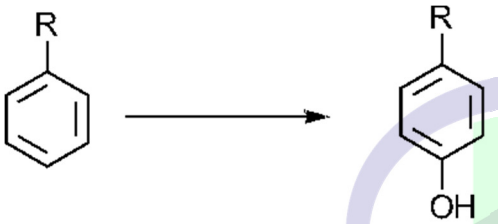
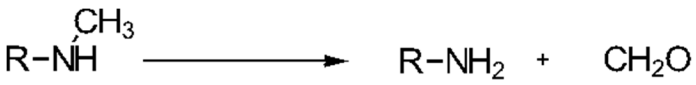
Biotransformation: Phase I and Phase II Metabolism

- **Xenobiotics**: Xenobiotics are unwanted chemicals in the environment and the diet that the body is exposed to. Polar xenobiotics are easily eliminated by the body through urine, sweat, etc. However, lipophilic xenobiotics require a sophisticated detoxicification system that can convert them to more polar entities so that they can be eliminated. This conversion is known as biotransformation or simply drug metabolism.
- **Biotransformation**: The goal of biotransformation is to convert lipophilic chemicals to more polar ones so that they can be eliminated more easily from the body. Most of the time this results in changes in the pharmacological activity of a drug. Although we ordinarily think of drug metabolism as a process that inactivates drugs, that is not always the case. In fact, biotransformation can have four possible outcomes. These are:
    - Conversion of an active drug to an inert metabolite
    - Conversion of an active drug to an active metabolite
    - Conversion of an active drug to toxic metabolite
    - Conversion of an inactive drug (prodrug) to an active drug
- The process of biotransformation involves a series of intracellular enzymatic reactions. That means that the drugs that are being transformed by these reactions must be imported into the cells either through passive diffusion or by transporters. The vast majority of metabolic enzymes are in the liver, but they are also found in other major organs throughout the body, so the GI tract, lungs, kidneys, skin, and even the brain have some capacity to metabolize drugs. Biotransformation is carried out by two distinct groups of enzymatic reactions that are known as Phase-I and Phase-II. Most of the time drugs undergo Phase-I reactions first and then Phase-II reactions, but it is possible that a drug undergoes either only Phase I or

only Phase II reaction. Very rarely, a drug might undergo Phase II reaction before Phase I reaction.

- **Phase I and Phase II Reactions**:
- **Phase-I Reactions**: Phase I reactions involve "functionalization" of drugs, meaning that either a new functional group is added or an existing functional group is "unmasked" in order to make the drug more water soluble. This is done through a combination of reactions involving oxidation, reduction, and hydrolysis.
    - **Oxidation**: Oxidation involves addition of an oxygen group to make the chemical more water soluble. Here we have an example of an aromatic ring undergoing oxidation, which in this case is hydroxylation.
    - 
    - **N-dealkylation**: Oxidative N-dealkylation is another form of oxidation reaction. In these reactions, secondary or tertiary amines undergo dealkylation. In this case, oxygen is added to the alkyl group that's being removed and not the parent compound. Having lost the alkyl group, the parent molecule becomes more water soluble, and the alkyl group is also converted to a more soluble ketone or aldehyde. For example, imipramine is an antidepressant that is converted to desimipramine through oxidative N-dealkylation.
    - 
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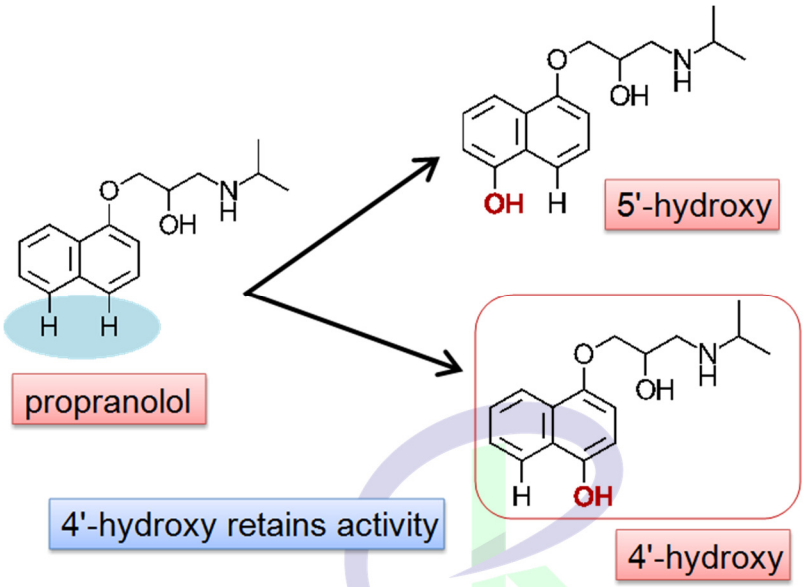
- Example (Propranolol): As mentioned above, biotransformation or drug metabolism does not always result in inactivation of a drug. For example, propranolol undergoes ring hydroxylation at two different ring positions resulting

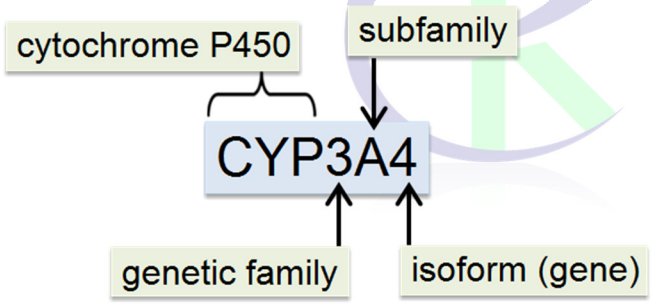
in two different metabolites. These are 4-hydroxypropranolol and 5-hydroxypropranolol. .Both metabolites are now more water soluble than propranolol itself. Interestingly though, of the two metabolites, the 4-hydroxypropranolol actually retains pharmacological activity, so in this case metabolism does not completely inactivate a drug.



- **Microsomal enzymes**: Phase I reactions are carried out by enzymes that reside in microsomes. Microsomes are small self-forming vesicles that are associated with smooth endoplasmic reticulum. Thuse, enzymes that carry out phase I reactions are grouped together as microsomal enzymes. There are many types of microsomal enzymes involved in phase I reactions, but over 95% of the reactions are carried out by a family of enzymes known as Cytochrome P450 enzymes. These are often referred to as simply P450 enzymes or CYP. The remaining 5% of the reactions are carried out by flavin monooxygenases, monoamine oxidases, and alcohol dehydrogenases.
- **Cytochrome P450 enzymes (CYPs)**: All CYP enzymes consist of a core heme protein that is similar to that in hemoglobin. When bound to carbon monoxide, these enzymes absorb light at 450nm, hence the name Cytochrome P450. The liver is the major site where these enzymes are found, but they can be found in many

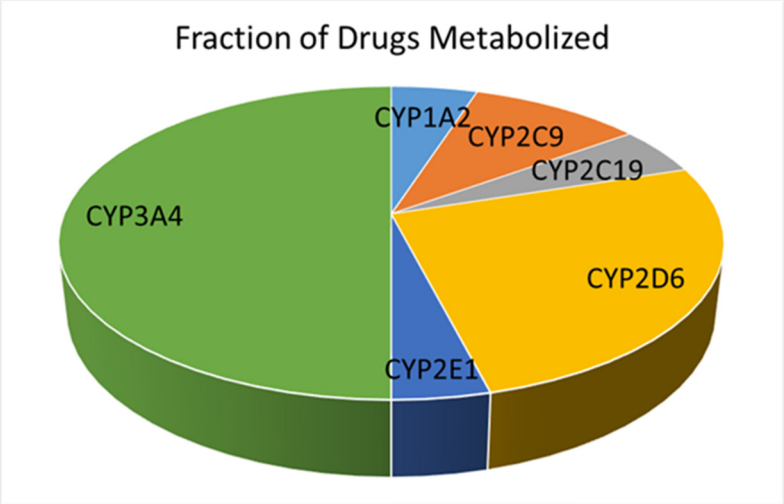
other organs as well. Even though all CYP enzymes contain the same core heme group, they have different surrounding protein structure so that they have different substrate specificity. However, compared to most other enzymes which work on one enzyme one substrate principle, CYP enzymes show a great deal of flexibility, which is important because only a few types of these enzymes have to deal with literally thousands of different types of toxins.

- **CYP Nomenclature**: As far as the nomenclature of these enzyme goes, the CYP prefix is followed by a number representing the genetic family, followed by a letter that represents the sub-family, and finally another number that indicates the actual isoform or gene. So for example, CYP3A4 is a CYP enzyme of genetic family 3, subfamily A, and isoform 4. The nomenclature is simply based on genetic relationship between different enzymes and in no way indicates any functional relationship between enzymes..



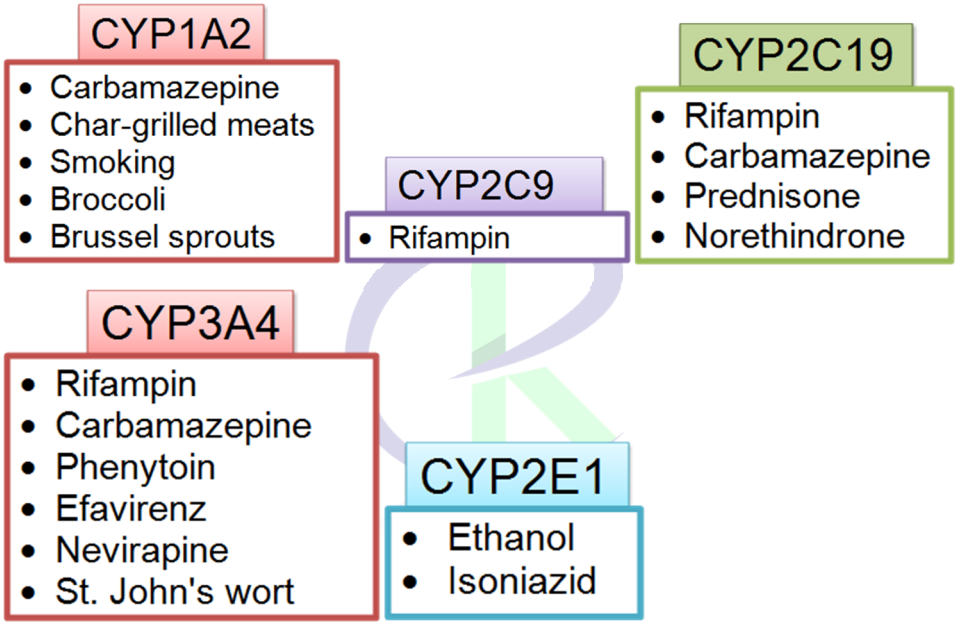
- **CYP Enzymes**: CYP enzymes are ubiquitous in a sense that they are found in yeast, plants, and all animals. There are about 60 different types of CYPs found in humans but only a handful are involved in drug metabolism. Most of them are actually involved in biocatalysis of steroids and lipids. As far as drug metabolism goes, we only have to remember six enzymes listed here because they are responsible for metabolizing more than 75% of the drugs in the market. These enzymes include CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. Among the six common CYPs that we are normally concerned with, CYP3A4 is the major enzyme because it

is responsible for metabolizing roughly half of the drugs. A third of the drugs are metabolized by CYP2D6 making it the second major enzyme. The remaining CYPs metabolize a small fraction of drugs each, but it doesn't make them less important because often they are critical for inactivating drugs that are not affected by CYP3A4 or CYP2D6. A drug may be subject to metabolism by more than just one enzyme, so it's not just one drug one enzyme relationship. Another important point is that some drugs act as substrates as well as inhibitors of CYP enzymes, which gives rise to significant drug-drug interactions.



- **CYP3A4**: CYP3A4 is mostly present in the liver but is also expressed in significant quantity in the GI tract within the intestinal mucosa where it plays an important role in the first pass effect which we discussed earlier. Within this same subfamily, there are other isoforms 3A5 and 3A7 but they play a relatively minor role as there is a great deal of substrate overlap with 3A4. When it comes to 3A4, one important dietary factor is grapefruit juice because flavonoids in grapefruit juice are potent inhibitors of this enzyme. This would lead to decreased 3A4 activity and increased plasma levels of many drugs.
- **CYP3A4 Substrates**: CYP3A4 metabolizes a wide range of drugs. Common substrates include macrolide antibiotics such as erythromycin and

clarithromycin, statins (particularly lovastatin and atorvastatin), HIV protease inhibitors, benzodiazepines, and calcium channel blockers. An extensive list of substrates and inhibitors of 3A4 and other enzymes will be posted in a separate handout.

- **CYP Inducers**: An interesting feature of CYP enzymes is that the levels of the enzymes can increase in response to a variety of drugs as well as dietary and environmental factors. Some of these inducers act on specific enzyme while others induce more than one enzyme. CYP2D6 is not inducible to any significant extent. .
  - 

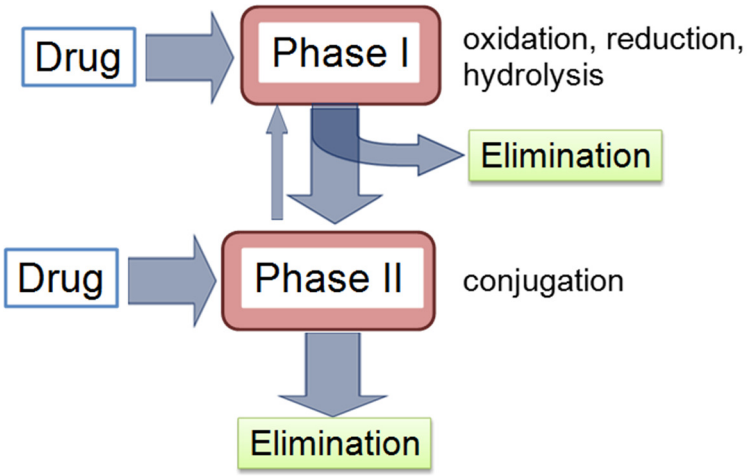
- **CYPs & Drug Interactions**: CYPs are the major source of drug-drug interactions because they not only metabolize multiple drugs but are also inhibited and induced by many drugs. A simplest form of drug-drug interaction occurs when there two drugs that are metabolized by the same CYP. This competition leads to less than optimal metabolism of both drugs and the plasma levels of these drugs are higher than what they would be if the drugs are taken individually. More seriously, though, a drug might simply inhibit a CYP and decrease the metabolism of its substrates. This would lead to multiple drug interactions. For example, the antifungal drug

ketoconazole is a potent inhibitor of CYP3A4, so this will affect all drugs that are normally metabolized by CYP3A4

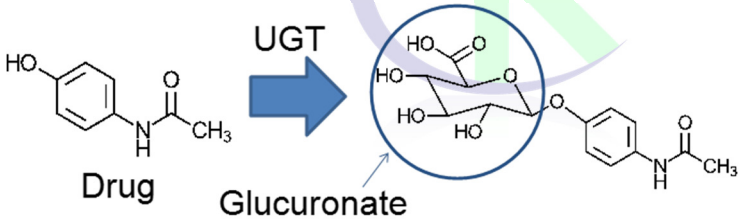
- **CYPs: Genetics & Environment**: When it comes to CYPs, it's not just the drug-drug interactions that we have to worry about. There are many dietary and environmental factors that can affect CYP-dependent drug metabolism. For example, grapefruit juice inhibits CYP3A4, chargrilled meats and smoking can induce 1A2, while ethanol induces 2E1. In addition, there are pollutants and pesticides that can induce or inhibit CYPs. Complicating things even further, CYPs are also subject to genetic variability. Individuals from different ethnicities may differ in their ability to metabolize certain drugs because they have variant forms of certain CYPs. This is particularly true when it comes to CYP2C9, CYP2C19, and CYP2D6. A patient who has a CYP that is not very efficient at metabolizing drugs would be considered a poor metabolizer. On the other hand, there might be a patient from a different ethnic background who has a form of CYP that is highly functional and efficient, so that patient may be considered an extensive metabolizer. So the genetic variability can have an important impact on drug therapy outcome.

Phase II Metabolism

- **Phase I and Phase II Summary:** Recall that Phase I reactions involve functionalization of drugs through oxidation, reduction, and hydrolysis reactions. Once drugs have undergone Phase I metabolism, they become more polar and are easier to eliminate. Metabolites of many drugs undergo Phase II metabolism subsequent to phase I metabolism. Of course, some drugs undergo only Phase I or only Phase II metabolism.



- **Phase II Metabolism:** Phase II metabolism involves conjugation of drugs or their metabolites with highly polar molecules. This renders these drugs water soluble and are easy to eliminate through the renal or biliary pathways. In these conjugation reactions, drugs can be attached to any of the five polar compounds, including glucuronic acid, glutathione, sulfate, acetyl, and glycine groups. Generally speaking, phase II metabolism renders the drugs or their metabolites more water soluble. What is different about Phase 2 reactions is that unlike in Phase I where drug metabolites often retain pharmacological activity, Phase 2 reactions pretty much inactivates the drug completely, so it's a more dramatic change in the structural and physiochemical properties of the drug. Another difference is that Phase II reactions are detoxification reactions, meaning that the resulting metabolites do not have any toxic side effects. Remember, this was not always the case in Phase I reactions, as sometimes Phase I reactions yield metabolites that may be pharmacologically inactive but are nevertheless toxic to the body. Since Phase II reactions involve conjugation of drugs to other compounds, these reactions involve a series of enzymes known as transferases, because they transfer these polar compounds onto the drugs. These transferases are found in multiple locations but are generally present cytosol and in microsomes.

- **Conjugation Reactions:** There are 5 major conjugation reactions that conjugate drugs to (1) glucuronic acid, (2) glutathione, (3) acetyl, (4) glycine, and (5) sulfate groups. All these reactions render the target drug more water soluble.
    - **Glucuronidation:** Among the most common Phase 2 reactions is Glucuronidation. This reaction involves conjugation of a drug with glucuronic acid, which is essentially a glucose molecule with an extra carboxyl group. The enzyme involved is UDP-Glucuronosyl Transferase or simply UGT. This enzyme takes molecule of Glucuronic acid from UDP-glucuronic acid and transfers it to a target drug. The resulting drug conjugate has a dramatically increased water solubility. Glucuronidation is important not only for drug elimination but also for bilirubin excretion. Normally, bilirubin, which results from the breakdown of red blood cells, is not very soluble in water and is difficult to remove. Once it is conjugated to glucuronic acid, though, bilirubin is eliminated by both urinary and biliary excretion.
    - 

    - **Glutathione Conjugation**: Another common Phase 2 reaction involves conjugation of drugs with a glutathione or GSH. Glutathione is essentially a tripeptide made up of Glutamate, Cysteine, and Glycine. The reaction is carried out by a group of enzymes known as glutathione-S-transferases or GST. In addition to removal of drugs, these enzymes play a critically role in neutralizing oxidative stress as well as carcinogenic chemicals before high enough levels build up. So the enzymes play a key role in cancer prevention.
    - **Acetyl, Glycine, and Sulfate Conjugation**: The remaining 3 reactions involve transfer of either, an acetyl group, glycine, or sulfate. These reactions

play a relatively minor role compared to glucuronic acid and glutathione conjugation. Acetylation is carried by an enzyme known as NAT or N-acetyl transferase also known as simply acetylase. Glycine transferases conjugate certain drugs with the glycine amino acid. Finally, sulfation involves conjugation of drugs with a sulfate molecule. All these reactions render the target drug more water soluble.