophilic atoms may also bind to protein-cysteines, conjugation with glutathione prevents their covalent binding to proteins. Therefore, conjugation with GSH is an important protective mechanism against reactive electrophiles, such as NAPBQI.

**Examples:** Compounds with electrophilic (electron deficient, partially positive) carbon atoms

Note: the carbon with (+) sign is electron-deficient (contains a partial positive charge), thus it is electrophilic, therefore reactive with GSH, whose SH group is electron-rich (nucleophilic).

**Acrolein,**

a reactive electrophilic aldehyde that causes hemorrhagic cystitis in CP-treated patients

**Cyclophosphamide (CP)**

(antitumor drug, a nitrogen-mustard)

**Acetaminophen**

= paracetamol

**N-acetyl-p-benzo-quinoneimine**

(NAPBQI)

**ELECTROPHILIC METABOLITE**

MAY CAUSE HEPATIC NECROSIS
ACETYLATION

**Enzymes:** N-acetyltransferases (NAT)

**Cosubstrate:** Acetyl coenzyme A (Ac-CoA)

![Chemical structure of Acetyl Coenzyme A (Ac-CoA)](image)

**Examples:**

- **Isoniazid** (antituberculotic)
  - **NAT2**
  - **Ac-CoA**
  - **Acetylissoniazid** (inactive metabolite)

**Others:**
- **NAT1 substrates:** p-Aminobenzoic acid, p-Aminosalicylic acid, Sulfamethoxazole
- **NAT2 substrates:** Dapsone, Procainamide, Hydralazine, Sulfamethazine
METHYLATION

**Enzymes:** Methyltransferases (MT)

**Cosubstrate:** S-adenosylmethionine (SAM)

![Reaction scheme]

**Examples:**

- **O-methylation**
  - L-Dopa (antiparkinson drug, dopamine precursor)
  - Reaction: \( \text{L-Dopa} \xrightarrow{\text{COMT, SAM}} \text{3-O-Methyl-L-Dopa} \)
  - Reagents: entacapone, COMT, SAM

- **N-methylation**
  - Histamine
  - Reaction: \( \text{Histamine} \xrightarrow{\text{HMT, SAM}} \text{N-Methylhistamine} \)

- **S-methylation**
  - 6-Mercaptopurine (antitumor drug)
  - Reaction: \( \text{6-Mercaptopurine} \xrightarrow{\text{TPMT, SAM}} \text{6-Methylmercaptopurine} \)

Note: In patients with thiopurine methyltransferase (TPMT) deficiency (like in patients treated with allopurinol – see earlier), 6-mercaptopurine elimination is slow. In such patients, this drug may not cause apoptosis of leukemic cells only (which is desirable), but may also induce apoptosis of normal bone marrow cells as well!
### 1. TYPICALLY: ACTIVE DRUG → INACTIVE METABOLITE

- warfarin → 7-hydroxy-warfarin  
  CYP2C9
- phenytoin → 4-hydroxy-phenytoin  
  CYP2C9
- theophylline → 1- or 3-methylxanthine  
  CYP2A1
- morphine → morphine-3-glucuronide  
  UDP-GT
- acetaminophen → acetaminophen-glucuronide  
  UDP-GT
- isoniazide → acetyl-isoniazide  
  NAT2

### 2. EXCEPTIONALLY: ACTIVE METABOLITE IS FORMED

**a. Inactive parent compound (PRODRUG) → active metabolite**

- cyclophosphamide → phosphoramidate mustard  
  CYP2B6
- tamoxifen → 4-hydroxy-tamoxifen  
  CYP2D6
- parathion → paraoxon  
  CYP3A4
- terfenadine → alcohol → acid  
  CYP3A4
- chloral hydrate → trichloroethanol  
  ADH (rev.), AR
- sulfasalazine → 5-aminosalicylic acid  
  Azo-reductase
- oxcarbazepine → 10-hydroxy-carbazepine  
  AK-reductase
- lovastatin (lactone) → lovastatin (free acid)  
  Paraoxonase
- enalapril (ester) → enalaprilate (free acid)  
  Esterase
- fenofibrate (ester) → fenofibrate (free acid)  
  Esterase
- ezetimibe → ezetimibe-glucuronide  
  UDP-GT
- minoxidyl → minoxidyl-sulfate  
  SULT
- ethacrynic acid (EA) → EA-cysteine  
  GST→GGT, DP

**b. Active parent compound → active metabolite**

- phenylbutazone → γ-OH-phenylbutazone  
  CYP2D6, 2C9
- carisoprodol → meprobamate  
  CYP2C19
- risperidone → 9-OH-risperidone (paliperidone)  
  CYP2D6
- imipramine → desmethyl-imipramine  
  CYP2D6
- codeine → morphine  
  CYP2D6
- diazepam → nordiazepam → oxazepam  
  CYP → CYP
- morphine → morphine 6-glucuronide  
  UDP-GT

**c. „Non-toxic” parent compound → toxic metabolite**

- acetaminophen → N-acetyl-p-benzoquinoneimine  
  CYP2E1
- halothane → trifluoroacetyl chloride  
  CYP2E1
- cyclophosphamide → acrolein  
  CYP3A4, 2B6
- methanol → formic acid  
  ADH → ALDH
- ethylene glycol → glycolic-, glyoxylic- oxalic acid  
  ADH → ALDH
- doxorubicin → doxorubicinol  
  AK-reductase
BIOTRANSFORMATION OF DIAZEPAM, FIRST INTO ACTIVE PHASE-I METABOLITES (NORDIAZEPAM, OXAZEPAM), AND FINALLY INTO INACTIVE OXAZEPAM-GLUCURONIDE

**PHASE-I biotransformation**

Diazepam
\[\text{Cl} \quad \text{N} \quad \text{O} \quad \text{C} \quad \text{H}_3\]
\[\text{T}_{1/2} = 40 \text{ hrs}\]

- **N-demethylation**
  - CYP2C19
  - CYP3A4

Nordiazepam
\[\text{Cl} \quad \text{N} \quad \text{N} \quad \text{C} \quad \text{H}_3\]
\[\text{active metabolite}\]

- **Hydroxylation**
  - CYP

Oxazepam
\[\text{Cl} \quad \text{N} \quad \text{N} \quad \text{C} \quad \text{H}_3\]
\[\text{T}_{1/2} = 8 \text{ hrs}\]

**PHASE-II biotransformation** (conjugation)

- **UDP-GT**
- **UDP-GA**

Oxazepam-glucuronide
\[\text{Cl} \quad \text{N} \quad \text{N} \quad \text{C} \quad \text{H}_3\]
\[\text{active metabolite}\]

**Examples of drugs whose active metabolites are also drugs:**
- diazepam VALIUM > oxazepam SERAX
- risperidone RISPERDAL > paliperidone INVEGA
- terfenadine TELDANE* > fexofenadine TELFAST
- sulfasalazine SALAZOPYRIN > mesalazine SALOFALK
- enalapril ENALAPRIL tabl > enalaprilate VASOTEC inj

* Withdrawn - see later

**HYDROXYLATION OF RISPERIDONE TO AN ACTIVE METABOLITE**

Risperidone
\[\text{F} \quad \text{O} \quad \text{N} \quad \text{N} \quad \text{C} \quad \text{H}_3\]
\[\text{active metabolite}; \text{RISPERDAL}, \text{T}_{1/2} = 3 \text{ hrs}\]

- **CYP2D6**

9-hydroxy-risperidone (active)
\[\text{F} \quad \text{O} \quad \text{N} \quad \text{N} \quad \text{C} \quad \text{H}_3\]
\[\text{T}_{1/2} = 25 \text{ hrs}\]

NOTE that both risperidone and its metabolite, paliperidone, are sold as drugs.
BIOTRANSFORMATION OF CYCLOPHOSPHAMIDE INTO THERAPEUTICALLY ACTIVE METABOLITE (PHOSPHORAMIDE MUSTARD), SOME INACTIVE METABOLITES, AND A TOXIC METABOLITE (ACROLEIN)

NOTE: Steps 1 and 2 represent N-dealkylation, starting with hydroxylation on the C atom linked to the N atom, then chain breaking at the arrow, and forming an aldehyde. Here the aldehyde is aldophosphamide.

Liver:
damage to the sinusoidal endothelial cells, causing sinusoidal obstruction sy. (SOS)

Bladder:
damage to the urothelial cells, causing hemorrhagic cystitis

NOTE: Antitumor effect and general toxic effect of antitumor drugs e.g., bone marrow depression
Terfenadine is INACTIVE as an antihistamine (it is a prodrug). As a K⁺ channel inhibitor, terfenadine inhibits repolarization of cardiomyocytes and is arrhythmogenic. It has caused polymorphic ventricular extrasystoles (called torsades de pointes) especially when the patient also received a CYP3A4 inhibitor drug, such as ketoconazole or erythromycin.

CYP3A4 inhibitors (e.g., erythromycin, ketoconazole) increased the arrhythmogenic effect of coadministered terfenadine and have caused several fatalities by precipitating polymorphic ventricular extrasystole (torsades de pointes). Now terfenadine is withdrawn and replaced with fexofenadine (TELFAST).
AN EXCEPTIONAL WAY FOR ACTIVATION OF A DRUG:
THE PRODRUG MINOXIDIL FORMS AN ACTIVE SULFATE CONJUGATE

The vast majority of sulfate conjugates are highly water-soluble (due to their anionic charge), pharmacologically inactive and readily excreted (e.g., acetaminophen-sulfate).

In contrast, minoxidyl-sulfate, owing to inner salt formation which masks the + and – charges of this conjugate, is poorly water-soluble, pharmacologically active and slowly excreted.

On a similar basis, the sulfate conjugate of 5-hydroxy-triamterene (the metabolite of the diuretic triamterene) is also an active and poorly water-soluble; in fact it may cause crystalluria by being precipitated out from the urine (see under Diuretics).
AN EXCEPTIONAL WAY FOR ACTIVATION OF MORPHINE:
BY CONJUGATION WITH GLUCURONIC ACID AT THE 6-OH GROUP
The superactive nature of Mo-6-glucuronide is explained by inner salt formation
(like for minoxidyl-sulfate, see above)

Morphine-3-glucuronide
**INACTIVE**
cannot form an inner salt

Morphine-6-glucuronide
**ACTIVE**
can form an inner salt

Morphine-6-glucuronide
**SUPERACTIVE**
can form an inner salt

AN EXCEPTIONAL WAY FOR ACTIVATION OF EZETIMIBE:
BY FORMATION OF A GLUCURONIDE THAT REACHES AND INHIBITS
THE INTESTINAL CHOLESTEROL TRANSPORTER

Ezetimibe
**active metabolite**
produced in the enterocytes and the liver

Ezetimibe-glucuronide (EG)
Mrp-2 transporter
Bile, intestinal tract

THE SERUM CHOLESTEROL LEVEL DECREASES
DETOXIFICATION OF ACETAMINOPHEN (PARACETAMOL) BY GLUCURONIDATION (OR SULFATION) AT ITS HYDROXYL GROUP AND TOXIFICATION OF ACETAMINOPHEN BY CYP2E1-CATALYZED DEHYDROGENATION TO A REACTIVE ELECTROPHILIC QUINONEIMINE (NAPBQI).

![Chemical diagram showing the detoxication of acetaminophen by glucuronidation or sulfation at its hydroxyl group and toxification by CYP2E1-catalyzed dehydrogenation to a reactive electrophilic quinoneimine (NAPBQI).]

**You must know this!**

NAPBQI IS NORMALLY DETOXIFIED BY CONJUGATION WITH GLUTATHIONE. HOWEVER, WHEN ACETAMINOPHEN IS OVERDOSED, NAPBQI IS PRODUCED IN SO LARGE QUANTITIES THAT GLUTATHIONE BECOMES CONSUMED AND DEPLETED. THEN NAPBQI BINDS COVALENTLY TO THIOL GROUPS IN HEPATOCELLULAR PROTEINS, INACTIVATING THESE PROTEINS (enzymes, transporters, mitochondrial e-transport proteins, etc.) AND ULTIMATELY CAUSING LIVER NECROSIS.

Alcoholists are hypersensitive to acetaminophen-induced liver injury, because (1) in the alcoholic’s liver the amount of CYP2E1 is increased, therefore more NAPBQI is formed, and because (2) in the alcoholic’s liver the amount of glutathione is decreased, therefore glutathione is more readily depleted, allowing NAPBQI to react with protein-thiols.

NAPBQI = N-acetyl-para-benzoquinoneimine
Part B. ALTERATIONS IN BIOTRANSFORMATION

1. BIOLOGICAL ALTERATIONS IN BIOTRANSFORMATION - summary

- **Age-related alterations** - Neonatal immaturity of biotransformation

- **Genetic alterations:**
  - *Mutation* of genes encoding drug metabolizing enzymes
    → the mutant gene may code for an inactive or unstable enzyme protein
      → **POOR** or **SLOW** metabolizer phenotype
    Such individuals may be **hyperreactive** to the drug
      if the active parent compound is *slowly* converted into inactive metabolite.

  - *Multiplication* of genes encoding drug metabolizing enzymes
    → the gene in multiple copies codes for multiple amounts of enzyme protein
      → **EXTENSIVE** or **RAPID** metabolizer phenotype
    Such individuals may be **non-reactive** to the drug
      if the active parent compound is *rapidly* converted into inactive metabolite.

NEONATAL IMMATURETY OF BIOTRANSFORMATION summary

<table>
<thead>
<tr>
<th>UDP-GT immaturity</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Bilirubin → Physiological jaundice (see FIG)</td>
</tr>
<tr>
<td>• Chloramphenicol → Grey baby syndrome (see FIG)</td>
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<tr>
<th>Immature glycine conjugation</th>
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</thead>
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<tr>
<td>• Benzyl alcohol → Gaspung syndrome (see FIG)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Immature CYP, UDP-GT</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Diazepam → Floppy infant syndrome (see FIG)</td>
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</tbody>
</table>
**UDP-GT immaturity:** physiological jaundice, chloramphenicol-induced grey baby sy.

Bilirubin (not excreted into bile) → Bilirubin diglucuronide (excreted into bile)

At high concentration, CL inhibits mitochondrial protein synthesis, causing:
> cardiomyopathy
> myopathy

= GREY BABY SYNDROME

**Immature glycine conjugation:** benzyl alcohol-induced gasping syndrome

CYP Cytochrome P450
ADH Alcohol-dehydrogenase
AOX Aldehyde-oxidase
BCS Benzoyl-CoA-synthetase
BC:GT Benzoyl-CoA:glycine-N-acyl-transferase

BA accumulation → acidosis → gasping

LOW CAPACITY STEP IN NEONATES

Note: The use of benzyl alcohol is prohibited in neonatology care units.
**Immature CYP, UDP-GT: diazepam-induced floppy infant syndrome (FIS)**

FIS develops in a baby whose mother received diazepam before delivery.

- **diazepam**
  - Active: [VALIUM]
  - Half-life: $T_{1/2} = 40$ hrs

- **nordiazepam**
  - Active
  - Half-life: $T_{1/2} = 8$ hrs

- **oxazepam**
  - Active: [SERAX]

- **oxazepam-glucronide**
  - Inactive
  - UDP-GT
  - UDP-GA
  - Glucuronic acid

**SLOW STEPS IN NEONATES**

**Immature alcohol dehydrogenase:** delayed elimination of ethanol from the baby monkey whose mother received ethanol before delivery

**Interpretation:**

**Before delivery,** ethanol elimination in the mother-fetus “unit” was entirely carried out by ADH in the maternal tissues. **After delivery,** the ethanol that remained in the baby can only be eliminated at a very slow rate because ADH is barely expressed in the neonatal tissues. This also happens to diazepam in *floppy infant sy* (see above).
GENETIC ALTERATIONS IN BIOTRANSFORMATION

I. Mutation of genes encoding drug metabolizing enzymes
   → the mutant gene may code for an inactive or unstable enzyme protein
   → POOR metabolizer (hyperreactive) phenotype

1. CYP deficiencies
   • CYP2C9 deficiency
     ↓ Warfarin hydroxylation → Bleeding
   • CYP2C19 deficiency
     ↓ Diazepam N-demethylation → Prolonged sedation
   • CYP2D6 deficiency
     ↓ Debrisoquin hydroxylation → Pronounced hypotension
     ↓ N-dealkylation of TCADs → Pronounced sedation, toxicity
     ↓ N-dealkyl. of antipsychotics → Tardive dyskinesia
       (involuntary movements, tics)

2. Carboxylesterase (pseudocholinesterase) deficiency
   Succinylcholine → Prolonged paralysis

3. NAT2 deficiency → slow acetylator phenotype
   Isoniazide → ↑ Neurotoxicity, hepatotoxicity
   Procainamide, hydralazine → ↑ Systemic lupus erythemathodes

4. Thiopurine methyltransferase deficiency
   6-Mercaptopurine → ↑ Myelotoxicity

5. Dihydropyrimidine dehydrogenase deficiency
   5-Fluorouracyl → ↑ Myelotoxicity

II. Multiplication of genes encoding drug metabolizing enzymes
   → the gene in multiple copies codes for multiple amounts of enzyme protein
   → EXTENSIVE metabolizer (non-reactive) phenotype

Multiplication of CYP2D6 gene → ultrarapid metabolizer phenotype

Psychotropic drugs, e.g.,
   - TCADs (imipramine, nortriptyline),
   (most are CYP2D6 substrates)
   - SSRIs (fluoxetine, paroxetine) and
   - Others (clozapine, mianserine)
   are all ineffective at regular doses
2. CHEMICAL ALTERATIONS IN BIOTRANSFORMATION - summary

- Overexpression of enzymes by inducers
- Inhibition of enzymes by inhibitors

Cytochrome P450 enzymes are often induced or inhibited by certain drugs or other chemicals. CYP inducers and inhibitors thus may significantly affect the fate and the effect of other drugs that are biotransformed by CYP enzymes.

CYP superfamily organization:

- **CYP families** – labeled with numbers; only CYP1, CYP2 and CYP3 families are involved in biotransformation of drugs. (CYP7A, for example, is cholesterol 7α-hydroxylase, the rate limiting enzyme of bile acid synthesis.)
- **CYP subfamilies** – labeled with capital letters, e.g. CYP2B, CYP2C, CYP2E
- **CYP family members** – labeled with numbers after the letter of the subfamily, e.g., CYP2C9, CYP2C19, CYP2D6, CYP3A4

Of all the CYPs, CYP3A4 is the most abundant in human liver (35% of total) and in human small intestinal mucosa cells (80% of total intestinal CYP).

- Some drugs are biotransformed largely or exclusively by one CYP. For example:
  - Phenytoin and warfarin are hydroxylated by CYP2C9
  - Halothane is oxidatively debrominated by CYP2E1

- Other drugs may be biotransformed by several CYPs. For example:
  - Acetaminophen is dehydrogenated by CYP2E1 (mostly), CYP1A2 and CYP3A4
  - Diazepam is N-demethylated by CYP2C19 and CYP3A4
  - Dextromethorphan is O-demethylated by CYP2D6 and CYP3A4

A drug that is biotransformed by multiple CYPs, is typically less susceptible to pharmacokinetic interactions by CYP inhibitors, which often inhibit one specific CYP enzyme, and therefore CYPs that remain uninhibited may still eliminate the drug.
ENZYME INDUCTION:
Induction means increased synthesis of the enzyme protein.

Induction is usually caused by increased gene transcription, which is mediated by ligand-activated transcription factors, such as aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR) and pregnane X receptor (PXR). (see the table on the next page).

The inducer is a drug or other chemical which binds as a ligand to the ligand-activated transcription factor (intracellular receptor) and activates it. The activated transcription factor (with its dimerizing partner) binds to a cognate sequence in the regulatory region of one or more CYP genes, thus promoting the transcription of such genes, ultimately increasing CYP protein synthesis.

Inducers may induce multiple CYP members. For example, phenobarbital induces CYP2 members (except CYP2D) and CYP3A4, whereas ethanol induces only CYP2E1 (called ethanol-inducible CYP form; although CYP2E1 is not truly induced by ethanol but is stabilized by it.)

THE MECHANISM OF CYP3A4 INDUCTION BY DRUGS:
Increased CYPA4 gene transcription by activated pregnane X-receptor (PXR)

*Ligands: rifampicin, phenobarbital, hyperforin (St. John's wort), etc.
PXR = Pregnane X receptor
RXR = Retinoid X receptor
<table>
<thead>
<tr>
<th>CYP family</th>
<th>Drug substrates</th>
<th>Inducer chemicals</th>
<th>Inducer receptors (TFs)</th>
<th>Inhibitors (often competing substrates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A1: barely expressed, unless induced.</td>
<td>Drugs: omeprazole</td>
<td>AHR: Aryl hydrocarbon receptor</td>
<td>A2: ciprofloxacin fluvoxamine cimetidine acyclovir</td>
</tr>
<tr>
<td></td>
<td>A2: caffeine, theophylline, tizanidine, ondansetron, acetaminophen</td>
<td>Others: PAHs (charcoal-broiled meat, smoking), TCDD, indole-3-carbinol (in brussels sprouts, broccoli, cabbage)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2</td>
<td>B6: cyclophosphamide, bupropion, ketamine</td>
<td>B6, C9, C19: phenobarbital rifampin</td>
<td>CAR: Constitutive androstane receptor</td>
<td>B6: clopidogrel ticlopidin C9: amiodarone (+D6), fluconazole C19: moclobemide</td>
</tr>
<tr>
<td></td>
<td>C19: omeprazole, diazepam, fluvastatin</td>
<td>(CYP2D6 is NOT inducible)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D6: Psychotropic drugs: SSRI (citalopram, fluoxetine, paroxetine), TCAD (amitriptyline, clomipramine, desipramine, imipramine, nortriptyline), clozapine, mianserine, mirtazapine, venlafaxine. Cardiovascular drugs: β-blockers (alprenolol, bufuralol, metoprolol, propranolol, timolol), encaïnide, flecaïnide, propafenone. Miscellaneous drugs: codeine, debrisoquine, dextromethorphan, phenformin</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dietary chemicals: naringenin: a flavonoid, bergamottin: a furano-cumarin (both present in grapefruit juice)</td>
</tr>
</tbody>
</table>
CONSEQUENCES OF CYP INDUCTION:

1. Enhanced inactivation of a drug after repeated administration
   Occurs when the drug is both a substrate and an inducer of a CYP; thus it accelerates its own biotransformation into inactive metabolites → diminished effect (self-induced tolerance)
   e.g. Barbiturates (both substrates and inducers of CYP2 and CYP3A4)
   Meprobamate (both substrate and inducer of CYP)

2. Enhanced inactivation of some coadministered drugs after repeated administration of the inducer
   Occurs when the inducer drug induces a CYP which transforms the coadministered drug into inactive metabolite; thus the inducer accelerates the biotransformation of the coadministered drug into inactive metabolites → diminished effect
   e.g. CYP2C9 inducers (phenobarbital, rifampin)
       + warfarin (anticoagulant, CYP2C9 substrate)
       → INSUFFICIENT ANTICOAGULANT EFFECT of warfarin!
       → THROMBOSIS, despite warfarin therapy!
   CYP3A4 inducers (phenobarbital, rifampin, phenytoin, carbamazepine)
   + an oral contraceptive (contains ethinyl estradiol, a CYP3A4 substrate)
   → INSUFFICIENT CONTRACEPTIVE EFFECT of the contraceptive
   → UNWANTED PREGNANCY, despite taking a contraceptive!
   CYP3A4 inducers (phenobarbital, rifampin, phenytoin, carbamazepine)
   + immunophyllin-binding immunosuppressive drug
     (e.g., cyclosporine A, tacrolimus, sirolimus; all are CYP3A4 substrates)
   → INSUFFICIENT IMMUNOSUPPRESSION
   → TRANSPLANT REJECTION, despite immunosuppressive therapy!

3. Enhanced toxification of a coadministered drug after repeated administration of the inducer
   Occurs when the inducer drug induces a CYP which transforms the coadministered drug into a toxic metabolite; thus the inducer accelerates the biotransformation of the coadministered drug into toxic metabolites → increased toxicity
   e.g. CYP2E1 inducer (ethanol) + acetaminophen overdose (CYP2E1 substr.)
       → increased formation of N-acetyl-p-benzoquinone imine (NAPBQI)
       → HEPATIC NECROSIS!
       Another cause of increased susceptibility of drinkers to paracetamol-induced liver injury: lower hepatic GSH → ↓detoxication of NAPBQI
   CYP2E1 inducer (ethanol) + halothane anesthesia
       → increased formation of trifluoroacetyl chloride
       → the probability for halothane HEPATITIS may increase!
CONSEQUENCES OF CYP INHIBITION:

1. Decreased inactivation of an active drug → increased therapeut. effect:

   e.g. a CYP2C9 inhibitor (e.g., amiodarone)
   + the oral anticoagulant warfarin → BLEEDING!

   a CYP3A4 inhibitor (e.g., itraconazole, clarithromycin)
   + the oral antidiabetic repaglinide (CYP3A4 substr.) → HYPOGLYCEMIA!

   a CYP3A4 inhibitor (e.g., ketoconazole)
   + cyclosporine A (CsA; an immunosuppressive drug; CYP3A4 substr.)
   → ↑ oral bioavailability and ↓ elimination of CsA
   → Two consequences (Two birds are killed with one stone!):
     • ↑ effectiveness (dose reduction → saving on the drug)
     • the antifungal ketokonazole prevents fungal infection of the immunosuppressed patient

2. Decreased inactivation of an active drug → increased adverse effect:

   e.g. a CYP3A4 inhibitor (e.g., itraconazole, clarithromycin, bergamottin)
   + a CYP3A4 substrate statin (not pravastatin, fluvastatin, rosuvastatin)
   → MYOTOXICITY! – see FIG.

   a CYP3A4 inhibitor (e.g., ketoconazole, erythromycin)
   + terfenadine (a withdrawn antihistamin; CYP3A4 substrate)
   → POLYMORPHIC VENTRICULAR ES! – see FIG.

   a CYP1A2 inhibitor (e.g., ciprofloxacin, fluvoxamine)
   + tizanidine (a central muscle relaxant α2-receptor agonist; CYP1A2 substr.)
   → EXCESSIVE SEDATION, HYPOTENSION! – see FIG.

3. Decreased activation of a prodrug → decreased therapeutic effect

   e.g. a CYP2D6 inhibitior (e.g., fluoxetine or paroxetine)
   + tamoxifen (an antiestrogen prodrug, CYP2D6 substrate)
   → decreased conversion into 4-hydroxytamoxifen (active antiestrogen)
      – see FIGURE

   Thus, CYP2D6 inhibitors can compromise the effectiveness of tamoxifen against estrogen-dependent breast cancers!

4. Decreased toxification of a drug → decreased toxic effect

   e.g. CYP2E1 inhibitior (disulfiram)
   + halothan (an inhalation anesthetic, CYP2E1 substrate)
   → decreased conversion into trifluoroacety chloride (reactive metab.)

   Thus, disulfiram pretreatment before halothane anesthesia could be used to decrease the likelihood of halothane hepatitis.
GRAPEFRUIT JUICE (contains bergamottin which inhibits CYP3A4 as well as the OATP transporter-mediated hepatic uptake of statins) INCREASES THE PLASMA CONCENTRATION OF SIMVASTATIN (SV; A PRODRUG) AND ITS ACTIVE METABOLITE SIMVASTATIN ACID SEVERAL FOLD. (Lilja et al., Clin. Pharmacol. Ther. 64: 477-483, 1998.)

**BIOTRANSFORMATION PATHWAYS FOR THE CHOLESTEROL-LOWERING DRUG SIMVASTATIN INTO ACTIVE SIMVASTATIN ACID BY PARAOXONASE AND INACTIVE METABOLITES BY CYP3A4**

**CYP3A4 inhibitors**, such as constituents of grapefruit juice (naringenin and bergamottin) as well as drugs (itraconazole, verapamil, erythromycin, etc.) may increase the myotoxicity of statins by diminishing their elimination by CYP3A4.

**OATP inhibitors**, such as the grapefruit juice constituent bergamottin and the fibrate-type triglyceride-lowering drugs (e.g., gemfibrozil), may also increase the myotoxicity of statins by diminishing their hepatic uptake mediated by OATP.
Terfenadine (TELDANE) (withdrawn) is INACTIVE as an antihistamine (it is a prodrug). As a K⁺ channel inhibitor, terfenadine inhibits repolarization of cardiomyocytes and is arrhythmogenic. It has caused polymorphic ventricular extrasystoles (called torsades de pointes) especially when the patient also received a CYP3A4 inhibitor drug, such as ketoconazole or erythromycin.

CYP3A4 inhibitors (e.g., erythromycin, ketoconazole) increased the arrhythmogenic effect of coadministered terfenadine and have caused several fatalities by precipitating polymorphic ventricular extrasystole (torsades de pointes). Now terfenadine is withdrawn and replaced with fexofenadine (TELFAST).
**Tizanidine**, a centrally acting muscle relaxant, is rapidly biotransformed by CYP1A2. This is responsible for both its hepatic presystemic elimination ($F = 0.7$) and rapid elimination ($T_{1/2} = 2.5$ hrs) of tizanidine. Therefore, **drugs that inhibit CYP1A2** (e.g., fluvoxamine, ciprofloxacin, rofecoxib) can increase the AUC of tizanidine several fold. At high level, tizanidine, which is a $\alpha_2$-receptor agonist like clonidine, can cause marked hypotension, bradycardia and sedation.

**CYP2D6 inhibitors** (e.g., fluoxetine or paroxetine) can compromise the therapeutic effectiveness of **tamoxifen** against estrogen-dependent breast cancer because they inhibit the conversion of tamoxifen into **4-hydroxytamoxifen**, the active antiestrogen metabolite of tamoxifen.
THERAPEUTIC USE OF ENZYME INHIBITORS

CYP inhibitors:
- **Ketoconazol** – to increase effectiveness of cyclosporine (to save on the drug)
- **Disulfiram** – to decrease the likelihood of
  - halothane hepatitis or
  - acetaminophen-induced hepatic necrosis

MAO inhibitors:
- **MAO A inhibitors** (e.g., moclobemide) – to treat major depression
- **MAO B inhibitors** (e.g., selegiline) – to treat Parkinson’s disease

COMT inhibitors:
- **Entacapone** – to treat Parkinson’s disease

XO inhibitors:
- **Allopurinol** – to decrease uric acid formation (in gout)
  (BUT: it decreases the elimination and thus increases the toxicity of 6-mercaptopurine!)

Alcohol dehydrogenase inhibitors:
- **Fomepizol, ethanol** – to treat methanol or ethylene glycol intoxication

Aldehyde dehydrogenase (ALDH) inhibitors:
- **Disulfiram** – to induce „acetaldehyde syndrome”
  and thus promote alcohol withdrawal

Note, disulfiram is not only an irreversible ALDH inhibitor, but a CYP inhibitor as well.

Drugs with unwanted ”**disulfiram-like effect**”:
- Some cephalosporins: cefamandol, cefoperazone
  (Their metabolite, 1-methyltetrazole-5-thiol, inhibits aldehyde dehydrogenase, like disulfiram does.)
- Chloramphenicol
- Metronidazole
- Procarbazine