PHARM 630

Metabolic Pathways

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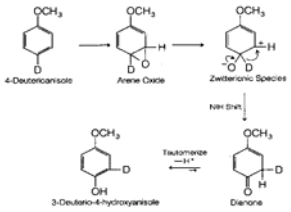
Phase I Reactions

I. Oxidation

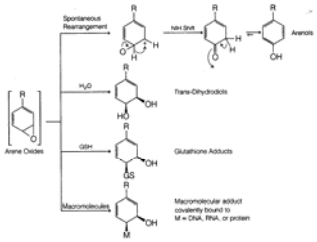
A. Main Reaction types catalyzed by MFO:

1. Aromatic Oxidation (hydroxylation)

- Xenobiotics containing aromatic moieties are susceptible to aromatic oxidation.
- For mono-substituted benzene compounds, para-hydroxylation usually predominates.
- Aromatic hydroxylation reactions proceed through an epoxide intermediate called an arene oxide.



- The arene oxide formed during aromatic hydroxylation is usually unstable and rearranges using the NIH shift.
- However, some are stable enough to be isolated. The importance of this is arene oxides are of significant toxicological concern.
- Epoxides are electrophilic and very reactive.



Detoxification of arene oxides:

1. The most important detoxification reaction for arene oxides is the spontaneous rearrangement to the corresponding arenols by NIH shift

2. Hydration (nucleophilic attack of water on the epoxide) of arene to yield inactive trans-dihydrodiol metabolites. This catalyzed by epoxide hydrases.
3. Sulfahydryl group present in glutathione to yield the corresponding glutathione adduct. This catalyzed by glutathione S-transferase

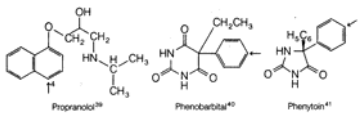
- Because electrophilic and reactive nature, arene oxides may also undergo spontaneous reaction with nucleophilic functionalities present on biomacromolecules. Such reaction lead to modified protein, DNA and RNA structure.

- In humans, aromatic hydroxylation is one of the major routes of metabolism for xenobiotics containing an aromatic group.
- Substituents attached to a ring will influence ease of hydroxylation.

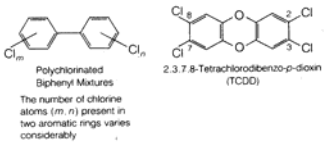
General rule:

- Hydroxylation proceeds most readily in activated rings. (electron rich)
- Aromatic rings with e- withdrawing groups are more resistant to oxidation (Cl, -$^{+}$NR3, COOH, SO_2NHR).

Examples of aromatic oxidation



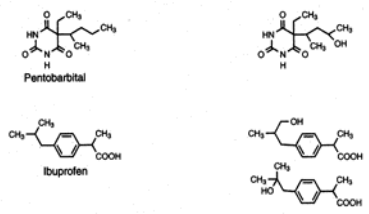
What happens if the oxidation metabolism is blocked?



- These environmental pollutants are resistant to aromatic oxidation because of the numerous electronegative chlorine atoms present in the aromatic ring.
- They are lipophilic in nature.
- Health hazard.

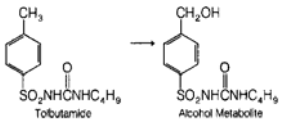
2. Aliphatic Oxidation of Carbon Atoms

   In drugs having straight or branched alkyl chains, you can observe oxidation of the terminal methyl group (ω oxidation) or the penultimate carbon atom (next to last or ω-1).



3. Benzylic Oxidation of Carbon Atoms

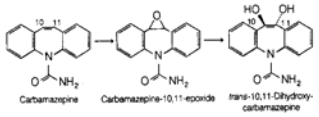
   A methyl group attached to an aromatic ring is susceptible to oxidation forming the alcohol.

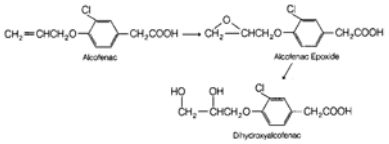


4. Oxidation of Olefins (Carbon-Carbon Double Bonds

- Oxidation of carbon-carbon double bonds leads to the corresponding epoxide.
- These epoxides are generally more stable than arene oxides.
- These epoxides are susceptible to hydration forming a diol.

Examples of oxidation of olefins



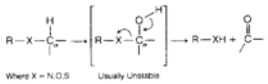


5. Oxidation of Carbon-Heteroatom (N,O,S) Molecules

- N and O functionalities are commonly found in most drugs and foreign compounds, whereas S functionalities occur only occasionally.

- Carbon heteroatom systems can undergo two types of oxidative metabolism:

1. Hydroxylation of the carbon atom attached directly to the heteroatom. The resulting intermediate is usually unstable and decomposes with the cleavage of the C-heteroatom bond.



2. Direct oxidation of the heteroatom (nitrogen and sulfur only).

N.B. Oxidative N-, O- and S- dealkylation as well as oxidative deamination reactions, fall under this mechanistic pathway.

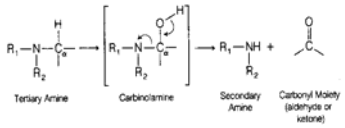
5.1 Oxidation of Carbon-Nitrogen Molecules

i) Tertiary Aliphatic Amines

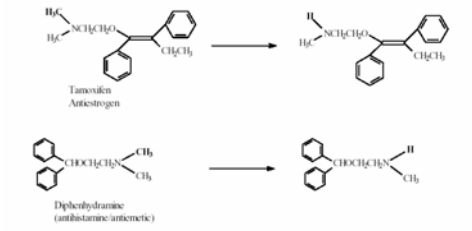
Oxidative removal of alkyl groups (particularly methyl groups) from teriary amines. This reaction is commonly referred to as oxidative N-dealkylation.

When the tertiary amine contains several different substituents, the smaller groups are preferentially removed.

The initial step involves α-carbon hydroxylation to form a carbinolamine intermediate, which is unstable and undergo spontaneous heterolytic cleavage of the C-N bond to give secondary amine and carbonyl moiety.

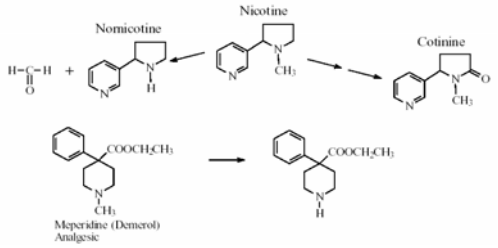


Examples of oxidation of tertiary aliphatic amines



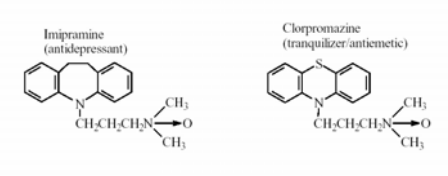
ii) Alicyclic Tertiary Amines

α-Carbon oxidation of nicotine generates lactam intermediates. Cyclic tertiary amines also undergo dealkylation.



iii) N- Oxidation of Tertiary Amines

- It is difficult to determine the true extent of N oxidation because N-Oxides are easily reduced back to the parent tertiary amine.



iv) Primary and Secondary Amines

Secondary amines are susceptible to N-dealkylation, deamination and N-Oxidation.

(* N-oxidation occurs to a much lesser extent for secondary amines)

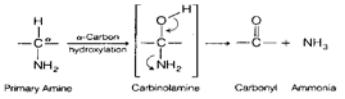
- Dealkylation of secondary amines occurs through the carbinolamine pathway. The difference is this pathway yields a carbonyl leaving group and a primary amine.

Primary amines are susceptible to oxidative:

1) Deamination

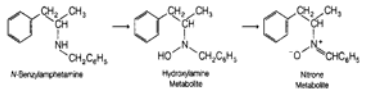
Involves initial α-carbon hydroxylation to form a carbinolamine. Carbinolamine then undergoes carbon nitrogen cleavage to the carbonyl metabolite and ammonia.

(* If α -carbon hydroxylation can not occur, then oxidative deamination is not possible)



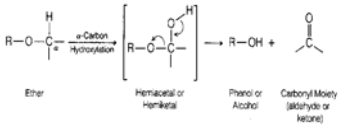
2) N-Oxidation

N-Hydroxylation of secondary amines generates the corresponding N-hydroxylamine products which are susceptible to further oxidation

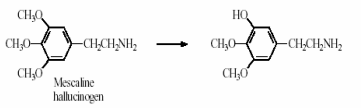


5.2 Oxidation of Carbon-Oxygen Molecules

- You can also get the oxidative removal of O-alkyl groups. This is called O-dealkylation.
- As with the nitrogen system, metabolism involves oxidation of the α-carbon.



Small alkyl groups are rapidly removed to uncover the alcohol.



5.3 Oxidation of Carbon-Sulfur Molecules

Carbon sulfur groups are susceptible to:

a) sulfur dealkylation*

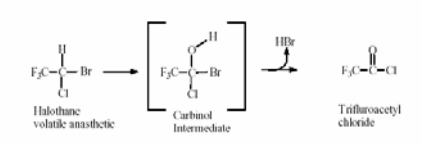
b) desulfuration*

c) sulfur oxidation (Xenobiotics which contain organic sulfur commonly undergo oxidation).

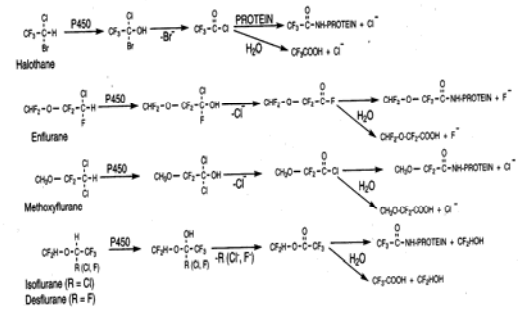
* not common

5.4 Oxidation of Carbon-Halogen Molecules

Halogen containing xenobiotics can undergo oxidative dehalogenation.



Examples of Oxidation of Carbon-Halogen Molecules



B. Main Reaction types catalyzed by other systems:

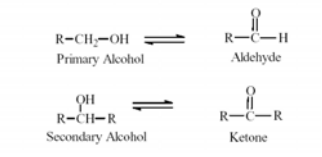
1. Alcohols and Aldehydes Oxidation

- Oxidation is catalyzed by alcohol & aldehyde dehydrogenases (ADH; ALDH)
- 3 major classes for each
- NAD+ is preferred co-factor for both

ADH

- Zinc-containing; cytosolic; highest levels in liver
- Class I – Small aliphatic alcohols (ethanol)
- Class II – Larger aliphatic & aromatic alcohols
- Class III – Long chain & aromatic alcohols

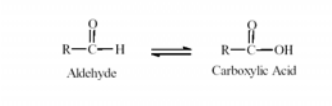
- Oxidative metabolism yields alcohols which can be further oxidized.
- Primary alcohols are oxidized to aldehydes while secondary alcohols are converted to ketones.



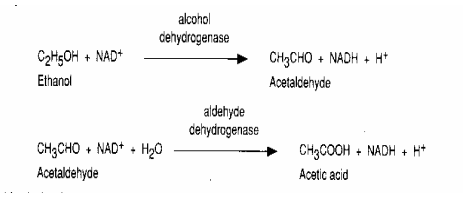
ALDH

- Class I – Cytosolic, diverse substrates
- Class II – Mitochondrial, simple aldehydes
- Class III – Cytosolic (gastric/extrahepatic)

Aldehyde dehydrogenases like xanthine oxidase and aldehyde oxidase can oxidize an aldehyde to its corresponding acid.

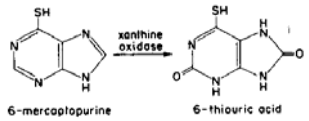


Example of ADH and ALDH oxidation



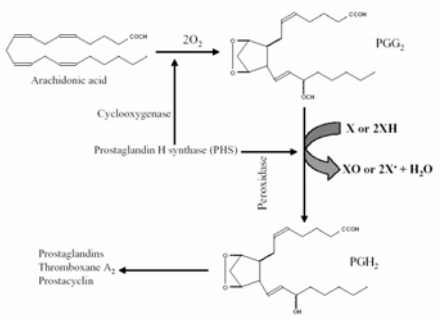
2. Purine oxidation

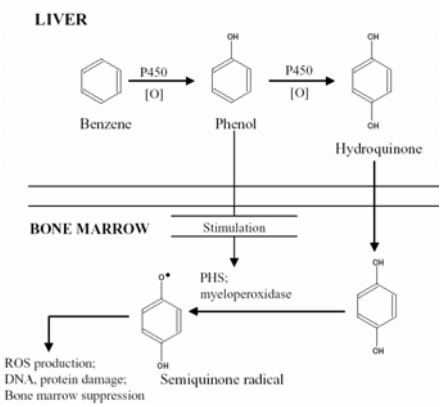
- Catalyzed by xanthine oxidase
- A major pathway for the metabolism of several purine derivatives (e.g. 6-mercaptopurine, theophylline, caffeine).



3. Peroxidase-dependent co-oxidation

- Reduction of hydrogen peroxide or lipid hydroperoxide coupled to oxidation of a xenobiotic by a peroxidase
- Prostaglandin H synthase
- Myeloperoxidase





2. Reduction

Reduction of certain functional groups can play an important part in xenobiotic metabolism.

In general:

a) Carbonyl group reduces to an alcohol.

b) Nitro group reduces to amino derivatives.

c) Azo group reduces to amino derivatives.

d) N-oxides reduce to tertiary amines.

e) Sulfoxides reduce to sulfide.

Xenobiotics with:

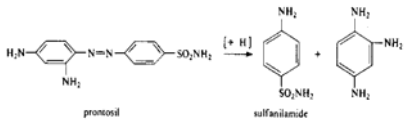
- RCHO
- R2C=O
- R2S=O
- RSSR
- Quinone
- *N*-oxide
- RCH=CHR
- RN=NR
- RNO2

Are all candidates for reduction

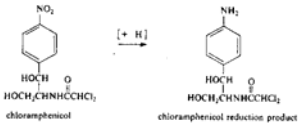
1. Azo- (RN=NR) & Nitro- (RNO2) reduction

- Intestinal Microflora
- Cytochrome P-450
- NAD(P)H Quinone Oxidoreductase (DT-Diaphorase)

Azo- reduction

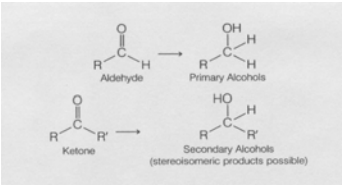


Nitro- reduction



2. Carbonyl (R2C=O; RCHO) reduction

- Carbonyl reductases
- NADPH-dependent
- Blood/cytosolic distribution



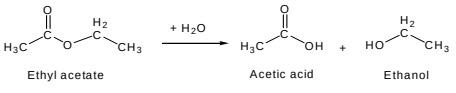
3. Hydrolysis

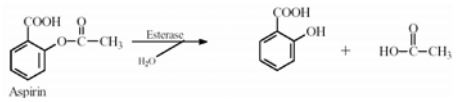
Hydrolytic Reactions

- Very important metabolic pathway because of the presence of hydrolytic enzymes in many tissues and plasma.
- This knowledge is used to make prodrugs which can enhance drug taste, solubility, or absorption.

1. Hydrolysis of Esters

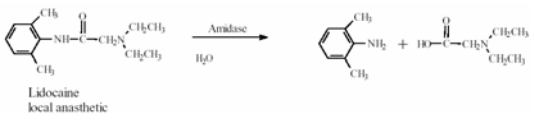
- This reaction is carried out by non-specific esterases found in the liver, kidney, plasma, intestine, and plasma.
- This reaction will occur quickly and easily. The products will be COOH, alcohols, or phenols.





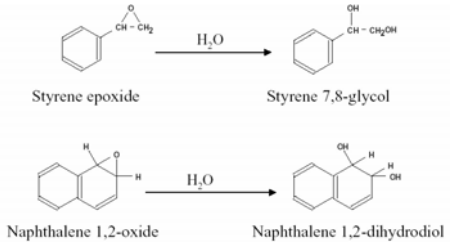
2. Hydrolysis of Amides

- This reaction is mediated by liver microsomal amidases, esterases and deacylases.
- The products of this reaction will be amines and COOH.



3. Epoxide Hydrolase

- Hydrolysis of epoxide to a dihydrodiol
- Detoxification of reactive electrophilic epoxides.



Phase II Reactions

Phase II Reactions (Conjugation)

A biotransformation that conjugates (attaches) small endogenous molecules to xenobiotics or phase I metabolites (conjugation).

- Phase I reactions do not always produce hydrophilic or pharmacologically inactive metabolites.
- Conjugation reactions are utilized to convert metabolites to even more water soluble products to facilitate their removal from the body or inactivation.

Removal

Conjugation reactions accomplish this by attaching small, polar, ionizable, endogenous molecules to the metabolite or xenobiotic.

1. Glucuronic acid, 2. Sulfate, 3. Amino acid (Glycine, Glutamine)

Inactivation

Conjugation reactions can be used to stop a molecules pharmacological activity (toxicity) without changing solubility.

4. Acetyl, 5. Methyl, 6. Glutathione

1) Glucuronic Acid Conjugation (Glucuronidation)

This is the most common conjugative pathway in drug metabolism for the following reasons:

1. The body has a readily available supply of D-glucose-1-phosphate which it can convert to D-glucuronic acid.
2. Numerous functional groups can be attached to glucuronic acid (flexibility).
   - OH, -NH-R, - COOH, - SH, - NH2
3. Glucuronic acid greatly increases water solubility of the conjugated product making it readily excretable.
   - Conjugate MW <350 excreted in urine
   - Conjugate MW >350 via bile to feces

Glucuronidation con't

The key enzyme responsible for transfering glucuronic acid to a suitable acceptor is called UDP-glucuronosyl transferase (UGT).

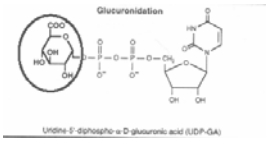
- * Many endogenous compounds are substrates for this enzyme:

- Bilirubin, Bile Acids and Steroid hormones

- Glucuronides formed in the liver with a MW>350 can be excreted into the small intestine through bile.

Enterohepatic Recirculation: The process by which a drug (conjugate) is excreted by the bile into the small intestine where it is cleaved and reabsorbed across the gut wall back into the systemic circulation.

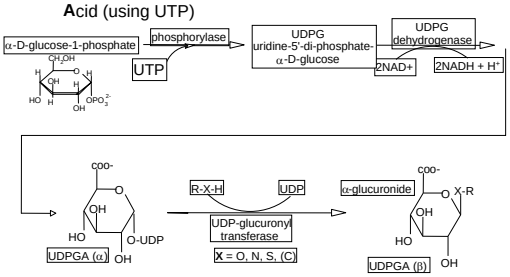
Glucuronidation: the mechanism



The functional group which reacts with, or is transferred to the xenobiotic is highlighted.

Glucuronidation: the mechanism

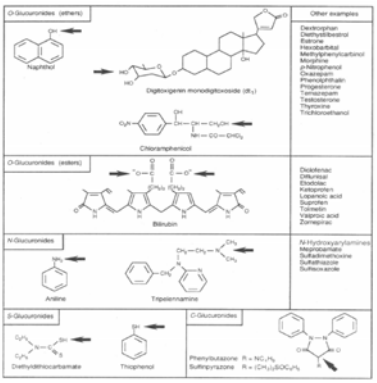
- Requires activation to **U**ridine **Di**phosphate **G**lucuronic **A**cid (using UTP)



Glucuronidation: what are the products?

- Alcohols and phenols ➔ ether glucuronides
- Aromatic COOH (+ some others) ➔ ester glucuronides
- Aromatic amines ➔ N-glucuronides
- Sulfhydryls ➔ S-glucuronides
- Can hydrolyze ➔ parent molecule

Glucuronidation: examples

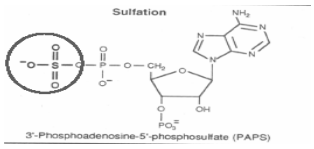


2) Sulfate Conjugation

- Conjugation with sulfate occurs primarily with phenols.
- Sulfate will conjugate with alcohols, aromatic amines, and N-hydroxy compounds as a minor metabolite.
- The amount of sulfate available for conjugation is limited because the body uses it to conjugate endogenous compounds.
- In general, formation of a sulfate conjugate requires two steps:

1) Activation of inorganic sulfate.
2) Transfer of activated sulfur.

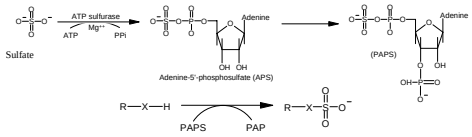
Sulfatation: the mechanism



The functional group which reacts with, or is transferred to the xenobiotic is highlighted.

Sulfatation: the mechanism

Requires activation to
Phospho**a**denosine **p**hospho**s**ulfonate



- Very active, but low concentration
  - low concentration of xenobiotic-sulfate
  - high concentration of xenobiotic-glucuronide

Sulfation con't

Two general classes of Sulfotransferases exist in tissue fractions:

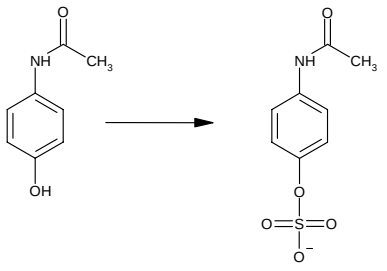
a) Cytosolic enzymes which are important in xenobiotic metabolism.

- These are the product of a multigene family (SULT).

b) Membrane bound form which is involved in the sulfonation of proteins.

Sulfation-example

Acetaminophen



3) Conjugation with Amino Acid (Glycine and Glutamine)

The amino acids glycine and glutamine are utilized by mammals to conjugate carboxylic acids.

- aromatic acids
- arylalkyl acids

The quantity of amino acid conjugates formed is small.

- Competition from glucuronidation and sulfation.
- Limited availability of amino acids in the body.

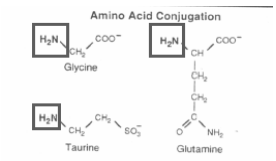
Amino Acid Conjugation con't

In general, formation of an amino acid conjugate requires:

1) Activation of the carboxylic acid (xenobiotic) with ATP and coenzyme A.

2) Transfer of acyl xenobiotic to glycine or glutamine.

Amino acid conjugation: the mechanism



The functional group which reacts with, or is transferred to the xenobiotic is highlighted.

Amino acid conjugation: the mechanism

Amino acids like acetyl can form CoA derivatives which then react with endogenous amines to form cojugates

+ ATP →
+ CoASH → + AMP
+ → + CoASH

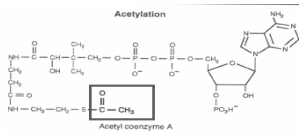
ATP PPi CoASH AMP glycine CoASH

Benzoic acid → Adenosyl benzoic acid → Hippuric acid

4) Acetylation

- An important pathway for conjugating drugs with a primary amino group.
- The amide derivatives from this reaction do not increase water solubility. Their function is to decrease pharmacological activity.
- The acetyl group utilized for this reaction comes from Coenzyme A.
- Catalyzed by N-acetyl transferase **(NAT)**

Acetylation: the mechanism



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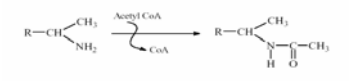
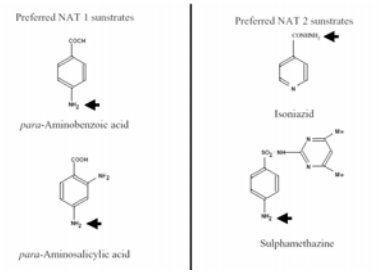


Acetylation-examples

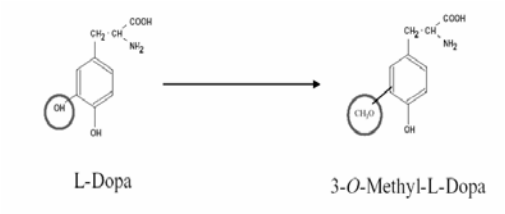


5) Methylation

- Addition of a methyl group to a xenobiotic is a minor pathway in drug metabolism. This reaction is almost exclusively for the synthesis of endogenous compounds.
- The methyl conjugates will not have increased water solubility. Their function is to stop pharmacological activity.
- In humans, you can find N, O, and S methyl conjugates.

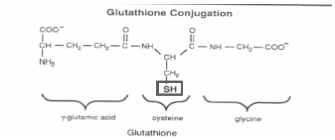
5) Methylation-example

O-methylation



6) Conjugation with Glutathione (GSH)

- GSH is a tripeptide (L-glutamyl-L-cysteine-glycine).



- GSH is the most abundant non-protein thiol in the cell.
- GSH is synthesized in all mammalian cells (predominantly in liver).

- There is a limited supply of GSH in a cell.
- The key functional element of GSH is the reactive thiol (nucleophilic sulfhydryl) that is responsible for many intracellular functions.

  a) Protects cell against damage from oxygen radicals and reactive metabolites from endogenous metabolic pathways.

  b) Facilitates membrane transport of metabolites.

  c) Protects cell against damage from reactive, electrophilic xenobiotics which can manifest their toxicity by reacting covalently with nucleophilic groups in proteins and nucleic acids.

Glutathione Conjugarion con't

- The enzyme that catalyzes the transfer of GSH to an electrophilic compound is *Glutathione–S–Transferase*.

  - Only requirement for this enzyme is an electrophilic compound that can react with the nucleophilic GSH.

  - Does not require activation.

  - This reaction can occur non-enzymatically.
- Once the tripeptide (GSH) has been attached to an acceptor molecule, it can be enzymatically cleaved.

Glutathione Conjugarion: the mechanism