Why Do Drug’s Get Metabolized?

• Metabolism is the process of preparing foreign chemicals for removal from the body

• Drug action is usually terminated by metabolic processes

• The rate of metabolism is one factor in determining the duration of action of drugs

• Metabolism often occurs in two steps
Phase I vs. Phase II

- Phase I metabolism involves chemical transformations, usually catalyzed by enzymes, including oxidations, reductions, hydrolyses, and other reactions that prepare the drug for elimination from the body.

- Most often the products of phase I metabolism are more polar than the drug molecule.
OH
-C- \[\text{oxidation}\] \rightarrow -C-OH
alcohol

-OH
-C- \[\text{reduction}\] \rightarrow -C-

-NR, OR, and SR \[\text{oxidative dealkylation}\] \rightarrow NH\textsubscript{2}, OH, and SH

O
-C- \[\text{reduction}\] \rightarrow OH

-N=N- \[\text{azo reduction}\] \rightarrow -NH\textsubscript{2}

-NO\textsubscript{2} \[\text{nitro reduction}\] \rightarrow -NH\textsubscript{2}

O
-C-OR \[\text{Hydrolysis of ester}\] \rightarrow -C-OH

O
-C-NHR \[\text{Hydrolysis of amide}\] \rightarrow -C-OH + -NHR
$\Delta^1$-THC

7-Hydroxy-$\Delta^1$-THC

$\Delta^1$-THC-7-oic Acid

R = Glucornyl group
Sites of Drug Biotransformation:

- **Extrahepatic microsomal enzymes** (oxidation, conjugation)
- **Hepatic microsomal enzymes** (oxidation, conjugation)
- **Hepatic non-microsomal enzymes** (acetylation, sulfation, GSH, alcohol/aldehyde dehydrogenase, hydrolysis, ox/red)
Cellular localisation of metabolic enzymes

- Endoplasmatic reticulum (ER) of intestinal- and liver cells contain P450
- Cytosol contains Phase II metabolic enzymes
oxidations

- Many Phase I oxidations are mediated by cytochrome P450 enzymes.

- Membrane bound proteins - found on the endoplasmic reticulum.

- Heme-containing proteins – porphyrin ring coordinating iron at the active site.

- Many iso-forms with different substrate specificities:
  - Major human CYP’s: 1A2, 2C9, 2C19, 2D6, 3A4
Structure of P450

Figure 1 Structure of P450 CYP2C9. a, Overall fold of CYP2C9, coloured from blue at the N terminus to red at the C terminus. The haem group is depicted as a ball-and-stick model in the centre of the molecule, flanked by helices I and L. There is a slight distortion in helix I, close to the haem. The substrate access channel is widely acknowledged to involve the loops between helices B and C, and helices F and G. The figure was produced using Molscript (http://www.ks.uiuc.edu/Molscript). b, View of Arg 97 and the haem group (shown at the bottom). Arg 97 is held in position by hydrogen bonds (indicated by dashed lines) to the haem propionates and to the carbonyl oxygen atoms of Val 113 and Pro 367. Figures 1b–4b were produced using Aesop 2.5 (M. Noble, unpublished work).
• **Cytochrome P450 system:**

• Cytochromes are hemoproteins (heme-thiolate) that function to pass electrons by reversibly changing the oxidation state of the Fe in heme between the 2+ and 3+ state and serves as an electron acceptor–donor.

• P450 is not a singular hemoprotein but rather a family of related hemoproteins. Over 1000 have been identified in nature with ~50 functionally active in humans with *broad substrate specificity*

• - CYPs are in smooth endoplasmic reticulum in close association with NADPH-CYP reductase in 10/1 ratio

• The reductase serves as the electron source for the oxidative reaction cycle
Cytochrome P450 Isoforms (CYPs) - An Overview

• NADPH + H⁺ + O₂ + Drug → NADP⁺ + H₂O + Oxidized Drug

• Carbon monoxide binds to the reduced Fe(II) heme and absorbs at 450 nm (origin of enzyme family name)

• CYP monooxygenase enzyme family is major catalyst of drug and endogenous compound oxidations in liver, kidney, G.I. tract, skin, lungs

• Oxidative reactions require the CYP heme protein, the reductase, NADPH, phosphatidylcholine and molecular oxygen
• CYP plus arabic numeral (>40% homology of amino acid sequence, eg. CYP1)

• Subfamily - 40-55% homology of amino acid sequence; eg. CYP1A

• Subfamily - additional arabic numeral when more than 1 subfamily has been identified; eg. CYP1A2

• Italics indicate gene (CYP1A2); regular font for enzyme

• Comprehensive guide to human Cyps
Oxidative Processes

• Common oxidative metabolisms include:
  - Aromatic hydroxyations
  - Aliphatic hydroxylations - Epoxidation of alkenes
  - Dealkylation on heteroatoms
  - Oxidation of sulfide to sulfoxides to sulfones
  - Oxidation of imines to imine oxides
  - Oxidation of alcohols to aldehydes
  - Oxidation of aldehydes to carboxylic acids
Aromatic hydroxylation:-

Hydroxylation usually occurs at the Para-position. Most phenolic metabolites undergo further conversion to polar and water soluble glucuoronide or sulfate conjugate which are readily excreted.
Phenytoin $\rightarrow$ P-HydroxyPhenytoin $\rightarrow$ O-Glucuronide Conjugate
Hydroxylation occurs most readily on rings that are electron-rich, i.e. those that have electron-donating groups directly attached to the ring (OH, OCH3, NH2, alkyl groups)
**Arene Oxides**

- Spontaneous Rearrangement
  - NIH Shift
    - Arenols

- H₂O
  - trans-Dihydriodiols

- GSH
  - Glutathione Adducts

- Macromolecules
  - Macromolecular adduct covalently bound to
    - M = DNA, RNA, or protein
Dihydrodiol metabolites have been reported in the metabolism of several aromatic hydrocarbons.
Enzymatic conjugation with glutathione plays an important role not only in detoxification of arene oxides but also in detoxification of a variety of other chemically reactive and potentially toxic intermediates.
Benzo[a]pyrene → 7,8-Oxide → 7,8-trans-Dihydrodiol

deoxyribose → Covalently Bound Deoxyguanosine
Benzo[a]pyrene Adduct → (+)-7,8-Diol-9,10-epoxide
Metabolic Oxidation of Alkenes

The oxidative metabolism of olefins (alkenes) is identical to that of aromatics except that the epoxides that are formed have little tendency to undergo rearrangement.

The initially-formed epoxides are usually not observed as metabolites, unless structural features of the molecule make them stable.

Most typically, they are attacked by epoxide hydrolase to give trans-dihydrodiols.
Oxidation of Olefins:

Epoxides are minor products owing to further conversion to 1,2-diol
Oxidation of Olefins:

\[
\text{Styrene} \xrightarrow{\text{Styrene Oxide}} \quad \text{GSH} \xrightarrow{\text{Covalent binding to proteins, nucleic acids}} \quad \text{Mercapturic Acid Derivative (major)} + \text{Mercapturic Acid Derivative (minor)}
\]
Metabolic Oxidation of Alkyl Groups

- Aliphatic carbons are also subject to metabolic oxidation. These oxidations can be categorized as:

  - Oxidation of aliphatic carbons that are adjacent to (at an α-position) a functional group
  - Oxidation of aliphatic carbons at or near the end of a chain of aliphatic carbons.
The product of these oxidation reactions is an alcohol.
The carbon undergoing metabolism must have at least one attached hydrogen.
The carbon adjacent to a functional group is the α-carbon. The carbons shown in bold-face type below are all α-carbons:
Metabolic Oxidation of Alkyl Groups

Oxidation of benzylic carbon atoms
Metabolic Oxidation of Alkyl Groups

Oxidation at carbon atom $\alpha$- to carbonyls and imines:

Diazepam → (3$S$) $N$-Methyloxazepam or 3-Hydroxydiazepam → Oxazepam

Flurazepam → Nimetazepam
Metabolic Oxidation of Alkyl Groups

**Oxidation at Aliphatic and alicyclic carbon atoms:**
Aliphatic carbon centers are subjected to mixed function oxidation:

(ω) **Oxidation:** - oxidation at the terminal methyl group.

(ω-1) **Oxidation at carbon atom next to the last carbon.**

![Chemical structures showing aliphatic and alicyclic oxidation reactions](image-url)
Metabolic Oxidation of Alkyl Groups

5-Hydroxyvalproic Acid

2-n-Propylglutaric Acid

ω Oxidation

ω-1 Oxidation

4-Hydroxyvalproic Acid

Amobarbital

3'-Hydroxyamobarbital
The cyclohexyl group is commonly found in many medicinal agents and is also susceptible to mixed function oxidation (alicyclic hydroxylation).
Metabolic Dealkylation

Oxidation involving carbon heteroatom systems:

*Hydroxylation of the α-carbon atom attached directly to the heteroatom (N, O, S), give unstable intermediate which decompose with the cleavage of the carbon- heteroatom bond.

\[
R-X-C_\alpha \rightarrow [R-X-C_\alpha + O] \rightarrow R-XH + C=O
\]

Where \( X = N, O, S \)

Usually Unstable
Metabolic Dealkylation

Hydroxylation or oxidation of the heteroatom (N,S) only.

N- Hydroxylation
N- Oxide formation
Sulfoxide formation
Sulfone formation
The C—N system is found in many drugs as amines or amides as in many naturally as well as in many drugs as
*Phenothiazines
*Antihistamines
*Tricyclic antidepressants
*β- Adrenergics
*Sympathomimetic phenyl ethylamine
*Barbiturates
*Benzodiazepines.
The nitrogen could be found as:

1) - aliphatic (tertiary, secoday, primary) and alicyclic (tertiary,secondary) amines.
2) - aromatic and heterocyclic nitrogen compounds
3) - amides.
The hepatic enzymes that are involved in N-metabolism are
1) - Hepatic cytp-450 mixed function oxidases which carry out the α-carbon hydroxylation reactions and
2) - Hepatic mixed function oxidase called amine oxidase or N-oxidase. These are NADPH dependent flavo proteins and do not contain cytp-450. It requires molecular oxygen and NADPH to carry out the N-oxidation.
Chlorpromazine

FMOs
CYP2D6

S-oxide

N-oxide
Metabolic Dealkylation
Tertiary aliphatic and alicyclic amines:

Oxidative removal of alkyl group (particularly methyl group) from tertiary aliphatic and alicyclic amines is carried out by hepatic cytochrome p-450 mixed function oxidase enzymes. This reaction is called oxidative N-dealkylation, which involves first α-carbon hydroxylation to give a carbinolamine (which is an unstable intermediate). This will undergo heterolytic cleavage of C–N bond to give a secondary amine and carbonyl moiety (aldehyde or ketone).

![Chemical structures showing the dealkylation process](image)
N-Oxide formation is complicated because of the susceptibility to undergo in vivo reduction back to the tertiary amine. Tertiary amines such as imipramine, morphine, and chlorpromazine reportedly form N-oxide products. In some instances, N-oxides possess pharmacological activity. A comparison of imipramine N-oxide with imipramine indicates that the N-oxide itself possesses antidepressant and cardiovascular activity similar to that of the parent drug.
Secondary amine either parent compound or metabolites are susceptible to oxidative N-dealkylation, oxidative deamination and N-oxidation but to a much lesser extent.

Metabolic Dealkylation
Oxidation of secondary and primary amines:

Secondary amine either parent compound or metabolites are susceptible to oxidative N-dealkylation, oxidative deamination and N-oxidation but to a much lesser extent.

Methamphetamine → Amphetamine → Phenylacetone
Metabolic Dealkylation

Ketamine \[\rightarrow\] Norketamine
N-oxidation of secondary aliphatic and alicyclic amines

N-oxidation of secondary aliphatic and alicyclic amines leads to several N-oxygenated products. N-hydroxylation of secondary amines generates the corresponding N-hydroxylamine metabolites. Often, these hydroxylamine products are susceptible to further oxidation (either spontaneous or enzymatic) to the corresponding nitrone derivatives.
N-oxidation of secondary aliphatic and alicyclic amines

*N*-Benzylamphetamine

Hydroxylamine Metabolite

Nitrene Metabolite

Phenmetrazine

*N*-Hydroxyphenmetrazine

Nitrene Metabolite
Metabolic Dealkylation

Primary amines:-

endogenous primary amines are metabolized by mono amine oxidase present in mitochondria of the cell.

Dopamine
Norepinephrine metabolised

Tryptamine by mono amine oxidase (MAO) inactive compound

Serotonine
Metabolic Dealkylation

Primary aliphatic amines (whether parent drugs or metabolites are bio-transformed by oxidative deamination through carbinolamine pathway or by N-oxidation.

Chemical Structures:
- **Amphetamine**
- **Phentermine**
- **p-Hydroxyphentermine**
- **N-Hydroxyphentermine**
- **Carbinolamine**
- **Phenylacetone**
N-oxidation of Primary aliphatic

Amphetamine → N-Hydroxyamphetamine → Imine

Phenylacetone

Oxime

Oxidation

H₂O

NH₂OH
Aromatic Amines and Heterocyclic Nitrogen Compounds:

\[ \text{Dapsone} \quad \text{R} = \text{H} \]
\[ \text{N-Acetyldapsone} \quad \text{R} = \text{CCH}_3 \]
\[ \text{N-Hydroxydapsone} \quad \text{R} = \text{H} \]
\[ \text{N-Acetyl-N-hydroxydapsone} \quad \text{R} = \text{CCH}_3 \]

Secondary Aromatic Amines
\[ \text{Secondary Hydroxylamine (secondary)} \]
\[ \text{Nitroine} \]
\[ \text{Hydroxylamine (primary)} \]
Tertiary aromatic amines:
Amides:

Diazepam → Carbinolamide → Desmethyl diazepam

(\text{CH}_3\text{CH}_2)_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{N} → \text{Flurazepam}

\text{Hexobarbital} \quad R_1 = \text{C}_6\text{H}_5, \quad R_2 = \text{CH}_3

\text{Mephobarbital} \quad R_1 = \text{CH}_3, \quad R_2 = \text{C}_6\text{H}_5

\text{Chlorpropamide} \quad R_1 = \text{SO}_2\text{NHCONHCH}_2\text{CH}_2\text{CH}_3
Cyclophosphamide → 4-Hydroxycyclophosphamide → 4-Ketocyclophosphamide

\[
\begin{align*}
\text{Phosphoramidic Acid} &\quad + \quad \text{Acrolein} \\
\text{Aldophosphamide} &\quad \rightarrow \quad \text{Carboxyphosphamide}
\end{align*}
\]
2-Acetylaminofluorene (AAF) → \( \text{N-Hydroxy AAF} \) → \( \text{O-Sulfate Ester of N-Hydroxy AAF} \)

\( \text{Nu} = \text{Nucleophile}\), e.g., DNA → Nitrenium Species
Metabolic Dealkylation

• Dealkylation of groups attached to oxygen and sulfur can also occur.

• The mechanism is identical to that of N-dealkylation.

• As before, the carbon directly attached to the O or S MUST have at least one hydrogen.
$$\text{R-O-C} \xrightarrow{\alpha\text{-Carbon Hydroxylation}} \begin{array} \text{R-O-C} \end{array} \xrightarrow{\text{Hemiacetal or Hemiketal}} \begin{array} \text{R-O-C} \end{array} \xrightarrow{\text{Phenol or Alcohol}} \text{R-OH} + \text{O=C}$$

- **Ether**
- **Hemiacetal or Hemiketal**
- **Phenol or Alcohol**
- **Carbonyl Moiety (aldehyde or ketone)**

**Codeine**

$$\text{CH}_3 \text{O} \text{O} \text{OH} \rightarrow \text{HO} \text{O} \text{OH} + \text{H} \text{C-C} \text{H}_3$$

**Morphine**

**Phenacetin**

$$\text{OCH}_2 \text{CH}_3 \rightarrow \text{OH} + \text{H} \text{C-C} \text{H}_3$$

**Acetaminophen**
Oxidation involving carbon-sulfur systems:
Carbon-sulfur functional groups are susceptible to metabolic:

S-dealkylation $\rightarrow$ α-carbon hydroxylation

desulfuration $\rightarrow$ $\begin{array}{c} \text{C=S} \\ \text{desulfuration} \end{array}$ $\rightarrow$ $\begin{array}{c} \text{C=O} \\ \text{desulfuration} \end{array}$

S-Oxidation $\rightarrow$ $\begin{array}{c} \text{S = O} \\ \text{S-Oxidation} \end{array}$
S-dealkylation

6-(Methylthio)-purine

6-Mercaptopurine
Desulfuration

Thiopental \[ \rightarrow \] Pentobarbital

Parathion \[ \rightarrow \] Paraoxon
S-Oxidation

Cimetidine $X = N\text{C} = N$
Metiamide $X = S$

Sulfoxide Metabolite

Oxisuran

Sulfone Metabolite

Dimethyl Sulfoxide

Dimethyl Sulfone
Oxidation of alcohols and aldehydes:-

Many oxidative processes (benzylic, allylic, alicyclic or aliphatic hydroxylation) generate alcohol or carbinol metabolite as intermediate products. These if not conjugated, they will further oxidized to aldehydes if primary alcohol or to ketones if secondary alcohols.
Oxidation of alcohols and aldehydes:

RCH₂OH \rightleftharpoons RCHO \rightleftharpoons RCOOH

Alcohol dehydrogenase

NAD⁺ NADH

Aldehyde dehydrogenase

NAD⁺ NADH

Examples:

Aldehyde oxidase

Xanthine oxidase

2° alcohol \rightleftharpoons Ketone

\downarrow

Conjugated
Oxidation of alcohols and aldehydes:-
Other oxidative biotransformation pathway: Aromatization or dehydrogenation

Norgesterol

17α-18-homoestraadiol
Aromatization or dehydrogenation
Oxidative dehalogenation:
Many halogen containing drugs are metabolized by oxidative dehalogenation egs:
**Reductive reaction:-**
Reductive processes play an important role in the metabolism of many compounds containing C=O, NO\(_2\) and –N=N groups.

**Reduction of aldehydes and ketones**

These reductions are carried out by aldo-keto reductases. They are found in liver and other tissue (kidney). They have broad substrate specificities and require NADPH as cofactor. Oxido-reductase enzymes that carry out both oxidation and reduction reactions also are capable of reducing aldehydes and ketones, alcohol dehydrogenase is a NAD\(^+\) dependent oxidoreductase which oxidizes ethanol and other aliphatic alcohols to aldehydes and ketones. This same enzyme system is capable of reducing carbonyl derivatives to their corresponding alcohols.

![Chemical structures](image-url)
Reductive reaction

An NADPH-dependent enzyme called nitro-reductase is the actual reducing agent.
Reductive reaction

Azo reduction

Reduction of N-oxide.

Miscellaneouse Reduction:-
Reduction of N-oxide.
Reductive reaction

Reduction of sulfur – containing functional groups:

\[ \text{Sulindac} \rightarrow \text{Sulindac Sulfide Metabolite} \]
Hydrolytic Reactions

Hydrolysis is the process of breaking bonds by the addition of water.

Functional groups that are most often metabolized by hydrolysis include esters (and lactones) and amides (and lactams).
Hydrolysis of esters and amides:

Hydrolytic enzymes that catalyze the cleavage of ester and amide linkages are present in many organs, various tissues and in plasma. The metabolic products formed (carboxylic acids, alcohol, phenols and amines) generally are polar and more susceptible to conjugation and excretion than the parent compounds (esters or amide drugs).
Amide hydrolysis appear to the mediated by liver microsomal amidases and esterase

Procainamide

\[
\begin{align*}
\text{H}_2\text{N} & - \text{C} & \text{O} \\
\text{amine} & \text{chain} & \text{amide} \\
\text{N} & \text{H} & \text{N} \\
\text{ester} & \text{group} & \text{ester} \\
\end{align*}
\]

\[\text{Amidase}\]

\[\text{slow Hydrolysis}\]

\[\text{H}_2\text{N} - \text{COOH}\]

Procaine

\[
\begin{align*}
\text{H}_2\text{N} & - \text{C} & \text{O} \\
\text{amine} & \text{chain} & \text{ester} \\
\text{N} & \text{H} & \text{N} \\
\text{ester} & \text{group} & \text{ester} \\
\end{align*}
\]

\[\text{Esterase}\]

\[\text{Rapid Hydrolysis}\]

\[\text{H}_2\text{N} - \text{COOH}\]
Phase II Metabolism

Conjugation Reactions
Drug conjugation pathways (phase II)
These are the most important xenobiotic biotransformation reactions.

Only after conjugation reaction have added an ionic hydrophilic moiety such as glucuronic acid, sulfate, or glycine to the xenobiotic, water solubility increased enough to make urinary elimination possible.

Many conjugative enzyme accomplish this objective, and these may show stereo specificity towards enantiomers when a racemic drug is administered.
N-acetylation or N-glucuronidation

O-glucuronidation or O-sulfation

acyl glucuronidation or amino acid conjugation
Glucuronide Conjugates

β-Glucuronic acid
Glucuronide Conjugates

♦ Among the most common phase II metabolites

♦ Body has large supply of glucuronic acid, which is made from D-glucose.

♦ Functional groups susceptible to glucuronidation include: **alcohols** and **phenols**, **carboxylic acids**, **amines**, **thiols**
The glucuronide group needs to be activated before its condensation with the substrate. The reaction between UDPGA and the compound is catalyzed by UDP-glucuronyl transferases, it’s a multigene family of isozymes located along the endoplasmic reticulum of the liver, epithelial cell of the intestine and other extra hepatic tissues. Its unique location in the endoplasmic reticulum along with CYP450 has important physiological effect in controlling levels of reactive metabolites present in these tissues.
α-D-glucose-1-phosphate \[\xrightarrow{\text{UTP PP phosphorylase}}\] Uridine-5-diphospho-D-glucose (UDPG)

UDPG \[\xrightarrow{\text{UDPG dehydrogenase}}\] 2NAD$^+$

2NADH + 2H

UDP-gluronyl-transferase

β-glucuronide conjugate

UDP-glucuronate
Amino salicylic acid

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{COOH} \\
\text{OH} & \\
\text{OH} &
\end{align*}
\]

\[
\text{HOOC} - \text{HN} - \text{COOH}
\]

**UDP-glucuronate**

\[
\begin{align*}
\text{HOOC} & \quad \text{COOH} \\
\text{OH} &
\end{align*}
\]
SULFATE CONJUGATION

The consequence of this conjugation is its increased aqueous solubility and excretion because the pK_a of the sulfate group is about 1-2. The sulfates conjugate are almost totally ionized in physiologic solutions. The cytosolic sulfotransferases are generally associated with the conjugation of phenolic steroids, bile acids, neurotransmitters and phenolic drugs.

\[
\text{SO}_4^{2-} + \text{ATP} \xrightarrow{1. \text{ATP-sulfurylase}} 3\text{-Phosphoadenosine-5\text{'}-phosphosulfate} + \text{ADP} + \text{PPi} \\
\xrightarrow{2. \text{APS-phosphokinase}} \text{(PAPS)}
\]

Sulfation pathways.
Phenacetin → N-Hydroxyphenacetin → O-Sulfate conjugate of N-Hydroxyphenacetin
CONJUGATION WITH AMINO ACIDS:

Conjugation with amino acids is an important metabolic route in the metabolism of the **carboxylic acids** prior to elimination. Glycine, the most common amino acid, forms water soluble ionic conjugates with aromatic, arylaliphatic and hetero cyclic carboxylic acids. These amino acid conjugates are usually less toxic than their precursor acids and are excreted readily into the urine and bile.

\[
\text{RCOOH} + \text{ATP} + \text{CoA} \xrightarrow{\text{acyl synthetase}} \text{R-CO-S-CoA} + \text{AMP}
\]

\[
\text{R-CO-S-CoA} + \text{R`-NH}_2 \xrightarrow{\text{transacylase}} \text{R-CO-NH-R`} + \text{CoASH}
\]

**Benzoic acid**

\[+ \text{NH}_2\text{CH}_2\text{COOH} \xrightarrow{\text{acyl synthetase}} \text{CONHCH}_2\text{COOH}
\]

**Glycine**

**Hippuric acid**
Mercapturic acids are S- derivatives of N- acetylcysteine synthesized from glutathione (GSH). The mercapturic acid pathway appears to have evolved as a protective mechanism against xenobiotic – induced hepato toxicity or carcinogenicity, serving to detoxify a large number of noxious substances that we inhale or that are produced daily in human body. Glutathione is a tripeptide (γ – glutamyl cysteinyl glycine) found in most tissues.
GLUTATHIONE OR MERCAPTURIC ACID CONJUGATESUTATHIO:-

\[
\text{Glutathione (GSH)} \\
\text{Glutathione S-transferase} \\
\text{Glutamyl-transferase} \\
\text{Cysteinyll-glycinase} \\
\text{Acetylase} \\
\text{Mercapturic acid derivative}
\]

Examples:

- Acrolein
- Nitroglycerin
- Benzyl chloride
- Styrene oxide

Glutathione and mercapturic acid conjugation pathways.
ACETYLATION:-
Acetylation is principally a reaction of amino groups involving the transfer of acetyl CoA to primary aliphatic and aromatic amines, amino acids, hydrazines or sulfonamide groups.
The acetyl group utilized in N-acetylation of xenobiotics is supplied by acetyl CoA. The transfer of this group to the amino substrate is carried out by soluble N-acetyl transferase present in the hepatic reticulendothelial system.

![Chemical reaction diagram]

Procainamide + Acetyl CoA $\xrightarrow{\text{transacetylase}}$ N-Acetylprocainamide
METYLATION:

It is a common biochemical reaction but appears to be of greater significance in the metabolism of endogenous compounds than for drugs and other foreign compounds. Methylation differs from other conjugation processes in that O-methyl metabolites formed may in some cases have as great or greater pharmacologic activity and lipophilicility than parent molecule (e.g. Norepinephrine to epinephrine). Methionine is involved in the methylation of endogenous and exogenous substrates because it transfers its methyl group via activated intermediate S-adenosyl methionine (SAM) to substrate under the influence of methyl transferases.
METYLATION:

ATP + Methionine $\xrightarrow{\text{Methionine adenosine transferase}}$ S-Adenosylmethionine + Pyrophosphate + Phosphate

$S$-Adenosylmethionine + RZH $\xrightarrow{\text{Methyl transferase}}$ RZ-CH$_3$ + $S$-Adenosylhomocysteine

(where Z is O, NH, or S)

Methylation pathways.
FACTORS AFFECTING DRUG METABOLISM:-

1. Age differences
2. Species and strain differences
3. Sex differences
4. Enzyme induction
5. Enzyme inhibition
6. Stereochemical Aspects of Drug metabolism:-
Age: the enzyme system at birth especially in preterm baby are functionally immature and especially for oxidation and for conjugation with glucuronic acid.

- hyperbilirubinemia in infant is due to inability of the infant to glucuronidase bilirubine. The drugs like chloramphenicol is unable to get conjugated can cause fatal grey baby syndrome in neonates.

- After first weeks of life the drug metabolic capacity increases rapidly
In elderly metabolism is reduced because liver mass and liver blood flow are decreased. Metabolic inactivation of drugs is slowed.

Drugs persist for longer time and in higher concentration the must be lowered e.g. tricyclic antidepressants, antidysrhythmic drugs.
Species and strain differences
Different animal species may bio-transform a particular xenobiotic by similar or different metabolic pathways.

Phenytoin

S(-)-p-Hydroxyphenytoin

R(+)-m-Hydroxyphenytoin

(Man)

(Dog)
NH$_2$NH$_2$O

Oxidative Deamination

Phenylacacetone

(Man, Rabbit and Guinea pig)

Aromatic Hydroxylation

p-Hydroxyamphetamine

(Rat)

(Rat)
**Strain Difference**

Even with the same species there could be differences in metabolism (strain difference)

Even with the same species there could be differences in metabolism (strain difference)

- **Eskimos and Asians** Rapid acetylators so there will be inadequate therapeutic response

- **Egyptians and Mediterranean** Slow acetylators might show toxic effect
Sex differences:-
The rate of metabolism of xenobiotic show some variation between man and woman which may be due to endogenous sex hormone or hydrocortisone or their synthetic equivalence may affect the activity of the metabolic enzymes e.g. N-demethylation of erythromycin was significantly higher in female than males.

N-demethylation of mepridine was depressed during pregnancy and for woman taking oral contraceptives.
Enzyme induction:-

It is either increase in the amount of newly synthesized enzyme or increase in the activity of the enzyme both result in increase in the rate of drug metabolism. This will affect the pharmacokinetics and pharmacodynamic of a drug with clinical implication for the therapeutic actions of a drug and increased potential for drug interactions. As a result of induction, a drug may be either metabolized more rapidly to metabolites that are more potent, more toxic or metabolites that are less active than the parent drug, for example phenobarbitone induce microsomal enzyme so when given with warfarine it increases its rate of metabolism so decrease its anticoagulant affect.

The same happens with contraceptive pills enhance metabolism of estrogen.

Polycyclic aromatic hydrocarbons, pesticides, cigarette smoke all are inducers of microsomal cytp-450 enzyme.
## Clinical Examples: Induction

<table>
<thead>
<tr>
<th>Inducing Agent</th>
<th>Drug Affected</th>
<th>Potential Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette Smoke</td>
<td>Theophylline</td>
<td>Asthma Attacks</td>
</tr>
<tr>
<td>Rifampine</td>
<td>Coumadine</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>Tegretol</td>
<td>OCs</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>St. John`s Woat</td>
<td>Protease inhibitor</td>
<td>Increased Viral Load</td>
</tr>
</tbody>
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Enzyme inhibition

It is the decrease in the rate of metabolism of xenobiotics by using cytochrome P450 inhibitors. Cyp450s inhibitors are divided into:
- Reversible inhibition.
- Metabolite intermediate complexation of cyp450.
- Mechanism-based inhibition of cyp450.
Reversible Inhibition

This is the result of reversible interaction at the heme-iron active center of cyp450, or the lipophilic site on the apoprotein or both e.g cimitidine, diltiazem, quindine and some other drugs.
Cyp450 complexation inhibition:
Alkyl amine drugs have the ability to undergo cyp450 mediated oxidation to nitroso alkane metabolites. This process is called metabolite intermediate complexation.
Mechanism-based inhibition:

Certain drugs are oxidized by cyp450 generating metabolites that can bind irreversibly to the enzyme e.g. cyclophosphoamide and its conversion to acroline and phosphoramide mustared. Spironolacton and its thio-metabolite that alkylates heme, choramphenicol and its oxidative dechlorination to an acyl moiety that alkylates cytp450 apoprotein.
Drug interaction:-

phenylbutazone inhibit metabolism of S(-)warfarin
↓
Increase anticoagulant effect

Hemorrhage

chloramphenicol inhibits metabolism of phenytoin result in toxicity.

Grape fruit juice inhibits some of the cytp450 enzyme increasing the bioavailability of some drugs like felodipine a calcium channel blocker.

Herbal can interact with drugs too like licorice when taken with steroids can reduce their metabolism and elimination.
Food have a great effect on intestinal cyp450s enzyme, cauliflower, cabbage, spinach may induce some of cyp450 or inhibit others.

Two days eating cooked Brussels sprouts decrease the hydroxylation testosterone (8oz) of grape fruit juice decrease sulfoxidation of omprazole.
Stereochemical Aspects of Drug metabolism

In addition to the physicochemical factors that affect xenobiotic metabolism, stereochemical factors play an important role in the biotransformation of drugs. Most of the metabolizing enzymes show stereoselectivity when one stereoisomes enters into biotransformation pathway preferentially, but not exclusively. Metabolic stereochemical reactions can be categorized as follows:-

Substrate Stereo selectivity

It is the preference of one stereoisomer for metabolizing enzyme or metabolic process than the other e.g. the preferred decarboxylation of S-α-methyldopa to S-α-methyldopamine, with almost no reaction for R-α-methyldopa

\[
\text{S(-)-α-Methyldopa} \xrightarrow{\text{L-aromatic a.a. decarboxylase}} \text{S(-)-α-Methyldopamine}
\]
Product stereoselectivity

It is preferential formation of one stereoisomeric metabolite over the other. The reduction of ketones to stereoisomeric alcohols and the β hydroxylation of S-α-methyl dopamine to 1R:2S-α-methyl norepinephrine.
Regioselectivity
Selective metabolism of one of the similar functional groups that are positioned in different regions of the molecule.
Dobutamine
Extra hepatic metabolism:

Intestinal metabolism:
The intestinal mucosa is enriched with cypt450 isoforms, glucuronosyl transferases, sulfo transferases, and glutathione S-transferases. The highest concentration of cyp450s occurs in the duodenum with a gradual tapering into the ileum.
Lung metabolism:-
Lung may play an important role in the metabolic elimination or activation of injected or inhaled xenobiotics.

Nasal metabolism:-
Many metabolic enzymes are present in the nasal mucosa like cyp450, dehydrogenase and other conjugation enzymes. The nasal decongestant essences, anesthetics, alcohols, nicotine and cocaine have been shown to be metabolized by in vitro nasal cyp450.
Outcome of metabolism

*Toxic
Most chemical carcinogens of concern are relatively inert and require activation by xenobiotic metabolizing enzyme before they can undergo reaction with DNA or proteins.

*Pharmacologically active metabolites

These are important in patients with decreased renal function. Some of them have been synthesized and marketed as drug especially for such patients.

*Pharmacologically Inactive metabolites

*Pharmacologically Inactive compound converted to Pharmacologically active metabolites (Pro-drug)
PRODRUGS
Metabolism may convert a pharmacologically inactive drug into an active substance. This may be done deliberately to improve patient acceptability of the agent.

Advantageous:
1. Increased absorption
2. Relief of pain at the injection site
3. Elimination of unpleasant taste
4. Decrease toxicity
5. Increased chemical stability
6. Prolonged or shortened action
7. Decrease metabolic inactivation
Pro-drugs can be divided into:

Carrier linked pro-drugs

These are drugs that have been attached through a metabolically labile linkage to another molecule. The so-called promoiety which is not necessary for activity but may impart some desirable property to the drug. Such as increased lipid or water solubility or site-directed delivery.
Functional Groups in Prodrugs

\[
\text{Drug} \cdot \text{Promoiety} \xrightarrow{\text{enzyme}} \text{Drug} \cdot \text{OH} + \text{HO} \cdot \text{Promoiety}
\]

or

\[
\text{Promoiety} \cdot \text{Drug} \xrightarrow{\text{enzyme}} \text{Promoiety} \cdot \text{OH} + \text{HO} \cdot \text{Drug}
\]

- Types of esterase enzymes mediating the hydrolysis process
  - Ester hydrolase, Lipases, Cholesterol esterases, Acetylcholinesterase, Carboxypeptidase, Cholinesterase
  - Bacterial microflora enzymes
- Wide number of choices of promoiety alcohols available
  - Steric, electronic and hydrophobicity properties allow rate and extent of hydrolysis to be controlled
The succinate ester is inactive as antibacterial but this ester is prepared in order to increase water solubility for parental administration. The ester undergoes hydrolysis in plasma to give active drug and succinate.
Amine derivatives as prodrugs

Hetacillin $\rightarrow$ Water $\rightarrow$ Ampicillin + Acetone

Tetracycline $\rightarrow$ Iminium ion $\rightarrow$ Formaldehyde $\rightarrow$ Pyrrolidine

Rolitetracycline - A prodrug of tetracycline with increased water solubility
Azo Prodrugs

Sulfasalazine - Azulfidine® - Pharmacia & Upjohn
Sulfonamide antibiotic and antiinflammatory
Used to treat Ulcerative colitis, rheumatoid arthritis

Sulfapyridine + 5-aminosalicylic acid
Mutual Prodrug

Estramustine Sodium Phosphate
Emcyt® - Pharmacia & Upjohn

Sodium phosphate and Carbon dioxide

Nor-nitrogen mustard

Aziridine

Actual alkylating species
Bio precursor pro drugs

Such drugs contain no pro-moiety but rather relay upon metabolism to introduce the necessary functionality to create an active species.

Types of activation are
Oxidatives which is the most common
Reductive
Phosphorylation as antiviral agents
Bioprecursor Prodrugs

Do NOT contain a carrier or promoiety
- Contain latent functionality
- Metabolically or chemically transformed into an active drug
- Types of activation at are predictable
  • Oxidative (most common method)
  • Reductive
  • Phosphorylation (antiviral agents)
- Oxidation Example - Nabumetone - Relafen® - Smith Kline Beecham

Active form of the drug that inhibits Prostaglandin biosynthesis by cyclooxygenase
Reduction

Sulindac is a pro-drug converted to a metabolite that appear to inhibit cyclooxygenase system about 8 time as effective as aspirin.
Bioprecursor Prodrugs

Phosphorylation example -

Iodoxuridine - Herplex®
Allergan - lipid soluble!
Ophthalmic product for
Herpes simplex keratitis
Higher affinity for viral
kinases than mammalian
kinases but some toxicity

TWO mechanisms of action: 1. Inhibits DNA polymerase  2. Incorporated into DNA affording incorrect base pairing and template activity