Drug Metabolism
Phase 2 conjugation reactions

Medicinal chemistry
3rd stage
Phase II or Conjugation reactions

1. Glucuronic acid conjugation
2. Sulfate conjugation
3. Glycine and Glutamine conjugation
4. Glutathione (GSH or mercapturic acid) conjugations
5. Acetylation
6. Methylation
Glucuronic acid conjugation

- Glucuronidation is the most common conjugative pathway in drug metabolism for several reasons:

  a. A readily available supply of D-glucuronic acid (derived from d-glucose).
  b. Numerous functional groups that can combine enzymatically with G.A.
  c. Glucuronyl moiety when attached to xenobiotic substrates increases water solubility of the conjugated product.
UDP-Glucuronyl transferase
(Formation B-Glucuronide)
<table>
<thead>
<tr>
<th><strong>Oxygen Glucuronides</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydroxyl compounds</strong></td>
</tr>
<tr>
<td>Phenols: morphine, acetaminophen, p-hydroxyphenytoin</td>
</tr>
<tr>
<td>Alcohol: trichloroethanol, chloramphenicol, propranolol</td>
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<tr>
<td>Enols: 4-hydroxycoumarin</td>
</tr>
<tr>
<td>N-Hydroxyamines: N-hydroxydapsone</td>
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<tr>
<td>N-Hydroxyamides: N-hydroxy-2-acetylaminofluorene</td>
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<tr>
<td><strong>Carboxyl compounds</strong></td>
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<tr>
<td>Aryl acids: benzoic acid, salicylic acid</td>
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<tr>
<td>Arylalkyl acids: naproxen, fenoprofen</td>
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<thead>
<tr>
<th><strong>Nitrogen Glucuronides</strong></th>
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<tr>
<td>Arylamines: 7-amino-5-nitroindazole</td>
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<tr>
<td>Alkylamines: desipramine</td>
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<tr>
<td>Amides: meprobamate</td>
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<tr>
<td>Sulfonamides: sulfisoxazole</td>
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<tr>
<td>Tertiary amines: cyproheptadine, tripelennamine</td>
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</table>

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<thead>
<tr>
<th><strong>Sulfur Glucuronides</strong></th>
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</thead>
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<tr>
<td>Sulfhydryl groups: methimazole, propylthiouracil, diethylthiocarbamic acid</td>
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</table>

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<thead>
<tr>
<th><strong>Carbon Glucuronides</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>3,5-Pyrazolidinedione: phenylbutazone, sulfinpyrazone</td>
</tr>
</tbody>
</table>
O-Glucuronide (Hydroxyl) conjugation

Morphine

Acetaminophen

*p*-Hydroxyphenytoin

Trichloroethanol

Chloramphenicol

Propranolol

4-Hydroxycoumarin

N-Hydroxydapsone
Phenytoin

Phenytoin → $p$-Hydroxyphenytoin

$O$-Glucuronide Conjugate
Carboxyl- Glucuronide conjugation

N-Hydroxy-2-acetylaminofluorene

Benzoic acid, $R = H$
Salicylic acid, $R = OH$

Naproxen

Fenoprofen
N and S -Glucuronide conjugation

7-Amino-5-nitroindazole

Desipramine

Meprobamate

Sulfisoxazole

Cyproheptadine

Tripelennamine

Methimazole

Propylthiouracil

Diethylthiocarbamic Acid
C-Glucuronide conjugation

![Chemical structure](image)

Phenylbutazone, $R = \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$

Sulfinpyrazone, $R = \text{CH}_2\text{CH}_2\text{SC}_6\text{H}_5$
• In neonates and children, glucuronidating processes are often not developed fully.
• In such subjects, drugs (Chloramphenicol) and endogenous compounds (bilirubin).
• Gray baby syndrome is a rare but serious side effect that occurs in newborn infants (especially premature babies) following the accumulation of antibiotic chloramphenicol.
• This condition is due to a lack of glucuronidation reactions occurring in the baby, thus leading to an accumulation of toxic chloramphenicol metabolites.
Sulfate conjugation

- Conjugation of xenobiotics with sulfate occurs primarily with phenol, alcohol aromatic amines and N-hydroxy compounds.
Sulfate conjugation

Formation of Sulfate conjugation
O-sulfate conjugation

Sulfate conjugation is well developed and become main route of acetaminophen conjugation in pediatric group.

![Chemical Structures]

- Acetaminophen
- O-Glucuronide Conjugate
- O-Sulfate Conjugate
O-sulfate conjugation

\[\text{\(\alpha\)-Methyldopa} \rightarrow \text{Salbutamol (Albuterol)} \rightarrow \text{Terbutaline}\]
Phenacetin is metabolized to N-hydroxyphenacetin and subsequently conjugated with sulfate. The O-sulfate conjugate of N-hydroxyphenacetin binds covalently to microsomal proteins.

This pathway may represent one route leading to reactive intermediates that are responsible for the hepatotoxicity and nephrotoxicity.
Conjugation with Glycine, Glutamine and other Amino acids

- Glycine and glutamine are used by mammalian systems to conjugate carboxylic acids, particularly aromatic acids.

- In contrast with glucuronic acid and sulfate, glycine and glutamine are not converted to activated coenzymes.
Figure 3.14  • Formation of glycine and glutamine conjugates of phenylacetic acid.
Glycine conjugation

Brompheniramine → 3-(p-Bromophenyl)-3-(2-pyridyl)-propionic Acid → Glycine Conjugate
Glycine conjugation

\[
\begin{align*}
\text{Benzoic Acid, } R &= H \\
\text{Salicylic Acid, } R &= \text{OH} \\
\rightarrow \\
\text{Hippuric Acid, } R &= H \\
\text{Salicyluric Acid, } R &= \text{OH}
\end{align*}
\]
Phase 2 conjugation reactions
GSH or Mercapturic acid conjugations

- GSH conjugation is an important pathway for detoxifying chemically reactive electrophilic compounds.
- The GSH group reacts with electron-deficient compounds to form S-substituted GSH adducts.
- GSH is a tripeptide (γ-glutamyl-cysteinylglycine) found in most tissues.
- Xenobiotics conjugated with GSH usually are not excreted as such, but undergo further biotransformation to give S-substituted N-acetylcysteine products called mercapturic acids.
Figure 3.15 • Formation of GSH conjugates of electrophilic xenobiotics or metabolites (E) and their conversion to mercapturic acids.
Compounds that react with GSH do so by two general mechanisms:

a. Nucleophilic displacement at an electron-deficient carbon or hetroatom.

b. Nucleophilic addition to an electron-deficient double bond.

- Many aliphatic and arylalkyl halides (Cl, Br, I), sulfates (OSO$_3^-$), sulfonates (OSO$_2$R), nitrates (NO$_2$), possess electron-deficient carbon atoms that react with GSH to form GSH conjugates.

![Chemical Reaction Diagram]

\[ \text{GSH}^+ \xrightarrow{\delta} \text{CH}_2^- \xrightarrow{\delta} \text{X} \rightarrow \text{GS}^- \text{CH}_2^+ \text{HX} \]

- R = Alkyl, Aryl, Benzylic, Allylic
- X = Br, Cl, I, OSO$_3^-$, OSO$_2$R, OPO(OR)$_2$
• Nucleophilic displacement often is facilitated when the carbon atom is benzylic or allylic or when X is a good leaving group (halide, sulfate).
Example of heteroaromatic nucleophilic substitution.
• GSH conjugate products are metabolized to alcohol derivatives and glutathione disulfide (GSSG), instead are converted to mercapturic acids.
• The nucleophilic addition of GSH to electron-deficient carbon-carbon double bond, occurs mainly in compounds with α,β-unsaturated double bond.
Ethacrynic Acid
(note $\alpha,\beta$-unsaturated ketone moiety)

Glutathione adduct of Ethacrynic Acid

Mercapturic Acid Derivative
Metabolic oxidation of acetaminophen generates the chemically reactive intermediate N-acetylimidoquinone.
Acetylation constitutes an important metabolic route for drugs containing primary amino groups.

- Aromatic amines (ArNH$_2$)
- Sulfonamides (H$_2$NC$_6$H$_4$SO$_2$NHR)
- Hydrazines (-NHNH$_2$)
- Hydrazides (-CONHNH$_2$)
- Aliphatic amines.
Examples of Aromatic amines

Aromatic Amines

- Aniline
- p-Aminobenzoic Acid $R = H$
- p-Aminosalicylic Acid $R = OH$
- Procainamide
- Dapsone
Examples of reduction aryl nitro compounds to 7-amino metabolite.

Clonazepam, R = Cl
Nitrazepam, R = H

7-Amino Metabolite

7-Acetamido Metabolite or
N-Acetylated Metabolite
Sulfonamides

Sulfanilamide

Sulfamethoxazole

Sulfisoxazole

Sulfapyridine

Sulfamethazine
Hydrazines and Hydrazides

Hydralazine

Phenelzine

Isoniazid
Aliphatic Amines

Histamine

Mescaline

Bisdesmethyl Metabolite of 3S,6S-α-(−) Methadol
Acetylation polymorphism

• Present in several drugs (e.g., isoniazid and hydralazine), either rapidly or slowly acetylated with acetyl-CoA.
• Asians are rapid acetylators, whereas Egyptians and some Western European groups are mainly slow acetylators.
The antituberculosis drug isoniazid

- The plasma half-life of isoniazid in rapid acetylators ranges from 45 to 80 minutes, in slow acetylators the half-life is about 140 to 200 minutes.
Methylation

- Methylation reactions play important role in biosynthesis of many endogenous compounds (e.g., epinephrine and melatonin).
- Inactivation of numerous physiologically active biogenic amines (e.g., norepinephrine, dopamine, serotonin and histamine).
- Minor pathway for conjugating drugs.
- It doesn’t lead to polar or water soluble metabolites, except when it creates, a quaternary ammonium derivatives.
Norepinephrine, $R = \text{OH}$
Dopamine, $R = \text{H}$

$\text{COMT}$

Normetanephrine, $R = \text{OH}$
3-Methoxytyramine, $R = \text{H}$
Figure 3.17  ●  Conjugation of exogenous and endogenous substrates (RXH) by methylation.
Factors affecting drug metabolism

1. Age Differences

- In most fetal and newborn animals, undeveloped or deficient oxidative and conjugative enzymes.
- Infant possess poor glucuronidating ability because of a deficiency in glucuronyl transferase activity.

Example:
Hexabarbital (10mg/kg)
Tolbutamide
Chloramphenicol
2- Species and strain differences

Phenytoin

[Chemical Structure]

S(-)-p-Hydroxyphenytoin

(Dog)

R(+)-m-Hydroxyphenytoin
Amphetamine → Aromatic Hydroxylation → p-Hydroxyamphetamine

Oxidative Deamination → Phenylacetone

Oxidation → Benzoic Acid

(man, rabbit, guinea pig)
Figure 3.18 • Phenazopyridine metabolism in humans, guinea pigs, rats, and mice.
### 3- Enzyme induction and inhibition

<table>
<thead>
<tr>
<th>Agent</th>
<th>Substrates</th>
<th>Inhibitors</th>
<th>Inducers</th>
<th>Agent</th>
<th>Substrates</th>
<th>Inhibitors</th>
<th>Inducers</th>
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<tbody>
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<td>CYP 1A2</td>
<td>Amitriptyline</td>
<td>Cimetidine</td>
<td>Carbamazepine</td>
<td>Imipramine</td>
<td>Furosemide</td>
<td>Captopril</td>
<td>Bridge</td>
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<td>Ciprofloxacin</td>
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<td>(R)-Warfarin</td>
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<td></td>
<td>Phenytin</td>
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</tbody>
</table>

**TABLE 3.4 Clinically Significant Cytochrome P450-Based Drug–Drug Interactions**
• 4- Stereochemical aspects of drug metabolism:

➢ The two enantiomers present in racemic mixture may differ in pharmacological activity.
➢ Many drugs (e.g., warfarin, propranolol, and hexobarbital), often are administered as racemic mixtures in humans.
➢ Usually, one enantiomer tends to be much more active than the other.

➢ Example
➢ The (S)(-) enantiomer of warfarin is 5 times more potent as an oral anticoagulant than the (R)(+) enantiomer.
### Pharmacological active metabolites

**TABLE 3.6 Pharmacologically Active Metabolites in Humans**

<table>
<thead>
<tr>
<th>Parent Drug</th>
<th>Metabolite</th>
<th>Biotransformation Process</th>
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<tbody>
<tr>
<td>Acetohexamide</td>
<td>Hydroxyhexamamide</td>
<td>Ketone reduction</td>
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<tr>
<td>Acetylmethadol</td>
<td>Noracetylmethadol</td>
<td>N-Demethylation</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Nortriptyline</td>
<td>N-Demethylation</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>6-Mercaptopurine</td>
<td>Glutathione conjugation</td>
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<td>Carbamazepine</td>
<td>Carbamazepine-9,10-epoxide</td>
<td>Epoxidation</td>
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<tr>
<td>Chloral hydrate</td>
<td>Trichloroethanol</td>
<td>Aldehyde reduction</td>
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<td>Chlorpromazine</td>
<td>7-Hydroxychlorpromazine</td>
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<td>Clofibrate</td>
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<td>Cortisone</td>
<td>Hydrocortisone</td>
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<td>Diazepam</td>
<td>Desmethyldiazepam and oxazepam</td>
<td>N-Demethylation and 3-hydroxylation</td>
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