INORGANIC PHARMACEUTICAL CHEMISTRY

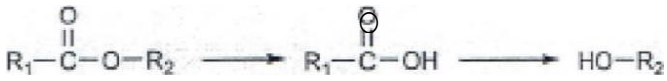
3RD STAGE

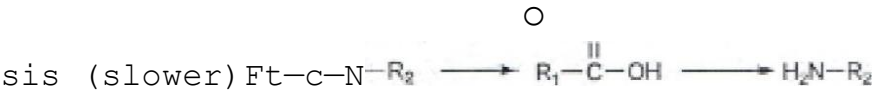
LEC 8&9

HYDROLYTIC REACTIONS

Hydrolysis of Esters and Amides

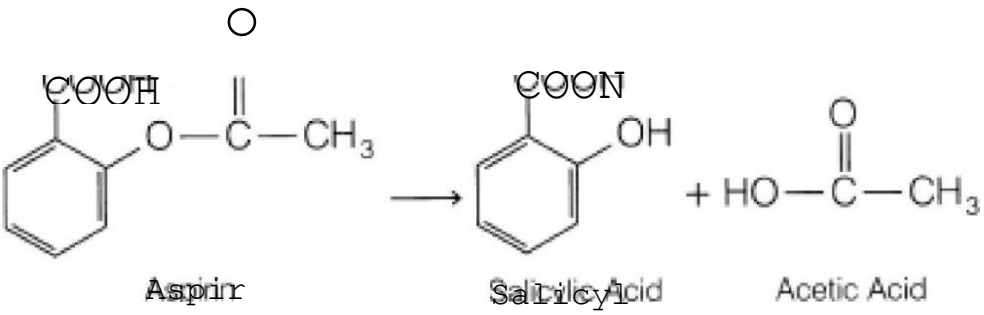
The metabolism of ester and amide linkages in many drugs is catalyzed by hydrolytic enzymes present in various tissues and in plasma. The metabolic products formed (carboxylic acids, alcohols, phenols, and amines) generally are polar and functionally more susceptible to conjugation and excretion than the parent ester or amide drugs. The enzymes carrying out ester hydrolysis include several nonspecific esterases found in the liver, kidney, and intestine as well as the pseudocholinesterases present in plasma.

Ester hydrolysis 

Amide hydrolysis (slower) 

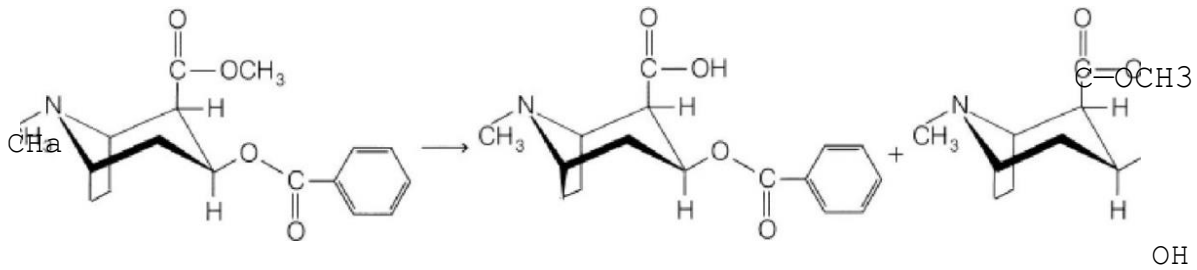
Amide hydrolysis appears to be mediated by liver microsomal amidases, esterases, and deacylases.

Hydrolysis is a major biotransformation pathway for drugs containing an ester functionality. This is because of the relative ease of hydrolyzing the ester linkage. A classic example of ester hydrolysis is the metabolic conversion of aspirin (acetylsalicylic acid) to salicylic acid.



(Acetylsalicylic acid)

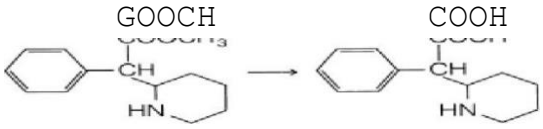
Of the two ester moieties present in cocaine, it appears that, in general, the methyl group is hydrolyzed preferentially to yield benzoylecgonine as the major human urinary metabolite. The hydrolysis of cocaine to methyl ecgonine, however, also occurs in plasma and, to a minor extent blood.



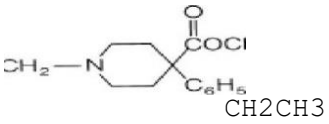
Cocaine Benzoyleogonine Methylecgonine

Methylphenidate is biotransformed rapidly by hydrolysis to yield ritalinic acid as the major urinary metabolite in humans. Often, ester hydrolysis of the parent drug leads to pharmacologically active metabolites.

For example, hydrolysis of diphenoxylate in humans leads to diphenoxylic acid which is, apparently, 5 times more potent an antidiarrheal agent than the parent ester.



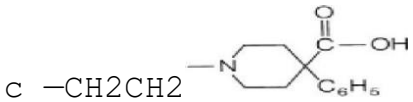
Methylphenidate Ritalinic Acid



CH2CH3

CCH

Dipher-routate

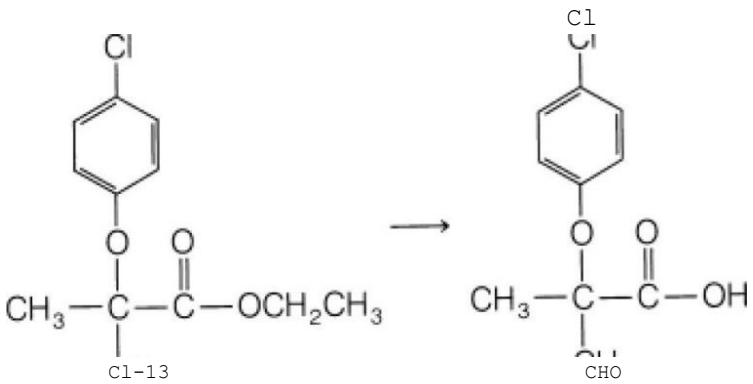


c —CH2CH2

Oiphenox•ylic Acid

(Difenoxin)

The rapid metabolism of clofibrate yields P-chlorophenoxyisobutyric acid (CPIB) as the major plasma metabolite in humans. Studies in rats indicate that the free acid CPIB is responsible for clofibrate's hypolipidemic effect

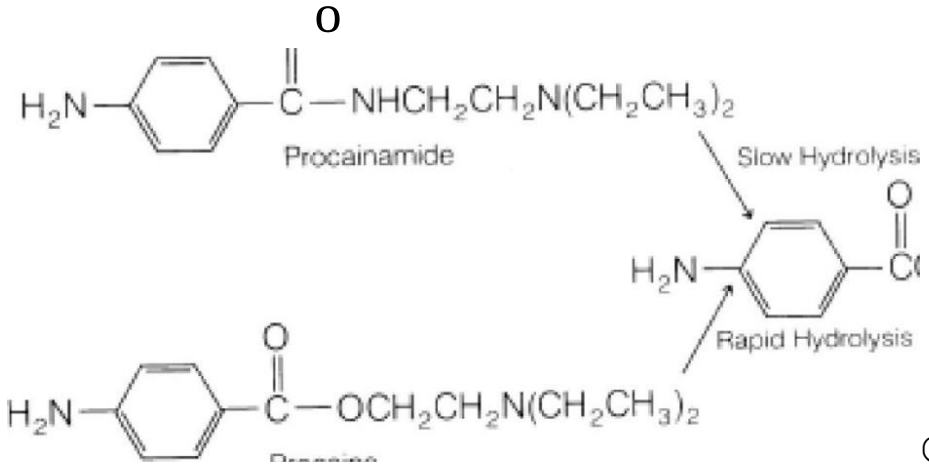


Cl-13 CHO

Clofibrate p.Chlorophenoxyisobutyric Acid

- Many parent drugs have been chemically modified or derivatized to generate so-called prodrugs to overcome some undesirable property (e.g., bitter taste, poor absorption, poor solubility. irritation at site of injection).
- The rationale behind the prodrug concept was to develop an agent that, once inside the biological system, would be biotransformed to the active parent dmg.
- The presence of esterases in many tissues and plasma makes ester derivatives logical prodrug candidates because hydrolysis would cause the ester prodrug to revert to the parent compound.
- Accordingly, antibiotics such as chloramphenicol and clindamycin have been derivatized as their palmitate esters to minimize their bitter taste and to improve their palatability in pediatric liquid suspension.

Amides are hydrolyzed slowly in comparison to esters Consequently, hydrolysis of the amide bond of procainamide is relatively slow compared with hydrolysis of the ester linkage in procaine

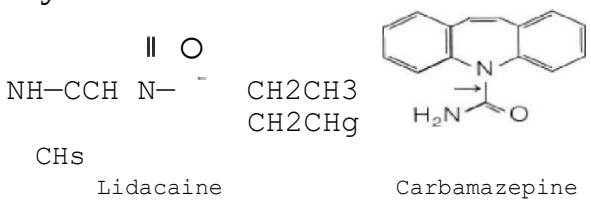


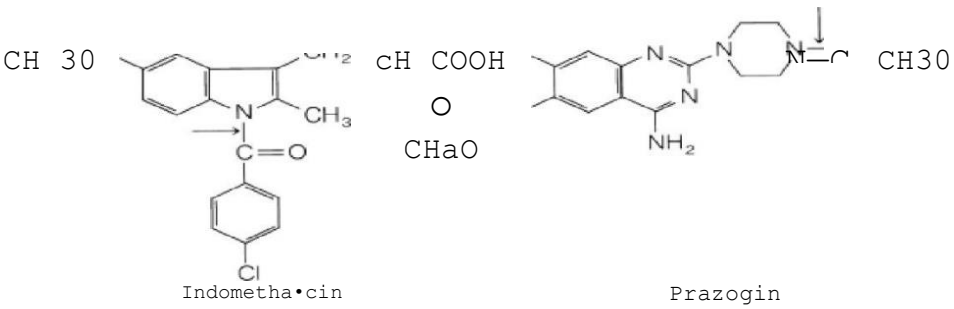
COH

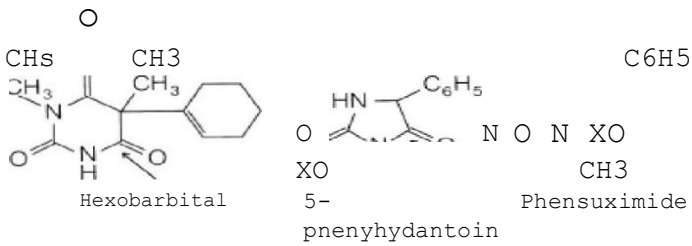
Procarne

Drugs in which amide cleavage has been reported to occur, to some extent, include lidocaine, carbamazepine, indomethacin, and prazosin. Amide linkages present in barbiturates (e.g., hexobarbital) as well as in hydantoins

(e.g. 5-phenylhydantoin and succinimides (phensuximide) are also susceptible to hydrolysis.







Miscellaneous Hydrolytic Reactions

- Hydrolysis of recombinant human peptide drugs and hormones at the N- or C-terminal amino acids by carboxypeptidase and aminopeptidase and proteases in blood and other tissues is a wellrecognized hydrolytic reaction. Examples of peptides or protein hormones undergoing hydrolysis include human insulin, growth hormone (GH), prolactin, parathyroid hormone (PTH),

- In addition to hydrolysis of amides and esters, hydrolytic cleavage of other moieties occurs to a mmor extent in drug metabolism, including the hydrolysis of phosphate esters (e.g., diethylstilbestrol diphosphate), sulfonylureas, cardiac glycosides, carbamate esters, and organophosphate compounds.

PHASE Il OR CONJUGATION REACTIONS

- Phase I or functionalization reactions do not always produce hydrophilic or pharmacologically inactive metabolites. Various phase Il or conjugation reactions, however, can convert these metabolites to more polar and water-soluble products.
- Many conjugative enzymes accomplish this objective by attaching small polar. and ionizable endogenous molecules, such as glucuronic acid, sulfate. glycinc. and glutamine to the phase I metabolite or parent xenohiotic.
- The resulting conjugated products are relatively water soluble and readily excretable. In addition, they generally are biologically inactive and nontoxic.

- Other phase Il reactions, such as methylation and acetylation. do not generally increase water solubility but mainly serve to terminate or attenuate pharmacological activity.

- The role of GSH is to combine with chemically reactive compounds to prevent damage to important biomacromolecules such as DNA, RNA and proteins.

- Thus, phase Il reactions can be regarded as truly detoxifying pathways in drug metabolism, with a few exceptions.
- A distinguishing feature of most phase Il reactions is that the conjugating group (glucuronic acid, sulfate, methyl and acetyl) is activated initially in the form of a coenzyme before transfer or attachment of the group to the accepting substrate by the appropriate transferase enzyme. In other cases, such as glycine and glutamine conjugation. the substrate is activated initially.
- Many endogenous compounds such as bilirubin, steroids. catecholamines, and histamine, also undergo conjugation reactions and use the same coenzymes. Although they appear to be mediated by more specific transferase enzymes.

1 . Glucuronic Acid Conjugation

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

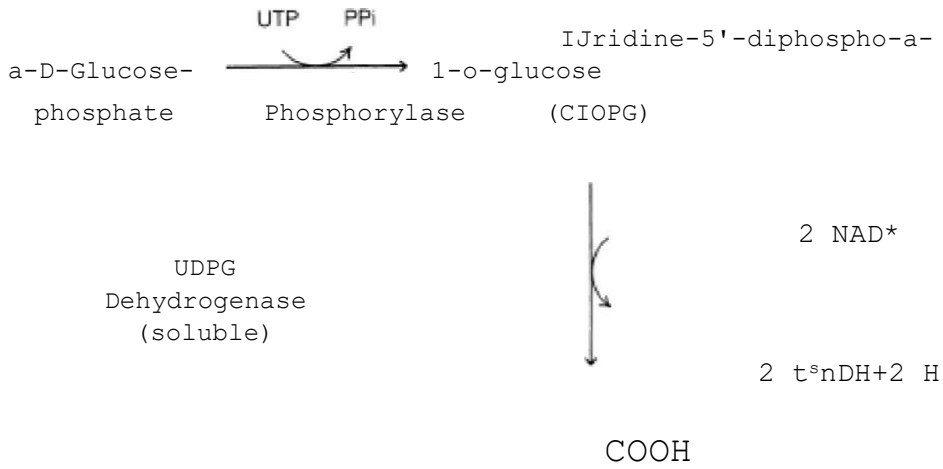
(a) a readily available supply of D-glucuronic acid (derived from Dglucose).

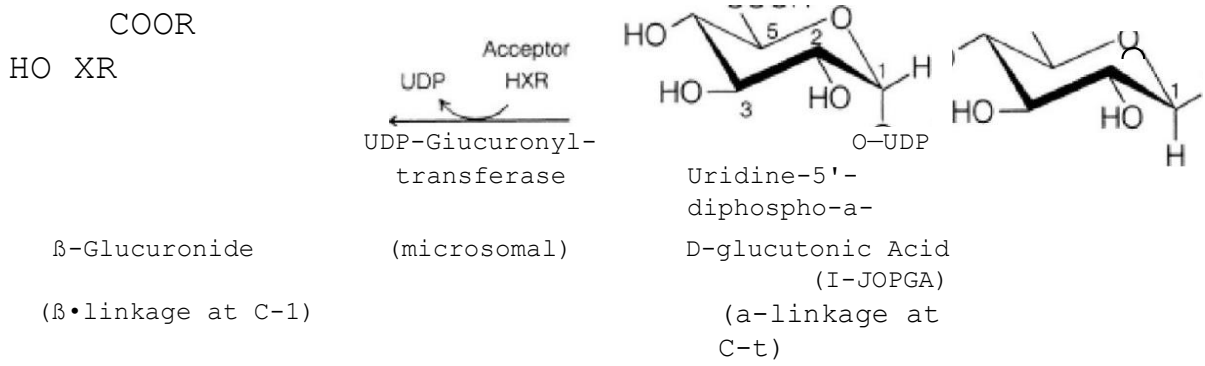
(b) numerous functional groups that can combine enzymatically with glucuronic acid, and

(c) the glucuronyl moiety (with its ionized carboxylate (pKa 3.2) and polar hydroxyl groups) which, when attached to xenobiotic substrates, greatly increases the water solubility of the conjugated products.

- Formation of ß-glucuronides involves two steps: synthesis of an activated coenzyme uridine-5 '-diphospho-a-Dglucuronic acid (UDPGA), and subsequent transfer of the glucuronyl group from UDPGA to an appropriate substrate)"
- The transfer step is catalyzed by microsomal enzymes called UDPglucuronyl transferase. They are found primarily in the liver but also occur in many other tissues. including kidney, intestine, skin, lung, and brain.

Formation of UDPGA and ß-glucuronide conjugates.





- Metabolic products are classified as oxygen-, nitrogen-, sulfur-, or carbon-glucuronide, according to the heteroatom attached to the C-l atom of the glucuronyl group.
- Two important functionalities, the hydroxy and carboxy, form Oglucuronides.
- Phenolic and alcoholic hydroxyls are the most common functional groups undergoing glucuronidation in drug metabolism.
- As we have seen phenolic and alcoholic hydroxyl groups are present in many parent compounds and arise through various phase I metabolic pathways.
- The carboxyl group is also subject to conjugation with glucuronic acid.

Table: 5.1 Types of Compounds Forming Oxygen Nitrogen, Sulfur, and Carbon Glucuronides

Oxygen Glucuronides
Hydroxyl compounds
Phenols: morphine, acetaminophen, p-hydroxyphenytoin
Alcohols: tricholoroethanol, chloramphenicol, propranolol
Enols: 4-hydroxycoumarin
N-Hydroxyamines.' N-hydroxydapsone
N-Hydroxyamides." N-hydroxy-2-acetylaminofluorene
Carboxyl compounds
Aryl acids: benzoic acid, salicylic acid

Arylalkyl acids: naproxen, fenoprofen

Nitrogen Glucuronides

Arylamines: 7-amino-5-nitroindazole

Alkylamines: desipramine

Amides: meprobamate

Sulfonamides: sulfisoxazole

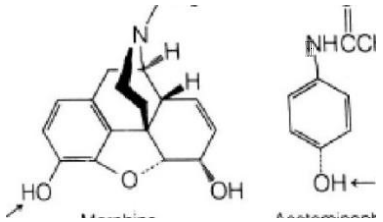
Tertiary amines cyproheptadine, tripelennamine

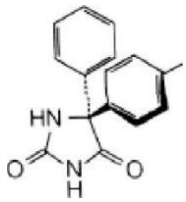
Sulfur Glucuronides

Sulfhydryl groups: methimazole, propylthiouracil, diethylthiocarbamic acid

Carbon Glucuronides

3,S-Pyrazolidinedione: phenylbutazone, sulfinpyrazone





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OH

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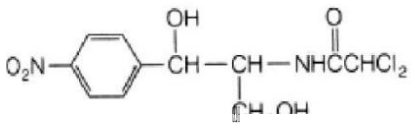
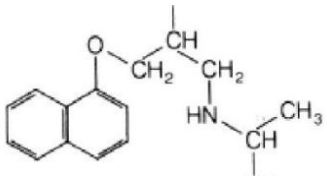
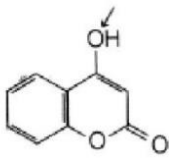
Motphine AcetamhopF,ecv p-Hydroxyphenytoin Trk:hlotoethaooa

OH







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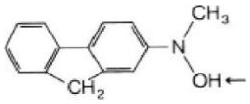
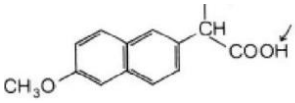
СНЭ

сглог-атрһеп'со' 4-Нуеохусоumari6 N-Ну•drcxydapsone



M•HyBroxy-2-acety4•

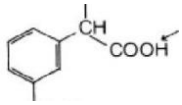
агп'гюьоэтег.е



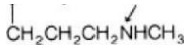
QC6Hs

Ветиз•с acid, H═ H

Sahcybc acid, a- он



Fenovoten



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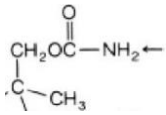
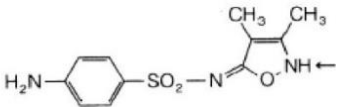
шертооатате

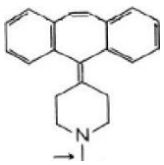
Sulfisoxazole

7.АтЙТО.5•

Des'pramine nitrohdazole







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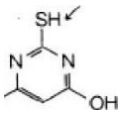


—SH

СН

NCH2CH2N СНЭ

ПСЗН,

снэ свньснг

СНЗ

СургоПер'аб(пе

Tripeler-mamine

azole

ptopylthiouraci

Dbethyl!hio-

саФсг%с

- Besides xenobiotics, a number of endogenous substrates, group notably bilirubin and steroids are eliminated as glucuronide conjugates, which are excreted primarily in the urine.
- As the relative molecular mass of the conjugate exceeds 300 Da, however, biliary excretion may become an important route of elimination.
- Glucuronides that are excreted in the bile are susceptible to hydrolysis by B-glucuronidase enzymes present in the intestine. The hydrolyzed product may be reabsorbed in the intestine, thus leading to enterohepatic recycling .

- ß-glucoronidase are also present in many other tissues, including the liver, the endocrine system, and the reproductive organs.
- Although the function of these hydrolytic enzymes in drug metabolism is unclear, it appears that, in terms of hormonal and endocrine regulation, ß-glucoronidase may liberate active hormones (e.g., steroids) from their inactive glucuronide conjugates.
- In neonates and children, glucuronidating processes are often not developed fully. In such subjects, drugs and endogenous compounds (e.g., bilirubin) that are metabolized normally by glucuronidation may accumulate and cause serious toxicity.
- For example, neonatal hyperbilirubinemia may be attributable to the inability of newborns to conjugate bilirubin with glucuronic acid.
- Similarly, the inability of infants to glucuronidate chloramphenicol has been suggested to be responsible for the gray baby syndrome, which results from accumulation of toxic levels of the free antibiotic.

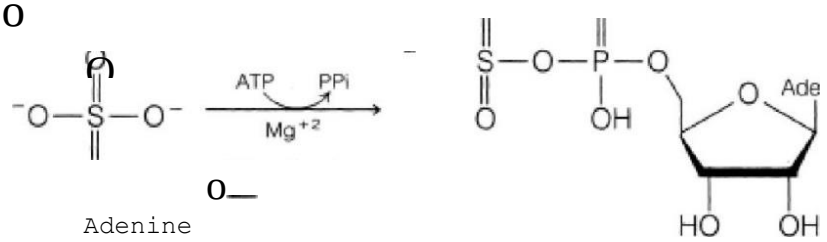
2. Sulfate conjugation

- Conjugation of xenobiotics with sulfate occurs primarily with phenols and, occasionally, with alcohols, aromatic amines, and N-hydroxy compounds.
- In contrast to glucuronic acid, the amount of available sulfate is rather limited. The body uses a significant portion of the sulfate pool to conjugate numerous endogenous compounds such as steroids, heparin, chondroitin, catecholamines, and thyroxine.

- The sulfate conjugation process involves activation of inorganic sulfate to the coenzyme 3'-phosphoadenosine - 5' phosphosulfat (PAPS).
- Subsequent transfer of the sulfate group from PAPS to the accepting substrate is catalyzed by various soluble sulfotransferases present in the liver and other tissues (e.g. kidney, intestine).

Formation of PAPS

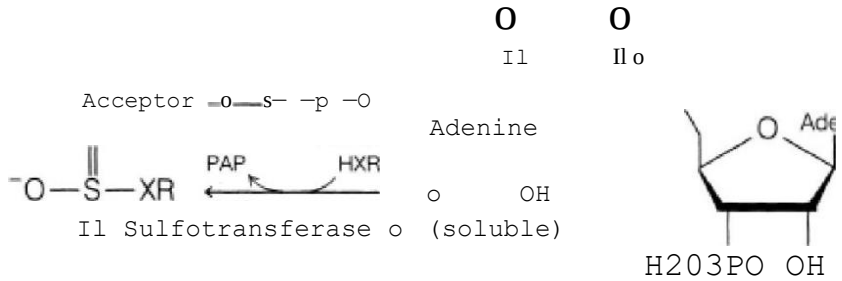
- o



Adenine

- ATP sulturylase
  Sulfate

Adenosine-5'-phosphosulfate (APS)



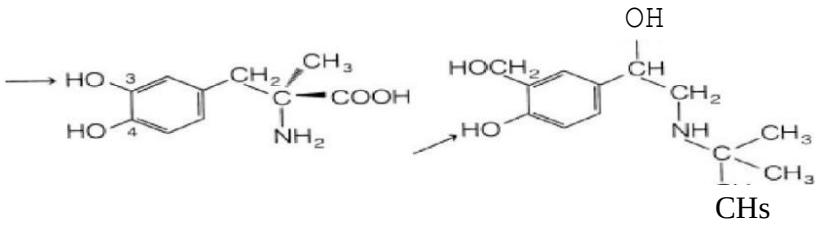
Il Sulfotransferase o (soluble)

H203PO OH

3'-Phosphoadenosine-5'-phosphosulfate (PAPS)

- Phenols compose the main group of substrates undergoing sulfate conjugation. Thus, drugs containing phenolic moieties are often

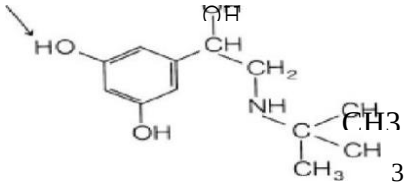
susceptible to sulfate formation. For example, the antihypertensive agent a-methyldopa (Aldomet) is metabolized extensively to its 3-0sulfate ester in humans.



a-Methytcsopa

CHs

Salbutan-wa
(Albuterol)

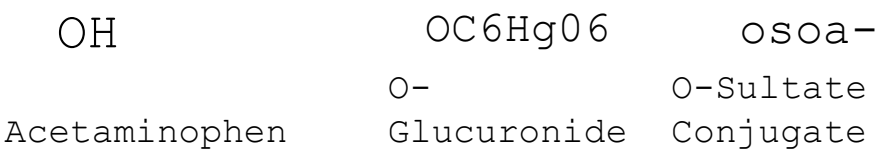


Terbutaline

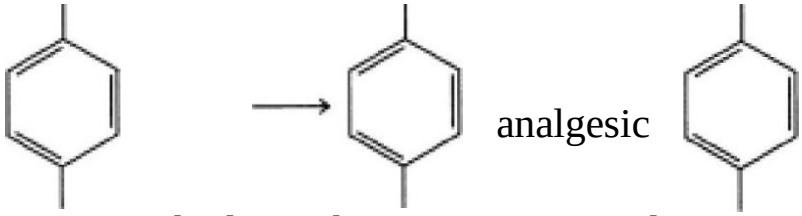
- In adults, the major urinary metabolite of the analgesic

acetaminophen is the O-glucuronide conjugate, with the concomitant O-sulfate conjugate being formed in small amounts. Interestingly, infants and young children (ages 3-9 years) exhibit a different urinary excretion pattern: the Osulfate conjugate is the main urinary product.

- The explanation for this reversal stems from the fact that neonates and young children have a decreased glucuronidating capacity because of



undeveloped glucuronyltransferases or low levels of these enzymes. Sulfate conjugation, however, is well developed and becomes the main route of acetaminophen conjugation in this pediatric group.

- The  analgesic Conjugate discontinued phenacetin is metabolized to Nhydroxyphenacetin and subsequently conjugated with sulfate. The osulfate conjugate of N-hydroxyphenacetin binds covalently to microsomal protein.
- This pathway may represent one route leading to reactive intermediates that are responsible for the hepatotoxicity and nephrotoxicity associated with phenacetin.

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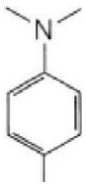
HO‖–o soCCHg



NH CCH 3 N—CCHa

OCH2CH3 OCH2CH3 OCH2Cl-13

Phenacetin N-Hydroxyphenacetin O-Sultate Conjugate of IV-Hydroxyphenacetin