

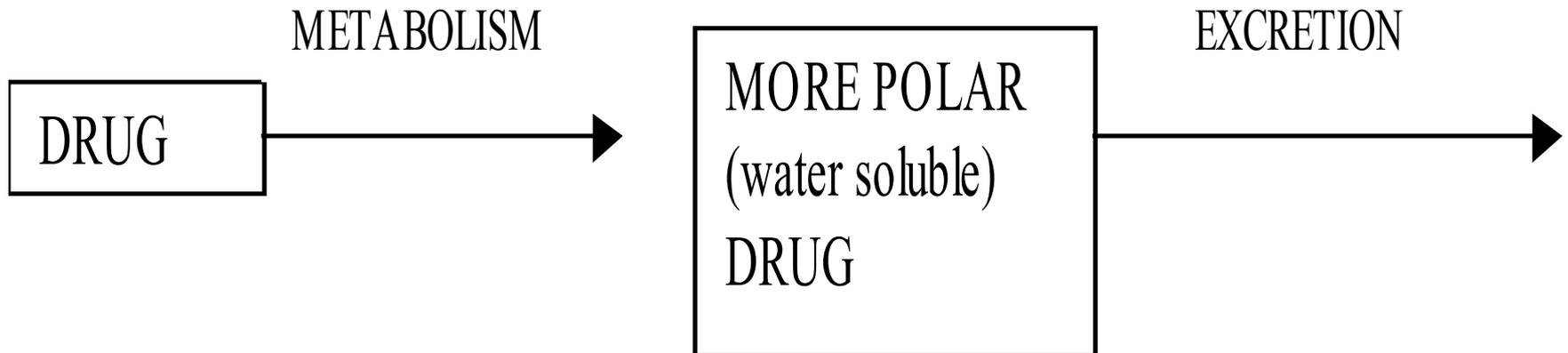
DRUG METABOLISM

- **To describe the various processes by which a drug may be metabolized in the body =>**
- **To understand the relationship between the parameters hepatic clearance, hepatic blood flow, fraction unbound, and free intrinsic clearance**

- **The body has another way of deactivating drugs in the body. This method of elimination is metabolism or biotransformation.**
- **Metabolic processes, in general, have the overall effect of converting drug molecules into more polar compounds. Again, in general, the effect of this should be to decrease tubular re-absorption in the kidney and thus increase drug elimination.**
- **Generally, it also means an immediate loss of pharmacological activity because transport into the site of action is hindered (less lipid soluble) or the molecule no longer fits into the receptor site.**
- **There are exceptions however, and a number of 'new' drugs have been discovered as active metabolites.**
- **Metabolism takes place by enzymatic catalysis. Most metabolism occurs in the liver (H) although other sites have been described, such as intestinal wall, lung, kidney (M), skin, blood, brain, testes, placenta, adrenals (L), nervous system (VL)**

Drug Metabolism

- The chemical modification of drugs with the overall goal of getting rid of the drug
- Enzymes are typically involved in metabolism



Consequences of Metabolism

- Drug Metabolism = Drug Inactivation
- The metabolite may have:
 - Equal activity of the drug
 - No or reduced activity of the drug
 - Increase activity (Prodrug)
 - Toxic properties, not seen with the parent drug

Metabolic reactions

There are four main patterns of drug metabolism. These are:

- 1) oxidation
- 2) reduction
- 3) hydrolysis
- -----
- 4) conjugation

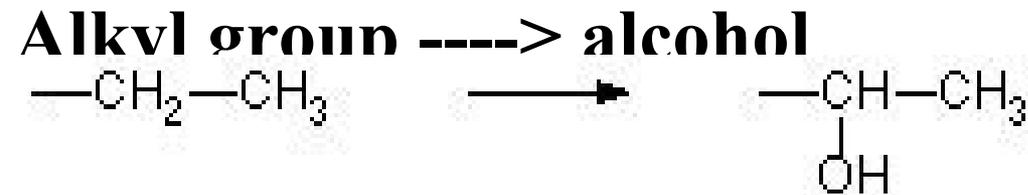
- The first three are often lumped together as phase I reactions, while the fourth process, conjugation, is called phase II metabolism.
- A common scheme in the overall metabolism of drugs is that metabolites are metabolized. In particular a drug maybe oxidized, reduced or hydrolyzed and then another group may be added in a conjugation step.
- A common cause of capacity limited metabolism is a limit in the amount of the conjugate added in the conjugation step.

Phase I

Oxidation

Oxidation is the addition of oxygen and/or the removal of hydrogen. Most oxidation steps occur in the endoplasmic reticulum. The most important enzymes: microsomal CYP-450

Common reactions include :-



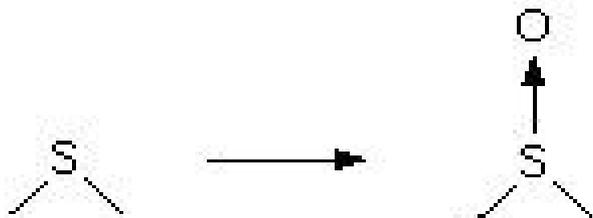
for example phenobarbitone

Aromatic ring ----> phenol



for example phenytoin

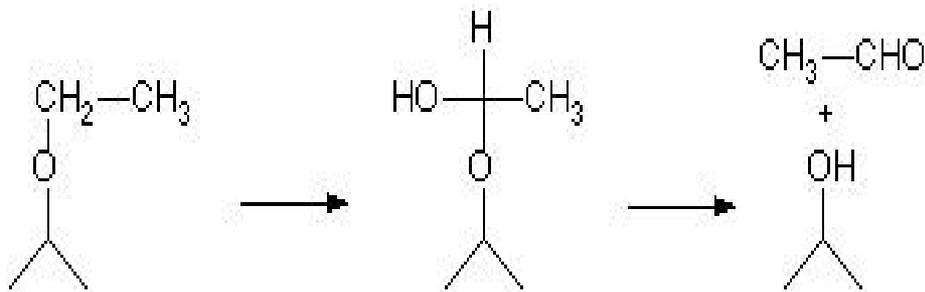
- **Oxidation at S or N**



sulfoxide

for example chlorpromazine

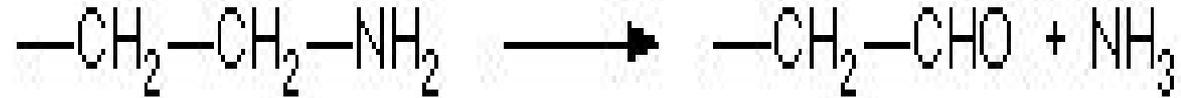
- **in two steps oxidative dealkylation is possible**



for example phenacetin

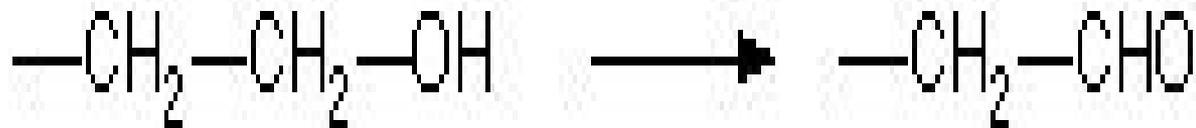
- Outside the microsomes - in liver and brain

- **Monoamineoxidase**



for example 5-hydroxytryptamine

- **Alcohol dehydrogenase - in liver, kidney, lung**



- **Reduction**

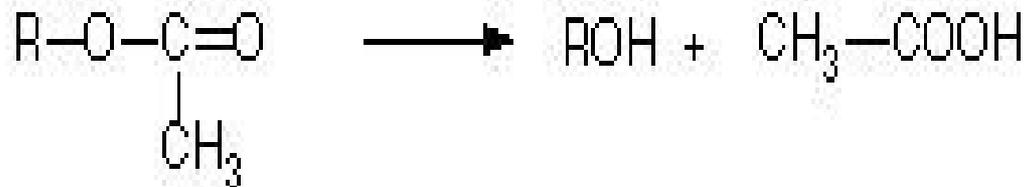
Add a hydrogen or remove oxygen
azo (-N=N-) or nitro groups (-NO₂) ----->
amines (-NH₂)

for example nitrazepam

- **Hydrolysis**

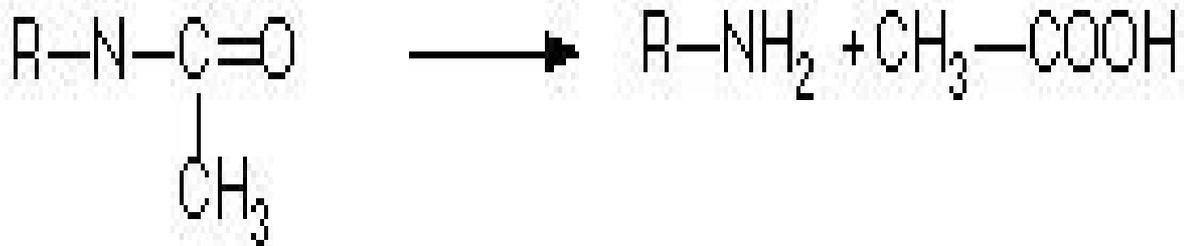
Addition of water with breakdown of molecule.
In blood plasma (esterases) and liver

Esters ---> alcohol and acid



for example aspirin to salicylic acid

- **Amides to amine and acid**



- for example procainamide

Phase II

Conjugation

Conjugation reactions involve the addition of molecules naturally present in the body to the drug molecule. The drug may have undergone a phase I reaction.

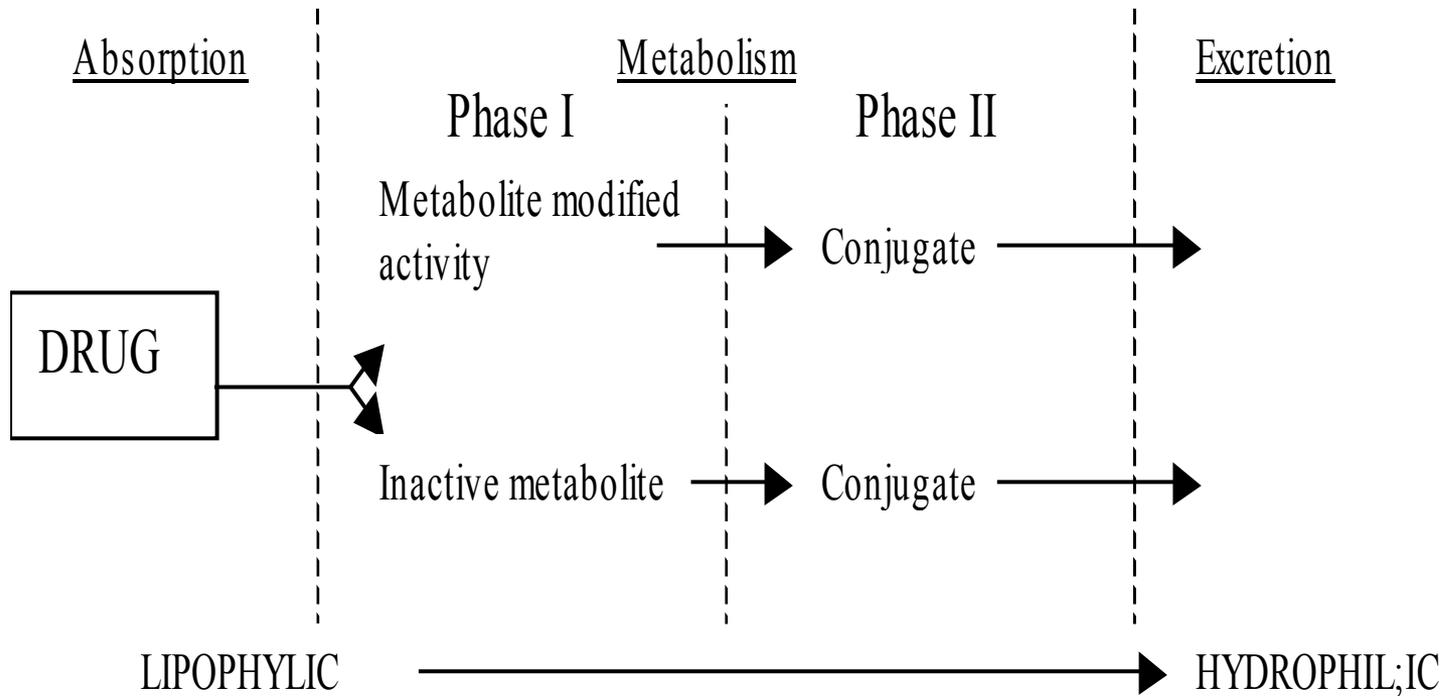
- **Glucuronidation.** This is the main conjugation reaction in the body. This occurs in the liver. Natural substrates are bilirubin and thyroxine. Aliphatic alcohols and phenols are commonly conjugated with glucuronide. Thus hydroxylated metabolites can also be conjugated for example morphine
- **Acylation.** Acylation, especially acetylation with the acetyl group, e.g. sulfonamides
- **Glycine.** Glycine addition ($\text{NH}_2\text{CH}_2\text{COOH}$) for example nicotinic acid
- **Sulfate.** Sulfate ($-\text{SO}_4$) for example morphine, paracetamol

Conclusion

- Phase I functionalization reactions introduce or expose a functional group on the parent drug
- Phase II conjugation reactions lead to covalent linkage of a functional group on the parent drug or phase metabolite with endogenously derived glucuronic acid, sulfate, methyl, glutathion, glycine/amino acids, acetate/acetyl, etc

Phase I and phase II metabolic reaction

- Scheme



- In most cases the metabolite is formed by production of a more polar group, for example C-H \rightarrow C-OH, or addition of a polar group, for example acetyl (CH₃COO-).

Generally the resultant metabolite is more water soluble, and certainly less lipid soluble. Less drug is reabsorbed from the kidney.

- Occasionally the metabolite is less water soluble. A significant example is the acetyl metabolite of some of the sulfonamides. Some of the earlier sulfonamides are acetylated to relatively insoluble metabolites which precipitated in urine, crystalluria. The earlier answer this was the triple sulfa combination, now the more commonly used sulfonamides have different elimination and solubility properties and exhibit less problems.

- In most cases the metabolites are inactive, however, occasionally the metabolite is also active, even to the extent that the metabolite may be the preferred compound to be administered. The original drug may take on the role of a pro-drug. For example:-
 - amitriptyline ---> nortriptyline
 - codeine ---> morphine
 - primidone ---> phenobarbital
- Drug metabolism can be quantitatively altered by drug interactions. This alteration can be an increase by induction of enzyme activity or a reduction by competitive inhibition.

Factors affecting biotransformation

- Race
- Age
- Sex
- Species
- Clinical or physiological condition
- Other drug administration
- Food
- First-pass (pre-systemic) metabolism

First-pass Effect

- The first-pass effect is the term used for the hepatic metabolism of a pharmacological agent when it is absorbed from the gut and delivered to the liver via the portal circulation.
- The greater the first-pass effect, the less the agent will reach the systemic circulation when the agent is administered orally

First-pass Effect

- Magnitude of first-pass hepatic effect:
Extraction Ratio (ER) : Cl_{liver} / Q

Q = hepatic blood flow (90 L/hr)

- Systemic drug bioavailability (F) may be determined from the extent of absorption (f) and the Extraction Ratio (ER):

$$F = f \times (1-ER)$$

Hepatic/Liver Clearance

- Extraction Ratio (ER): the extent to which a drug is cleared from blood in one liver passage
- Low ER ($\approx 0,1$: warfarin, diazepam, phenytoin).
In this case, changes in liver blood flow are less critical

Substrate, Inhibitors, Inducers

Substrate

- **High ER (≈ 1 : propranolol, meperidine), overall liver extraction of the drug is dependent on liver blood flow**
- **Is a compound that is metabolized by a given enzyme (> 1)**
- **Fluoxetine : CYP2D6 & CYP3A4**

Inhibitor

- **Compound that slow down the metabolism of a substrate by a given enzyme**
- **Fluoxetine slows down the metabolism of desipramine by CYP2D6 – fluoxetine acts as an inhibitor – desipramine levels will rise – toxicity, arrhythmia, death**

Inducer

- **Compound that speeds up the metabolism of a substrate by a given enzyme**
- **Carbamazepine speeds up the metabolism of clozapine (substrate) by CYP1A2 and CYP3A4 – carbamazepine acts as an inducer – clozapine plasma levels will fall. Conversely if carbamazepine discontinued, clozapine levels will rise – adverse effects such as an unanticipated seizure**

Induction

- A large number of drugs can cause an increase over time in liver enzyme activity. This in turn can increase the metabolic rate of the same or other drugs. Phenobarbitone will induce the metabolism of itself, phenytoin, warfarin, etc. Cigarette smoking can cause increased elimination of theophylline and other compounds. Dosing rates may need to be increased to maintain effective plasma concentrations
- Barbiturates, carbamazepine
- Shorten action of drugs or increase effects of those biotransformed active agents

Inhibition

- Alternately some drugs can inhibit the metabolism of other drugs. Drug metabolism being an enzymatic process can be subjected to competitive inhibition. For example, warfarin inhibits tolbutamide elimination which can lead to the accumulation of drug and may require a downward adjustment of dose
- Cimetidine
- Prolongs action of drugs or inhibits action of those biotransformed to active agents (pro-drugs)

Cytochrome P-450 (CYP)

Isoenzymes

- Comes from the wavelength of light (450 nm) that is absorbed by this enzyme
- CYP isoenzymes are responsible for oxidative metabolism (phase I) of many drugs, steroids and carcinogens
- Are a group of heme containing enzymes embedded primarily in the lipid bilayer of the endoplasmic reticulum of hepatocytes (liver cells)
- CYP metabolism also occurs in the small intestine, kidney, lung and brain
- >30 CYP human isoenzymes have been identified

Cytochrome P-450: Oxidative

- Addition of an oxygen atom
- Most common process (liver)
- General chemical equation:
- $-RH + NADPH + O_2 + H^+ \rightarrow ROH + NADP^+ + H_2O$
- Mixed function oxidases or monooxygenases located in the liver hepatocyte endoplasmic reticulum
- NADH, NADPH, O_2 = cofactor
- CYP-450 or cytochrom b5 enzymes need: heme proteins, iron containing porphyrin – binds O_2 , works on a large number of diverse compounds

Cytochrome P-450: oxidative

- Structural diversity due to: non specificity and isozymes – multiple forms of an enzymes
- Enzymes are inducible by various chemicals
- Exposure to heat increases the rate of enzyme production but not following Arrhenius
- Enzymes isolated by disruption of the liver cells

Cytochrome P-450: oxidative

- Isozym differ in protein structure
 - Different amino acid sequences
 - Produce different 3-D structures
 - Drug bound to the protein
- All activated oxygen chemistry occurs at the iron center heme with oxygen transfer to the protein bound substrate

Human Liver Drug CYPs

CYP enzyme	Level, % total
1A2	~ 13
1B1	< 1
2A6	~ 4
2B6	<1
2C	~ 18
2D6	Up to 2.5
2E1	Up to 7
2F1	
2J2	
3A4	Up to 28
4A, 4B	

Human Drug Metabolizing CYPs Located in Extrahepatic Tissue

CYP Enzyme	Tissue
1A1	Lung, kidney, GIT, skin, placenta, others
1B1	Skin, kidney, prostate, mammary, others
2A6	Lung, nasal membrane, others
2B6	GIT, lung
2C	GIT (small intestine mucosa), larynx, lung
2D6	GIT
2E1	Lung, placenta, others
2F1	Lung, placenta
2J2	Heart
3A	GIT, lung, placenta, fetus, uterus, kidney
4B1	Lung, placenta
4A11	Kidney

Factors Influencing Activity and Level of CYP enzymes

Nutrition	1A1; 1A2; 2E1; 3A3;3A4,5
Smoking	1A1; 1A2
Alcohol	2E1
Drugs	1A1; 1A2; 2A6; 2B6; 2C; 2D6; 3A3; 3A4,5
Environment	1A1; 1A2; 2A6, 1B, 2E1; 3A3, 3A4,5
Genetic Polymorphisme	1A, 2A6, 2C9, 19; 2D6; 2E1

Human Drug Oxidation

- More than 90% of human drug oxidation is due to 6 CYP isoenzymes
- These isoenzymes: 1A2, 2C9, 2C19, 2D6, 2E1, 3A4
- CYP2C19 and CYP2D6: extensive metabolizers (EMs) and Poor Metabolizers (PMs)
- PMs: develop adverse effect and/or toxicity from high levels of unmetabolized drugs
- EMs: to be non responders at the usual therapeutic dose range

Drug Interactions Involving Drug Metabolism

- The enzymes involved in the metabolism of drugs may be altered by **diet** and the **co-administration of other drugs and chemicals**
- *Enzyme Induction* – increase enzyme activity due to an increase in the amount of enzyme present – requires some onset time for the synthesis of enzyme protein: rifampicin 2 days; phenobarbital 1 week; carbamazepin 2-5 days up to 1 month/longer
- Smoking can change the rate of metabolism of many Tri Cyclic Antidepressant drugs through enzyme induction
- Benzopyrene – meat grilled, satay, insecticides-chlordane and drugs mentioned before – enzymes inducers

- *Enzyme Inhibition* – decrease enzymes activity due to substrate competition or due to direct inhibition of drug metabolizing enzymes, particularly one of several of the CYP-450 enzymes
- *SSRI* – Selective Serotonin Reuptake Inhibitors – inhibit the CYP2D6 system, resulting elevated plasma concentration of coadministered psychotropic drugs. *SSRI (fluoxetine, paroxetine, fluvoxamine), TCA and CYP2D6* – TCA level rise – toxic
- Fluoxetine causes 10x decrease in the clearance of imipramine and desipramine because of its inhibitory effect on hydroxylation

- Diet – affects drug metabolizing enzymes: plasma theophylline concentration and theophylline clearance in patients in high protein diet are lower than in patients in high carbohydrate diet
- Sucrose, glucose, fructose – decrease the activity of mixed function oxidases – slower metabolism rate and prolongation in hexobarbital sleeping time in rats
- Chronic adm 5% of glucose – affect sleeping time in subjects receiving barbiturates
- Grape fruit juices + saquinavir (protease inhibitor) – AUC increase 150%, 220% (concentrated)

- Naringin, a bioflavonoid in grapefruit juice, responsible for the inhibition of CYP3A4 in the liver and intestinal wall, which metabolizes saquinavir, resulting in an increase in AUC
- Ketoconazole, Ranitidine – increase AUC saquinavir by inhibition of the CYP-450 enzymes. In contrast, rifampicin greatly reduces AUC saquinavir due to enzymatic stimulation
- AUC increase with grapefruit juices: several sedatives and the anticoagulant coumarin
- Increase AUC is dangerous, pharmacokinetic of drugs with potential interactions should be closely monitored
- Triazolam, Midazolam, Cyclosporin, Coumarin, Nisoldipine, Felodipinein

Drug Interactions Affecting Mixed Function Oxidase Enzymes

Inhibitors of Drug metabolism	Example	Result
Acetaminophen	Ethanol	Increased hepatotoxicity in chronic alcoholics
Cimetidine	Warfarin	Prolonged of prothrombine time
Erythromycin	Carbamazepine	Decreased carbamazepine clearance
Fluoxetine	Imipramine	Decreased clearance of CAD
Fluoxetine	Desipramine	Decrease clearance of CADF.

Inducers

Inducers of Drug Metabolism	Example	Result
Carbamazepine	Acetaminophen	Increased acetaminophen metabolism
Rifampin	Methadone	Increase methadone metabolism, may precipitate opiate withdrawal
Phenobarbital	Dexamethasone	Decreased dexamethasone elimination half-life
Rifampin	Prednisolone	Increased elimination of prednisolone

In Cytochrome P-450 Isoforms and Examples

CYP1A2	Substrates-amitryptiline, imipramine, theophylline; induced by smoking
CYP2B6	Substrate-cyclophosphamide, methadone
CYP2C9	Metabolism of S-warfarin and tolbutamide CYP2C9. Substrates-NSAIDs-ibuprofen, diclofenac
CYP2C19	
CYP2D6	Many antidepressants, β -blockers are metabolized by CYP2D6
CYP2E	
CYP3A4, 5, 6	

Factors affecting biotransformation

- **Race**
- **Age – new born jaundice: kernicterus**
- **Sex - hormonal**
- **Species - genetic differences: man, monkey**
- **Clinical or physiological condition**
- **Other drug administration**
- **Food: protein, carbohydrate, fat, charcoal grilled meat**
- **First-pass (pre-systemic) metabolism**
- **Enzyme induction, inhibition**
- **Pregnancy, Disease states**
- **Circadian rhythm, Substrate stereoselectivity, Pharmacologically active metabolites**