PHASE II METABOLIC PATHWAYS
Phase I or functionalization Rx.s don’t always produce hydrophilic or pharmacologically inactive metabolites. Various phase II or conjugation rx.s, however, can convert these metabolites to more polar & water soluble products. Many conjugative enz.s accomplish this objective by attaching small, polar & ionizable endogenous molecules, such as, (Glucuronic acid, Sulfate, Glycine & Glutamine) to the phase I metabolite or parent xenobiotic.

Resulting conjugated products:
* Relatively water sol. & readily excreetable
* Biologically inactive & non toxic
Other phase II Rx.s such as:
Acetylation & Methylation

↓

don’t generally water sol. But mainly serve to terminate or attenuate pharmacological activity.

∧ role of GSH (Glutathione Conjugation) is to combine w- chemically reactive cpd.s to prevent damage to important biomacromolecules, such as DNA, RNA & protein.
Thus, phase II Rx.s can be regarded as truly detoxifying pathways in drug metabolism, with a few exceptions.
Phase II feature
Conjugating grp. (glucuronic acid, sulfate, methyl & acetyl) is activated in a form of a coenzyme and involves a transferase enzyme.
1- Glucuronic acid conjugation:
Glucuronida is most common pathway in drug metabolism for several reasons:
1- readily available supply of D-glucuronic acid (derived from D-glucose)
2- numerous functional groups that can combine enzymatically with glucuronic acid.
3- glucuronyl moiety (with its ionized carboxylate (pKa 3.2) (100% ionized) & polar OH groups with attached to xenobiotic substrate, greatly increase H₂O solubility of the conjugated product.
\[ \text{\(\alpha\)-d-Glucose-1-phosphate} \xrightarrow{\text{Phosphorylase}} \text{Uridine-5'}-\text{diphospho-\(\alpha\)-d-glucose (UDPG)} \]

\[ \text{UDPG Dehydrogenase (soluble)} \xrightarrow{2 \text{ NAD}^+} 2 \text{ NADH} + 2 \text{ H} \]

\[ \text{\(\beta\)-Glucuronide (\(\beta\)-linkage at C-1)} \xrightarrow{\text{UDP-Glucuronyl-transferase (microsomal)}} \text{Uridine-5'}-\text{diphospho-\(\alpha\)-d-glucuronic Acid (UDPGA) (\(\alpha\)-linkage at C-1)} \]
Uridine-5'-α-D-glucuronic Acid

Uridine-5’-diphospho-α-D-glucuronic acid (UDP-GA)

* microsomal enz. glucuronyl transferase conducts * dona of glucuronic acid from * endogenously synthesized UDPGA to various substrates to form glucuronide conjugates. Examples of such substrates r- morphine and acetaminophen.
<table>
<thead>
<tr>
<th>cpds forming O-, N-, S- &amp; C-glucuronides:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>uronides</strong></th>
<th><strong>N-Glucuronides</strong></th>
<th><strong>S- Glucuronides</strong></th>
<th><strong>C- Glucuronides</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydroxyl cpds</strong></td>
<td>Aryl amine: 7-amino-5-nitroindazole</td>
<td>Sulfhydryl grp.s: methimazole, propylthiouracil, diethylthiocarbamic acid</td>
<td>3,5- pyrazolidinedione: phenylbutazone, sulfipyrazone</td>
</tr>
<tr>
<td>Phenols: morphine, acetaminophen, p-hydroxyphenytoin</td>
<td>Alkyl amines: desipramine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohols: trichloroethanol, Enols: 4-hydroxycoumarin</td>
<td>Amides: meprobamate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-hydroxyamine: N-hydroxydapsone</td>
<td>Sulfonamides: sulfisoxazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-hydroxyamides: N-hydroxy-2-acetylaminofluorene</td>
<td>3 ry amines: cyproheptadine, tripeleamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Carboxyl cpds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aryl acids: benzoic acid, salicylic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arylalkyl acids: naproxen, phenoprofen</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chloramphenicol - Chloromycetin® - Parke Davis
Serious infections - monitor for blood dyscrasias

Acetaminophen
APAP
N-acetyl-p-aminophenol
Less frequent is glucuronidation of other hydroxyl groups such as

- Enol
- N-Hydroxylamine
- N-Hydroxylamide
Fenoprofen
Nalfon® - Ranbaxy
non-steroidal antiinflammatory

$\text{N-hydroxy metabolite of Dapsone}$
An "Orphan Drug"
Used in treatment of leprosy,
Pneumocystis carinii pneumonia,
brown recluse spider bites
Occasionally, **N-glucuronides** r- formed w- Ar. amines, Aliphatic amines, amides & sulfonamide. Glucuronida of Ar. & Aliphatic amines is generally a **minor pathway** in comparison w- **N-acetyla** or oxidative processes (e.g. **oxidative deamination**), 3 ry amines, such as & antihistaminic agents cyproheptadine(periactin) & tripelennamine, form interesting quaternary ammonium glucurononides metabolites.
*** b- \( \wedge \) thiol grp. (SH) does not commonly occur in xenobiotics, S-glucuronide products have been reported for only a few drugs. For instance, \( \wedge \) thiol grps present in methimazole(Tapazole), propylthiouracil & N,N-diethyldithiocarbamic acid(major reduced metabolite of disulfiram(Anabuse) undergo conjuga\( \wedge \) w- glucuronic acid.
forma of glucuronides attached directly to a C- atom is relatively novel in drug metabolism. Studies in humans have shown that conjugation of phenylbutazone (Butazolidin) & sulfinpyrazone (Anturane) yield corresponding C-glucuronides:
Endogenous substrates such as bilirubin & steroid r- eliminated as glucuronide conjugates, w- r- excreted l ry in urine. As relative molecular mass (M.Wt.) of conjugate exceeds 300 Da, however, biliary excre may become an important route of elimination. Glucuronides at r- excreted in bile r- susceptible to hydrolysis by β-glucuronidase enz.s present in intestine. Hydrolyzed product may be reabsorbed in intestine, thus leading to enterohepatic recycling
β-Glucuronidases are also present in many other tissue, including liver, endocrine system & reproductive organs. Although function of these hydrolytic enzymes in drug metabolism is unclear, it appears that, in terms of hormonal & endocrine regulation, β-Glucuronidases may liberate active hormones (e.g. steroids) from their inactive glucuronide conjugates.
In neonates & children, glucuronidating processes often not developed fully. In such subjects, drugs & endogenous cpds (e.g. bilirubin) may be metabolized normally by glucuronida may accumulate & cause serious toxicity. For example, neonatal hyperbilirubinemia may be attributable to inability of newborns to conjugate bilirubin with glucuronic acid. Similarly, inability of infants to glucuronidate chloramphenicol has been suggested to be responsible for gray baby syndrome, which results from accumulation of toxic levels of free antibiotic.
sulfate conj.:  
** Occurs primarily with phenols 
*** Rarely: alcohols, aromatic amines, and N-hydroxyl cpds. 
*** Catalyzed by sulfotransferases 
*** p- in liver, kidney and intestine 
** in contrast to glucuronic acid:  
a) amount of available sulfate is rather limited. 
b) body uses a significant portion of sulfate Pool to conjugate numerous endogenous cpds., such as steroids, heparine, catechol amine, chondroitin & thyroxine.  
Glucuronate conjugation often more competitive process
Sulfate $\xrightarrow{\text{ATP sulfurylase, Mg}^{2+}}$ Adenosine-5'-phosphosulfate (APS)

$\text{O-SO}_2^-\text{XR} \xrightarrow{\text{PAP, Sulfotransferase (soluble)}} \text{H}_2\text{O}_3\text{PO}_4^-\text{Adenine}$

$\text{O-SO}_2^-\text{XR} \xrightarrow{\text{PAP, Sulfotransferase (soluble)}} \text{H}_2\text{O}_3\text{PO}_4^-\text{Adenine}$
Acetaminophene, in infant & young (3-9 year), the O-sulfate conjugate is the main urinary product for acetaminophene, b- they have decrease glucuronidating capacity owing to undeveloped glucuronyl transferases or low level of these enz.s
Glucuronida\^ of phenols is frequently a competing rx.s & may be predominate as a conjugative route for some phenolic drugs, in adults, major urinaray metabolite of analgesic acetaminophen is the O-glucuronide conjugate & small amount O-SO$_3^-$, but the latter product represent the main urinary prod. In infant & young.
Phenols compose the main group of substrate undergoing sulfate conjugation.

- **alpha-Methylidopa**
  - *Aldomet*<sup>®</sup>
  - Antihypertensive

- **Salbutamol (Albuterol)**

  **3-O-Sulfate ester in human**

*beta-adrenergic bronchodilators*
Other functionalities, such as:

** alcohols (aliphatic alcoh. C1-C5, diethylene glycol)
** Ar.NH₂ (aniline, 2-naphthylamine) can also form sulfate conjugate, these rxns. have only a minor importance in drug metabolism.
** ^ sulfate conjugation of NH-OH & -CO-NHOH take place. O-sulfate ester conjugates of N-OH cpds. r- of considerable toxicological concern b- they can lead to reactive intermediates ^ at r- responsible for cellular toxicity.
Carcinogenic agent: N-methyl-4-aminoazobenzene & 2-acetylaminoazobenzene r- believed to mediate their toxicity through N-hydroxylation to corresponding N-OH cpds. sulfoconjugation of N-OH metabolites yields O-sulfate ester, w- presumably r- ultimate carcinogenic species. loss of SO42- from foregoing sulfate conjugates generates electrophilic nitrenium species, w- may react w- nu. grp. (SH, OH, NH2) p- in prot., DNA, RNA to form covalent linkages at lead to structural & functional altera of these crucial biomacromolecules. consequences of is r- cellular toxicity (tissue necrosis) or altera of genetic code, eventually leading to cancer.
Some evidence supporting the role of sulfate conjugation in metabolic activation of N-OH compounds to reactive intermediates comes from observations at the degree of hepatotoxicity & hepatocarcinogenicity of N-OH-2-acetyl-amino fluorene depend on the level of sulfotransferase activity in the liver.

*** Analgesic phenacetin ----NHOH------conjugate with sulfate----binds covalently to microsomal protein. This pathway represents one route leading to reactive intermediates responsible for hepatotoxicity & nephrotoxicity associated with phenacetin.

** Other pathways (Arene oxides) leading to reactive electrophilic intermediate routes are also possible.
UDP-α-D-Glucuronyltransferase

- Is also called glucuronyl transferase
- A microsomal enzyme
- Substrates are called aglycones
- Conducts phase 2 metabolic reactions
- Products are called glucuronides
- Glucuronides formed
  - RN-G; RO-G; RCOO-G; RS-G; RC-G
- Bilirubin is an endogenous substrate
- Induced by phenobarbital
Glucuronidation of Benzoic Acid

$\text{UDPGA + HO-C-}$

$\xrightarrow{\text{UGT}}$

$\text{COOH}$

$\text{O-C}$

$\text{Benzoic acid}$

$\text{Benzoyl glucuronide}$

$\text{UDPG} + \text{UDP}$

$\text{UGT}=\text{UDP-}\alpha-\text{D-Glucuronysyltransferase}$
Glucuronidation of Aniline

$\text{UDPGA} + \text{H}_2\text{N} - \text{C}_6\text{H}_4\text{NH}_2 \xrightarrow{\text{UGT}} \text{UDP} + \text{Aniline glucuronide}$
Glucuronidation of \( p \)-Hydroxyacetanilid
<table>
<thead>
<tr>
<th>Conjugate</th>
<th>Coenzyme Form</th>
<th>Groups Conjugated</th>
<th>Transferase Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine-5'-diphospho-α-D-glucuronic acid (UDPGA)</td>
<td><img src="image" alt="Glucuronide" /></td>
<td>-OH, -COOH, -NH₂, -NR₂, -SH, C-H</td>
<td>UDP-Glucuronosyltransferase</td>
</tr>
<tr>
<td>3'-Phosphoadenosine-5'-phosphosulfate (PAPS)</td>
<td><img src="image" alt="Sulfate" /></td>
<td>-OH, -NH₂</td>
<td>Sulfotransferase</td>
</tr>
</tbody>
</table>
Methylation is a relatively minor component to drug or xenobiotic metabolism but is rather important in the biosynthesis of endogenous compounds such as epinephrine and melatonin. Usually the cofactor SAM (S-adenosyl methionine) serves as a methyl donator. Methylation (like acetylation) differs from most conjugation reactions in that it produces products with lower hydrophilicity. The exception is methylation of tertiary or pyridine-type nitrogens resulting in a charged, quaternary ammonium salts:
Phase II Transformations

• **O-Methylation** occurs mostly with catechols and at the *meta* position.

\[ \text{isoproterenol} \rightarrow \text{methyltransferase} \rightarrow \text{SAM} \rightarrow \text{methylation product} \]

**N-Methylation** is less common but does occur. Heterocyclic nitrogen atoms are also candidates for methylation.
• **S-Methylation** is common for both aromatic and aliphatic sulfhydryl groups. Once they are formed, they may be further oxidized to **sulfoxides or sulfones** (Phase I transformations).
Morphine Metabolism

Morphine $\rightarrow$ Morphine-6-glucuronide (active metabolite)

Morphine $\rightarrow$ Morphine-3-glucuronide (inactive metabolite)

A small amount of morphine undergoes N-demethylation
Morphine Metabolism

Morphine

Morphine-3-glucuronide

Morphine-3-glucuronide is the major metabolite
Induction Of UDP-\(\alpha\)-D-Glucuronyl Transferase

- Induced by phenobarbital
- Induced by 3-methylcholanthrene
Glucuronidation in the Cat

- The cat can glucuronidate bilirubin but cannot glucuronidate phenolic compounds such as phenol and napthol
SULFATE CONJUGATION
Sulfate Conjugation

- Conducted by the soluble enzyme sulfotransferase
- Endogenous donor molecule to conjugation is 3’-phosphoadenosine-5’-phosphosulfate (PAPS)
- Conjugates are ethereal in character
- Noninducible
\[ \text{Phenacetin} \rightarrow \text{N-Hydroxyphenacetin} \rightarrow \text{O-Sulfate Conjugate of N-Hydroxyphenacetin} \]
Phenylacetic Acid

\[ \text{Phenylacetic Acid} \xrightarrow{\text{ATP, PPI}} \text{Phenylpropanal} \xrightarrow{\text{CoASH, AMP}} \text{An Acyl-CoA Intermediate} \]

Glycine or Glutamine

\[ \text{N-Acyltransferase} \]

Glycine Conjugate \( R = H \)
Glutamine Conjugate \( R = \text{CH}_2\text{CH}_2\text{CONH}_2 \)
Benzoic Acid $R=H$
Salicylic Acid $R=OH$
Hippuric Acid $R=H$
Salicyluric Acid $R=OH$
Brompheniramine → 3-(p-Bromophenyl)-3-(2-pyridyl)-propionic Acid → Glycine Conjugate
Isoniazid (R=H) or N-Acetylisoniazid (R=COCH₃) → Hydrolysis → Isonicotinic Acid → Glycine Conjugate
Mescaline \rightarrow 3,4-Dihydroxy-5-methoxyphenylacetic acid \rightarrow \text{Glutamine Conjugate}
Diphenhydramine \[\rightarrow\] Diphenylmethoxyacetic Acid \[\rightarrow\] Glutamine Conjugate
GSH $^+\delta$CH$_2$$^\delta$X $\rightarrow$ GS$^+\delta$CH$_2$ + HX

R = Alkyl, Aryl, Benzylic, Allylic
X = Br, Cl, I, OSO$_3^-$, OSO$_2$R, OPO(OR)$_2$
Electrophilic Substrate

$$\text{E} + \text{HS-C}_2\text{C-NH-C}_2\text{COH}$$

Glutathione

Glutathione Adduct or conjugate

$$\text{E-S-C}_2\text{C-NH-C}_2\text{CH}_2\text{COH}$$

$$\text{S-Transferase (soluble)}$$

$$\gamma$$-Glutamyl Transpeptidase (Microsomal)

Acetyl CoA

$$\text{E-S-C}_2\text{C-NH-C}_2\text{CH}_2\text{COH}$$

$$\text{N-Acetylase (Microsomal)}$$

Mercapturic Acid derivative

$$\text{O=C}$$

S-Substituted Cysteine derivative

$$\text{H}$$

Amino Acid (AA)

Glycine

$$\text{O=C}$$

$$\text{O=C}$$

Cysteinyl Glycinase (Microsomal)
Methyl Parathion

Pathway a

S-Methylglutathione

Pathway b

S-P-Nitrophenylglutathione

2,4-Dichloronitrobenzene
Azathioprine $\xrightarrow{\text{GSH}}$ 1-Methyl-4-nitro-5-(S-glutathionyl) imidazole + 6-Mercaptopurine
\[
\begin{align*}
\text{\textbf{\(
\begin{array}{c}
\beta \\
\alpha - \beta \text{-Unsaturated System}
\end{array}
\end{align*}}\right) \\
\xrightarrow{\text{Michael Addition}} \\
\text{Glutathione Adduct}
\end{align*}
\]
Ethacrynic Acid
(note α,β-unsaturated ketone moiety)

Glutathione adduct of Ethacrynic Acid

Mercapturic Acid derivative
Acetaminophen $\rightarrow$ N-Acetylimidoquinone $\rightarrow$ Mercapturic Acid Derivative
2-Hydroxy-17β-estradiol

Orthoquinone

Semi-quinone

GSH

SG
Aromatic Amines

- Aniline
- p-Aminobenzoic Acid $R = H$
- p-Aminosalicylic Acid $R = O^-$
- Procainamide
- Dapsone
Aliphatic Amines

Histamine

Mescaline

Bisdemethyl Metabolite of 3S,6S-(-) Methadol
Clonazepam, $R = \text{Cl}$
Nitrazepam, $R = \text{H}$

7-Amino Metabolite

7-Acetamido Metabolite or $N$-Acetylated Metabolite

Sulfanilamide $R = \text{H}$
Sulfamethoxazole $R = \text{N} - \text{O} - \text{CH}_3$
Sulfisoxazole $R = \text{O}$
Sulfamethazine $R = \text{N}$
Sulfapyridine $R = \text{N}$
Hydralazine $\rightarrow$ N-Acetylhydralazine $\rightarrow$ 3-Methyl-s-triazolo-[3,4-a]phthalazine
Isoniazid $\xrightarrow{N\text{-acetylation}}$ $N$-Acetylsalicylic acid $\xrightarrow{\text{Hydrolysis}}$ Acatyhydrazine + Isonicotinic acid

Liver Damage $\leftrightarrow$ Covalent Binding $\left[\begin{array}{c}
\text{Reactive intermediates} \\
\text{possibly, } \begin{array}{c}
\text{O} \\
\text{CH}_3C + \text{CH}_3C \cdot
\end{array}
\end{array}\right]$

N-oxidation
Cytochrome P-450
Mediated
Norepinephrine, $R = \text{OH}$
Dopamine, $R = \text{H}$

3-Methoxytyramine, $R = \text{H}$
Normetanephrine, $R = \text{OH}$

$\text{COMT}$
Morphine

Catechol Metabolite of Phenytoin

Terbutaline (not a substrate for COMT)

O-methylation

Codeine

Amantadine

Norephedrine
Phenylacetone → Oxidation → Benzoic Acid (man, rabbit, guinea pig)

Amphetamine → Oxidative Deamination

Aromatic Hydroxylation → p-Hydroxyamphetamine (rat)
Figure 4-18  Phenazopyridine metabolism in humans, guinea pigs, rats and mice.
Phenytoin → \(S(-)-5\text{-}(4\text{-Hydroxyphenyl})\text{-}5\text{-phenylhydantoin}\) + \(R(+)\text{-}5\text{-}(4\text{-Hydroxyphenyl})\text{-}5\text{-phenylhydantoin}\)
Diazepam, $R = \text{CH}_3$
Desmethyldiazepam, $R = \text{H}$

(3S) $N$-Methyloxazepam, $R = \text{CH}_3$
S(+)-Oxazepam, $R = \text{H}$
Pentazocine $\rightarrow$ trans-Alcohol $+ cis$-Alcohol
<table>
<thead>
<tr>
<th>Parent Drug</th>
<th>Metabolite</th>
<th>Biotransformation Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetohexamide</td>
<td>Hydroxyhexamide</td>
<td>Ketone reduction</td>
</tr>
<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>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Carbamazepine-9,10-epoxide</td>
<td>Epoxidation</td>
</tr>
<tr>
<td>Chloral hydrate</td>
<td>Trichloroethanol</td>
<td>Aldehyde reduction</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>7-Hydroxychlorpromazine</td>
<td>Aromatic hydroxylation</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>Chlorophenoxyisobutyric acid</td>
<td>Ester hydrolysis</td>
</tr>
<tr>
<td>Cortisone</td>
<td>Hydrocortisone</td>
<td>Ketone reduction</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Desmethyldiazepam and oxazepam</td>
<td>N-Demethylation and 3-hydroxylation</td>
</tr>
<tr>
<td>Digitoxin</td>
<td>Digoxin</td>
<td>Alicyclic hydroxylation</td>
</tr>
<tr>
<td>Diphenoxylate</td>
<td>Diphenoxylate acid</td>
<td>Ester hydrolysis</td>
</tr>
<tr>
<td>Imipramine</td>
<td>Desipramine</td>
<td>N-Demethylation</td>
</tr>
<tr>
<td>Mepobarbital</td>
<td>Phenobarbital</td>
<td>N-Demethylation</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>α-Hydroxymethylmetoprolol</td>
<td>Benzyllic hydroxylation</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>Acetaminophen</td>
<td>O-Deethylation</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>Oxybutazone</td>
<td>Aromatic hydroxylation</td>
</tr>
<tr>
<td>Prednisone</td>
<td>Prednisolone</td>
<td>Ketone reduction</td>
</tr>
<tr>
<td>Primidone</td>
<td>Phenobarbital</td>
<td>Hydroxylation and oxidation to ketone</td>
</tr>
<tr>
<td>Procainamide</td>
<td>N-Acetylprocainamide</td>
<td>N-Acetylation</td>
</tr>
<tr>
<td>Propranolol</td>
<td>4-Hydroxypropranolol</td>
<td>Aromatic hydroxylation</td>
</tr>
<tr>
<td>Quinidine</td>
<td>3-Hydroxyquinidine</td>
<td>Allylic hydroxylation</td>
</tr>
<tr>
<td>Sulindac</td>
<td>Sulfide metabolite of sulindac</td>
<td>Sulfoxide reduction</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>Mesoridazine</td>
<td>S-oxidation</td>
</tr>
<tr>
<td>Warfarin</td>
<td>Warfarin alcohols</td>
<td>Ketone reduction</td>
</tr>
</tbody>
</table>
The cytosolic enzyme sulfotransferase conducts the donation of sulfate from the endogenously synthesized PAPS to various substrates to form sulfate conjugates. An example of such substrate is acetaminophen.
Sulfate Conjugation of $\rho$-Hydroxyacetanilid

\[ \text{PAPS} + \text{PAP} \xrightarrow{\text{sulfotransferase}} \cdot \text{PAP} \]

\[ \text{PAP}: 3'\text{-phosphoadenosine- 5'\text{-phosphate}} \]
MINOXIDIL METABOLISM

MINOXIDIL → MINOXIDIL N-O-SULFATE

MINOXIDIL (inactive) → MINOXIDIL N-O-GLUCURONID (inactive metabolite)
Species Differences in Sulfate Conjugation

• Some species are deficient in the sulfate conjugation pathway
  – Pig
  – Opposum
N-ACETYLYATION
N-Acetyltransferase

- A soluble enzyme
- Isoniazid is a substrate
- Genetic variation occurs
  - Some individuals are fast acetylators
  - Some individuals are slow acetylators
- Acetyl coenzyme A is the endogenous donor molecule
Various acetylases, for example, choline acetylase and N-acetyltransferase, all soluble enzymes, conduct the transfer of the acetyl group of acetyl CoA to various substrates. For example, N-acetylation of isoniazid. Genetic polymorphism occurs with N-acetyltransferase.
N-Acetyltransferase

\[
\text{sulfanilamide} + \text{CoA-S-CCH}_3 \rightarrow \text{N}_4\text{-acetylsulfanilamide} + \text{CoA-SH}
\]
N-Acetyltransferase

- The dog cannot acetylate aromatic amino compounds because it lacks the appropriate isoenzyme of NAT
SUGAR CONJUGATION
Conversion of 6-Mercaptopurine to a Nucleotide

6-mercaptopurine + 5-phosphoribosyl 1-pyrophosphate (PRPP) → pyrophosphate

6-mercaptopurine nucleoside monophosphate
METHYLATION
Cytosolic enzymes such as catechol-O-methyl transferase (COMT) and phenylethanolamine-N-methyl transferase (PNMT) conducts the donation of the methyl group from the endogenously synthesized SAM to various substrates to form methylated conjugates. Norepinephrine is N-methylated by PNMT to form epinephrine. Norepinephrine, epinephrine, dopamine, and L-DOPA are O-methylated by COMT.
Methyltransferases

- A family of soluble enzymes that conducts
  - N-methylation; $\text{N-CH}_3$
  - O-methylation; $\text{O-CH}_3$
  - S-methylation; $\text{S-CH}_3$

- S-adenosylmethionine (SAM) is the endogenous donor molecule. It is demethylated to S-adenosylhomocysteine.
N-Methyltransferases

PNMT- Phenylethanolamine-N-methyltransferase

Norepinephrine $\xrightarrow{\text{PNMT}}$ Epinephrine

\[
\begin{align*}
\text{norepinephrine} & \quad \text{OH} \quad \text{OH} \quad \text{CHCH}_2\text{NH}_2 \\
\text{epinephrine} & \quad \text{OH} \quad \text{OH} \quad \text{CHCH}_2\text{NH}{-}\text{CH}_3
\end{align*}
\]
O-Methylation Of Catecholamines

COMT- catechol-O-methyltransferase
O-Methylation of Norepinephrine

COMT - catechol-O-methyltransferase

Norepinephrine

Normetanephrine
S-Methylation of 6-Mercaptopurine

TPMT - thiopurinemethyltransferase; some individuals are deficient in this enzyme that is critically important for the metabolism of this agent.
METABOLISM OF MERCAPTOPURINE (1)

6-Mercaptopurine \(\xrightarrow{TMPT}\) 6-Methylmercaptopurine

- **TMPT - Thiomethylpurine transferase**
  - Conducts S-methylation of the substrate
  - Found in RBC’s
  - Isoforms exist
    - active enzyme
    - inactive enzyme
AMINO ACID CONJUGATION
AMINO ACID CONJUGATION

\[ \text{RCOOH} + \text{CoA-SH} \xrightarrow{\text{Acid:CoA ligase, ATP}} \text{RCO-S-CoA} \]

\[ \text{RCO-S-CoA} + \text{NH}_2\text{CH}_2\text{COOH} \xrightarrow{\text{N-acyltransferase, Glycine}} \text{RCONHCH}_2\text{COOH} \]

Glycine conjugate

(mitochondria)
Multiple Metabolic Pathways Exist for Aspirin’s Metabolism

Hydolysis of aspirin produces salicylic acid, as seen in the next slide
Salicyluric Acid is the Glycine Conjugate of Aspirin

Salicylic acid, the glycine conjugate of salicyclic acid, is the main metabolite of aspirin. Approximately 76% of aspirin is metabolized through amino acid conjugation.
Acetyl Salicylic Acid (Aspirin) Metabolism

• Salicylic acid the hydrolytic product of acetyl salicylic acid. Salicylic acid is further metabolized.

• Salicyl uric acid is the glycine conjugate and the main metabolite of aspirin. About 75% of aspirin is metabolized by this pathway.

• Other metabolites of aspirin
  – the acyl glucuronide conjugate of salicylic acid (salicylic acid glucuronide)
  – the phenol glucuronide conjugate of salicylic acid (salicyl phenol glucuronide)
  – the ring hydroxylated product of salicylic acid (gentisic acid)
  – the ring hydroxylated product of the glycine conjugate (gentisuric acid)
TRANSULFURATION
TRANSULFURATION

\[ \text{CN}^- + \text{S}_2\text{O}_3^{2-} \rightarrow \text{CNS}^- + \text{SO}_3^{2-} \]

cyanide  thiosulfate  thiocyanate  sulfite

Mediated by mitochondrial thiosulfate sulfurtransferase (rhodanese)
GLUTATHIONE CONJUGATION
DRUG INTERACTION WITH GLUTATHIONE

GLUTAMIC ACID --- CYSTEINE --- GLYCINE

\[ D + SH \]

\[
\begin{align*}
\text{GLUTAMIC ACID --- CYSTEINE --- GLYCINE} \\
D & \quad SH \\
\text{CYSTEINE --- GLYCINE} \\
D & \quad SH \\
\text{CYSTEINE} \\
D & \quad SH \\
\text{ACETYL-N-Cysteine} \\
D & \quad SH \\
\text{mercapturate metabolite of drug}
\end{align*}
\]
MERCAPTURIC ACID FORMATION

- Conjugation of substrate to glutathione by the enzyme **glutathione transferase**
- Hydrolytic removal of glutamic acid by glutamyl transpeptidase
- Hydrolytic removal of glycine by cysteinyl glycine
- Acetylation of the cysteinyl substrate by **N-acetyltransferase** to form the N-acetylated cysteinyl conjugate of substrate; substrate referred to as a “mercapturate”
ACETAMINOPHEN METABOLISM
Bioactivation of Acetaminophen

**Acetaminophen**

- Activation by cytochrome P-450
- NADPH, O₂

**Postulated toxic intermediates**

- Nucleophilic cell macromolecules

**Conjugate**

- PAPS Sulfotransferase
- UDP-GA UDP-Glucuronosyl transferase

**Cell macromolecules**

- Mercapturic acid
ACETAMINOPHEN AND ITS PHASE II METABOLITES

The sulfate and glucuronide conjugates of acetaminophen are the major metabolites. High doses of acetaminophen can exhaust the metabolic pathways that produce these conjugates, allowing more of the parent drug to undergo the phase I metabolic pathway which is involved in bioactivation and toxication.
ACETAMINOPHEN AND ITS PHASE I METABOLITES

Acetaminophen $\xrightarrow{\text{CYP 450}}$ N-Hydroxy-acetaminophen $\xrightarrow{\text{spontaneous rearrangement}}$ N-Acetylbenzoquinoneimine $\xrightarrow{\delta^+}$ p-Aminophenol
The minor metabolite (4% of acetaminophen), N-hydroxyacetaminophen, is always produced by microsomal cytochrome P450. It rearranges to the electrophile N-acetylbenzoquinoneimine, which in turn reacts with the sulfhydryl group of glutathione. Acetaminophen mercapturic acid is the final metabolite. If tissue glutathione stores are depleted as a result of fasting, intake of excessive doses of acetaminophen or through induction of CYP2E1 as a result of chronic intake of ethanol, the quinone interacts with nucleophilic sites of cellular macromolecules, such as proteins. Liver necrosis is the result. Regular intake of acetaminophen during fasting or chronic ethanol intake should be avoided. N-acetylcysteine is the antidote for acetaminophen poisoning. It reacts with the electrophile. A small amount of acetaminophen is reported to undergo deacetylation to the phase 1 metabolite \( p \)-aminophenol.
N-ACETYLCYSTEINE FOR ACETAMINOPHEN TOXICITY

N-acetylbenzoquinoneimine

N-acetylcysteine

Acetaminophen mercapturic acid
CARCENOGENSIS
N-Hydroxylation of AAF is the first metabolic step towards the development of a carcinogenic agent.
Further Metabolism of N-HydroxyAAF Produces Cancer

N-HydroxyAAF undergoes phase II metabolism to the ultimate carcinogen. The glucuronide pathway is also involved in carcinogenesis.
CYP1A1 Converts Benzopyrene to a Carcinogen

Benzo(a)pyrene

P-450 + NADPH + O₂ → Epoxide hydrolase + H₂O

DNA → Major adduct

DNA → Ultimate carcinogen

+ P-450 + NADPH + O₂
Aflatoxin is metabolized to a carcinogenic agent.

\[ \text{AFLATOXIN B}_1 \quad (\text{AFB}_1) \quad \xrightarrow{\text{CYP 3A4}} \quad \xrightarrow{\text{or \ CYP 1A2}} \quad \text{AFB}_1-2,3-\text{OXIDE} \quad \xrightarrow{\text{DNA}} \]
FACTORS AFFECTING DRUG METABOLISM
ENZYME INDUCTION
EFFECT OF CHRONIC PENTOBARBITAL ON SLEEPING TIME IN RABBITS

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pentobarbital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleeping Time (Minutes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CORRELATION BETWEEN SLEEPING TIME AND PLASMA $T_{1/2}$ IN CHRONIC PENTOBARBITAL PRETREATED RABBITS

![Graph showing the correlation between sleeping time and plasma $T_{1/2}$ in chronic pentobarbital pretreated rabbits. The graph compares the control group with the PTB group.](image-url)
Factors Affecting Drug Metabolism

• Enzyme Induction - increased enzyme protein levels in the cell
  – Phenobarbital type induction by many drugs
  – Polycyclic hydrocarbon type induction by polycyclic hydrocarbons such as 3,4-benzopyrene and 3-methylcholanthrene
AGE
FACTORS AFFECTING DRUG METABOLISM

• Age
  – Neonates
  – Children
  – Elderly
DIET
Activated acyl or aroyl coenzyme A cosubstrate

Glycine and glutamine

\[
\begin{align*}
\text{R} & \quad \text{(Ar)} \quad \text{O} \quad \text{S} \quad \text{CoA} \\
\text{H}_2\text{N} & \quad \text{COOH} \quad \text{R} \quad \text{-COOH} \\
\text{Glutathione (GSH)}
\end{align*}
\]

Glycine

\textit{N}-acyltransferase

Glutamine

\textit{N}-acyltransferase

\[
\begin{align*}
\text{Ar-X, arene oxide, epoxide, carbocation or related} \\
\text{Glutathione S-transferase}
\end{align*}
\]

Acetyl coenzyme A

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{O} \quad \text{S} \quad \text{CoA} \\
\text{-OH, -NH}_2 \\
\text{Acetyltransferase}
\end{align*}
\]

\[
\begin{align*}
\text{S-Adenosyl methionine (SAM)}
\end{align*}
\]

Methyl

\[
\begin{align*}
\text{HOOC} & \quad \text{CH}_3 \quad \text{NH}_2 \\
\text{-OH, -NH}_2, -\text{SH}, \text{heterocyclic N} \\
\text{Methyltransferase}
\end{align*}
\]

\^ The bold-faced parts are transferred to the drug or metabolite.
FACTORS AFFECTING DRUG METABOLISM

• Diet
  – Charcoal broiled foods (contain polycyclic hydrocarbons that increase certain enzyme protein in cells)
  – Grapefruit juice (the active component is the furancoumarin 6,7-dihydroxybergamottin which inhibits a certain a group of microsomal enzymes)
GENETIC VARIATION
Some Enzymes That Exhibit Genetic Variation

– Pseudocholinesterase
  • typical enzyme
  • atypical enzyme
– N-Acetyltransferase (isoniazid is a substrate)
  • fast acetylation
  • slow acetylation
– Cytochrome P450 2D6
– Cytochrome P450 2C19
– TMPT -Thiomethylpurinetransferase
– Dihydropyrimidine Dehydrogenase
STATE OF HEALTH
FACTORS AFFECTING DRUG METABOLISM

• State of health
  – Hepatitis
  – Liver cancer
  – Cardiac insufficiency
  – Uremia
    • degree of protein binding
Changes In Drug Metabolism As A Consequence Of Hepatic Disease

From *Principles of Drug Action*
GENDER
FACTORS AFFECTING DRUG METABOLISM

• Gender
  – Most studies are performed in the rat. In general, male rats metabolize drugs faster than female rats
DEGREE OF PROTEIN BINDING
FACTORS AFFECTING DRUG METABOLISM

• Degree of protein binding
  – Conditions that displace bound drug from protein allows more of the drug to be accessible to the enzyme for which it serves as a substrate e.g. uremia, low plasma albumin
SPECIES VARIATION
FACTORS AFFECTING DRUG METABOLISM

• Species variation
  – Quantitative
  – Qualitative
Factors Affecting Drug Metabolism

• **Species variation**
  - Human beings metabolize amphetamine by deamination; rats and dogs metabolize the drug by aromatic hydroxylation
  - Guinea pigs have very little sulfotransferase activity, humans have substantial activity
  - Guinea pigs do not N-hydroxylate substrates; mice, rabbits, dogs do
  - Hexobarbital is metabolized at different rates by different species
HEXOBARBITAL METABOLISM RATE DIFFERS AMONG SPECIES

Animals were treated with identical doses of hexobarbital n= 8-10 animals per species
SUBSTRATE COMPETITION
Factors Affecting Drug Metabolism

• Substrate competition
  – Two or more drugs competing for the same enzyme can affect the metabolism of each other; the substrate for which the enzyme has the greater affinity would be preferentially metabolized