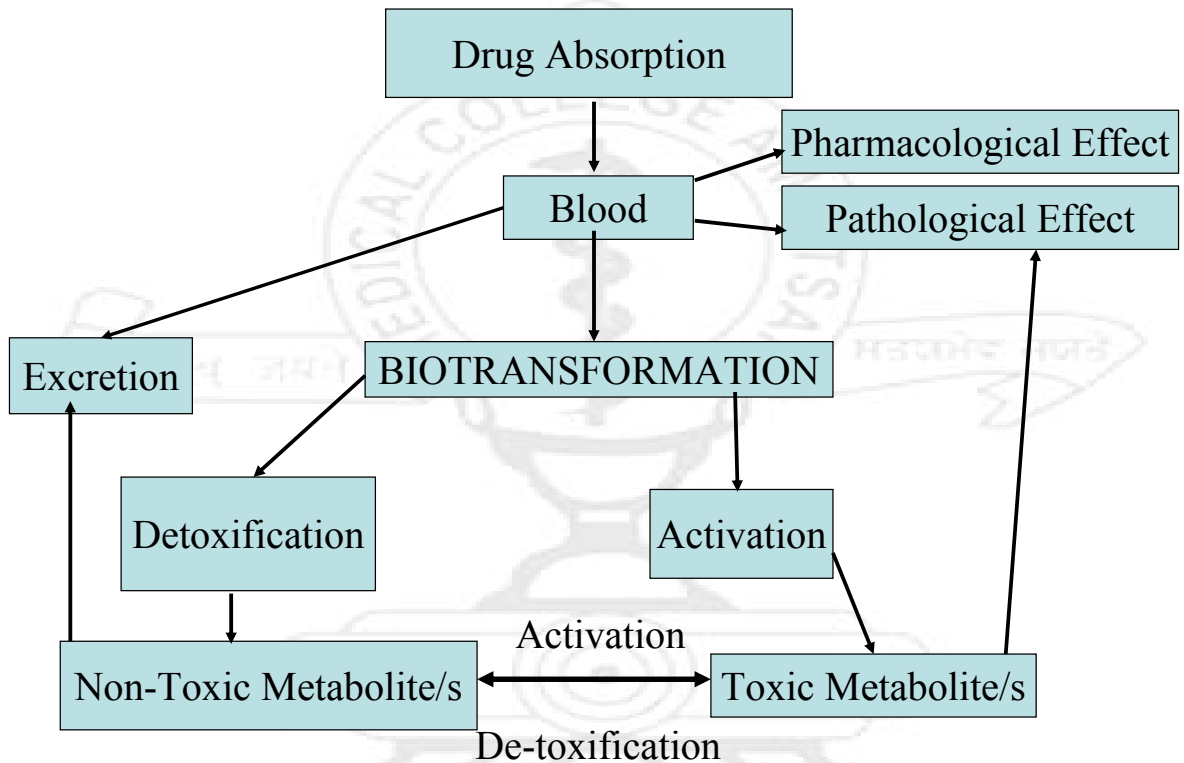
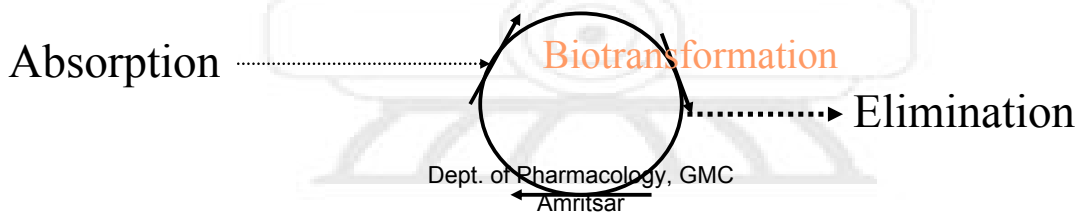


# Biotransformation



# Fate of Drugs

- Drugs are eliminated from the body by the aid of two processes-
  - Metabolism & Excretion  
(Biotransformation) (Elimination)
- Elimination is important or else the drug would stay in the body indefinitely!



3

# Background

- Drug molecules are processed by enzymes that have evolved to cope with endogenous compounds or those to which we are exposed.

## Xenobiotics

# Why Biotransformation?

- Most drugs are excreted by the kidneys
- For renal excretion drugs should:
  - have small molecular mass
  - be polar in nature
  - not be fully ionised at body pH
- Most drugs are complex and do not have these properties and thus have to be broken down to simpler products.

- Drugs are lipophilic in nature
- Thus readily pass across biological barriers –membranes
- Strongly bound to plasma proteins
- This property also stops them from getting eliminated
- They have to be converted to simpler hydrophilic compounds so that they are eliminated and their action is terminated.

Grease

BIOTRANSFORMATION

SALT

Water

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## Consequences

- Biotransformation can also result in bioactivation, which involves the production of reactive metabolites that are more toxic, mutagenic, or carcinogenic than their parent compound(s).
- Drugs may be converted to
  - less toxic materials
  - more toxic materials
  - materials with different type of effect or toxicity

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# When and How?

- Biotransformations occur between absorption and elimination from kidneys.
- Drugs administered orally can biotransform in the intestinal wall.
- Biotransformation reactions can be:
  - Phase I reactions
  - Phase II reactions

# Phase I reactions

- Catabolic in nature
  - e.g. Oxidation, Reduction, Hydrolysis
- End-products are chemically more reactive
- Metabolites are usually more polar than the parent drug.
- Introduce a reactive group – **Functionalisation**
- The functional group becomes the starting point for Phase II reaction.

# Phase I

- Cytochrome P450 is the major enzyme system (Oxidations, reductions, etc.)
- **Phase I metabolites may be:**
  - Inactive
  - Equally Active
  - More Active
  - Toxic
  - Activated - “prodrug”
- **Polar metabolites may be excreted.**

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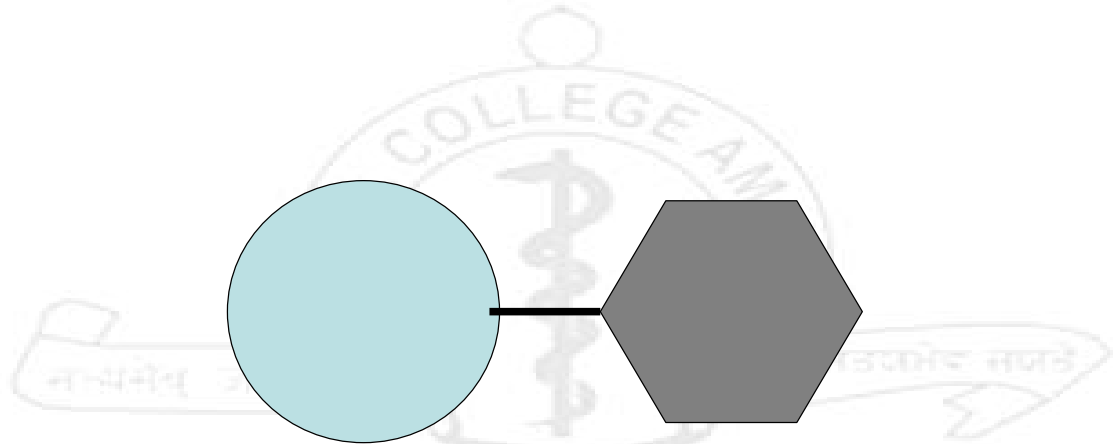
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# Phase II reactions

- Synthetic processes
- An endogenous substrate is coupled to an existing (or phase I formed) conjugation site
- Glucuronic acid, sulphuric acid, acetic acid, amino acids
- Forms a highly polar conjugate
- Phase II reactions usually occur after Phase I but can also take place earlier than Phase I

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**Parent Drug**

**Glucuronic acid**

**or**

**Sulfate**

**Phase I  
metabolite**

**Glutathione**

**others**

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13

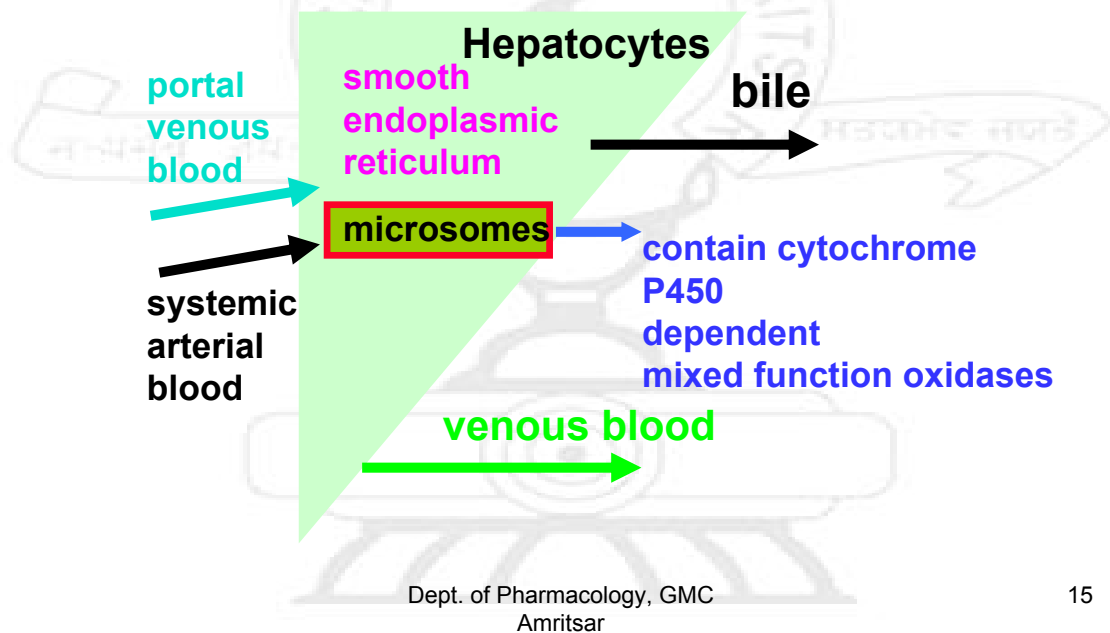
## Where?

- All tissues have the ability to biotransform drugs
- The liver is ideally placed to intercept natural ingested xenobiotics and has a major role in biotransformation
- GIT, Lungs, Skin and Kidneys

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14

# The liver



15

- Certain drugs when administered orally get absorbed from the intestine and are transported via the portal system to the liver.
- They are extensively metabolised – **First Pass Effect**- limits bioavailability
- Drugs given orally can also get metabolised
  - in intestine/
  - by intestine wall enzymes
  - by microorganisms in lower GIT

# Drug metabolizing enzymes

- Cytochrome P450 (CYP 450) is the major drug metabolizing enzyme system in the body.
- Comprised of multiple proteins.
- Active site or core of the enzyme system is a heme protein
- Also known as “mixed function monooxygenases”
- Liver and gut wall have the greatest concentration of P450
- Almost all tissues in the body have some P450 (Lungs, Kidney, Skin, Brain, etc)

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17

- Specificity is very low
- Many drugs, chemicals are metabolised by the CYP 450 enzyme system.
- Attributes
  - Enzyme Induction
  - Enzyme Inhibition
  - Genetic Polymorphism

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## Induction

- Reversible increase in enzyme concentration
- Resulting from administration of certain drugs
- Potential to increase rate of the “inducing” drug’s breakdown
- Leads to “Pharmacokinetic or Drug Disposition Tolerance”
- May increase the metabolism of other drugs taken concurrently

## Inhibition

- Some drugs can block P450 enzymes that metabolise other drugs
- May increase serum concentrations of second drug
- Can lead to toxicity
- Unlike induction, enzyme inhibition usually begins with the first dose of the inhibitor.
- Inhibition is maximal when the inhibitor reaches steady state (four to seven half-lives).

# Cytochrome P450 Enzymes

(mixed function monooxygenases)

- Major drug metabolising enzyme system in the body.
- Located in the smooth endoplasmic reticulum of various organs.
- In liver microsomes – Microsomal Enzymes
- liver and gut wall have the greatest concentration of P450
- Almost all tissues in the body have some P450 (Lungs, Kidney, Skin, Brain, etc)

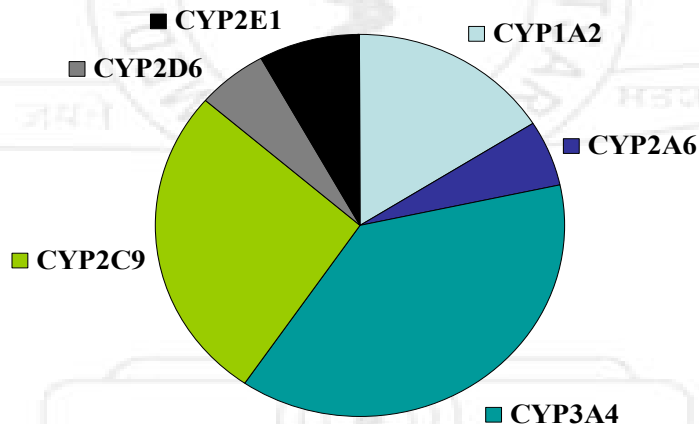
## ...CYP 450...

- Substrate specificity is very low
- Varied drugs, chemicals are metabolised by the CYP 450 enzyme system.
- Powerful oxidising property
- Lipid solubility is important for substrate.
- Attributes
  - Enzyme Induction
  - Enzyme Inhibition
  - Genetic Polymorphism

# Nature

- Haeme protein superfamily of related enzymes.
- Enzymes differ in Amino acid sequence.
- >74 CYP gene families based on amino acid sequence are known.
- Three families are responsible for drug metabolism
- CYP1, CYP2 & CYP3

# CYP Isoforms



# Induction

- Results from repeated exposure to certain xenobiotics including drugs.
- Increased rate of synthesis of enzyme or reduced rate of breakdown.
- Marked increase in rate of metabolism of drug-thus decrease in pharmacological effect.
- Leads to Pharmacokinetic or “Drug Disposition” Tolerance.

## ...Induction...

- May also increase the metabolism of other drugs taken concurrently
- Some drugs are metabolized to reactive products and may thus exhibit increased toxicity - Paracetamol.

# Inducers

- Some xenobiotics causing induction:
  - Polycyclic hydrocarbons-Epoxyde formation
  - Smoke- Tobacco
  - Charcoal – barbecued food
  - Plasticizers
  - Dioxin
- Drugs
  - Rifampicin
  - Ethanol
  - Carbamazepine

# Inhibition

- Some drugs can block P450 enzyme isoforms that metabolize other drugs
- May increase serum concentrations of second drug
- Can lead to toxicity
- Unlike induction, enzyme inhibition usually begins with the first dose of the inhibitor.
- Inhibition is maximal when the inhibitor reaches steady state (four to seven half-lives).

## ...Inhibition...

- Quinidine inhibits CYP2D6 but is not a substrate.
- Ketoconazole (antifungal) and known inhibitor of many enzyme isoforms.
- Clinically important as may lead to drug interactions.

## Other Phase I enzymes

- Not all Phase I reactions are CYP 450 based.
- Other enzymes:
  - Alcohol dehydrogenase-Ethanol
  - Xanthine oxidase
  - Monoamine oxidase-catecholamines
- Reduction reactions
- Hydrolytic reactions

# Reduction reactions-Phase I

- Nitroreductases
- Bacterial reductases
- Aldo-keto reductases
- NADPH-cytochrome-c-reductase

Remove O, add H, decrease  
valence

# Hydrolysis Reactions-Phase I

- Esterases
- Amidases
- Phosphatases
- Sulphatases
- Epoxide Hydrolase

Add water-expose groups for  
Phase II

# Phase II reactions-Enzymes

- Liver, lungs, kidney
- Most of these enzymes are in the cytosol
- Generally occur at a higher rate than Phase I reactions
- Uridine diphosphate glucuronyl transferase
- Sulphonyl transferase
- Glutathione S-transferase
- N-acetyltransferases + acetyl Co A (NAT +Co A)
- Methyltransferases

## ...Phase II...

- **Glucuronidation**
  - Major pathway
  - Catalyzed by UDP glucuronosyl-transferase
    - Requires UDP co-factor
  - Glucuronides are water soluble

## ...Phase II...

- Sulfation
- Sulfate conjugation products are water soluble
- Catalyzed by sulfotransferase
  - Transfer of sulfonate

## ...Phase II...

- Methylation
  - Important but minor pathway
  - Catalyzed by methyltransferases
- Acetylation
  - May or may not be more water soluble
  - Increases renal excretion
  - Catalyzed by N-acetyltransferase

## ...Phase II...

- Amino acid conjugation
  - Glycine conjugates
  - Taurine conjugates
- Glutathione Conjugation
  - Reaction catalyzed by glutathione s-transferase using glutathione thiolate

## Biotransformation-Conclusion

- Change the xenobiotic to a form that can be eliminated from the body
- Change the xenobiotic to a less biologically active form
- Bioactivation to more toxic forms can also occur
- Catabolic Phase I reactions are carried out by the CYP450 system
- Synthetic Phase II reactions are carried out by other enzymes



# Factors Modifying Biotransformation

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# Background

- Most patients are treated on similarities rather than as individuals.
- Same
  - Dose
  - Problem
  - Expected Result
- True for most drugs with a wide margin of safety

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40

# Examples

- One tablet Paracetamol for fever
- In impaired liver function (alcoholics, hepatitis)
  - can cause harm
- Two tablets of Aspirin for toothache
- In patients with renal disease, patients on other drugs like Warfarin
  - can be harmful

# Sources of variation

- Age
- Gender
- Environmental factors
- Diet
- Disease
- Drug-drug interactions
  - Enzyme induction
  - Enzyme inhibition
- Genetic factors
- Species difference
- Pregnancy

# Age

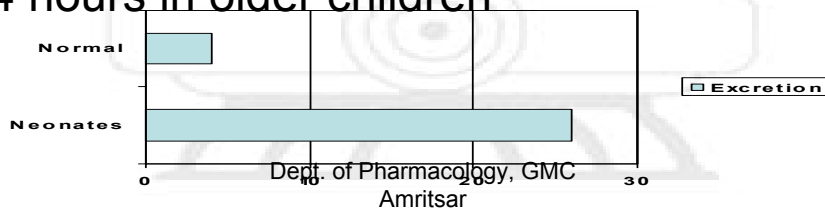
- Extremes of age are associated with disturbances in metabolism of drugs.
- In paediatric age group
  - Premature infants, neonates, children and adolescents cannot be treated like small adults.
- All these groups have special metabolic parameters.
- Foetus: CYP3A Sub-family only poor metabolism.
- Neonates virtually no Phase-2 enzymes

## ...Age...

- Hepatic biotransformation and enzyme activity is reduced in the early neonatal stages.
- There is decreased biotransformation of drugs and increased plasma levels and prolonged half life.
- Less developed excretory mechanisms.
- Malnutrition in children can impair metabolism.

## Gray-baby syndrome.

- Chloramphenicol toxicity leading to Inadequate glucuronidation due to diminished glucuronyl transferase activity
  - Immature kidney exhibits inadequate renal excretion of unconjugated drug and glucuronide conjugate.
  - Elimination half life 26 hours in neonates
  - 4 hours in older children



45

## Elderly Patients

- In patients > 65 years complex pharmacokinetic changes occur.
- Decrease in liver size and liver blood flow
- Activity of phase I pathways is reduced thus drugs predominantly metabolised by this path may show an exaggerated response.
  - eg. Diazepam as sedative
- Irregular eating habits (PCM) and vitamin deficiencies are associated with impaired metabolism

## ...Elderly...

- Diminished enzyme induction
- Drug drug interactions are more common
  - Larger number of drugs being prescribed.
  - Both induction and inhibition can result.
- Renal excretion of drugs and metabolites is impaired

## Gender

- Seen and documented in experimental animals.
- Usually associated with sex hormones.
- Menstrual cycle affects metabolism
- In humans a difference in metabolism of a few drugs has been reported:
  - Alcohol
  - Benzodiazepines
  - Some antiinflammatory drugs
  - Propranolol oxidation M>F.
  - Morphine
- N-Demethylation of erythromycin F>M.

# Pregnancy

- In pregnancy there is a concern for foetus
- Placenta high in **CYP1A** family if smoker.
- Consequences to foetus or neonate: teratogenicity, carcinogenicity, hepatotoxicity
- Can have profound induction in pregnancy.  
e.g., may have to increase anticonvulsants.

# Environmental factors

- Cigarette smoke leads to enzyme induction and increases the breakdown of drugs.
- Exposure to industrial chemicals, pollutants also alters metabolism.
- Clinical outcome:
  - Increase dose in smokers
  - Drugs with narrow safety margins should be given carefully.

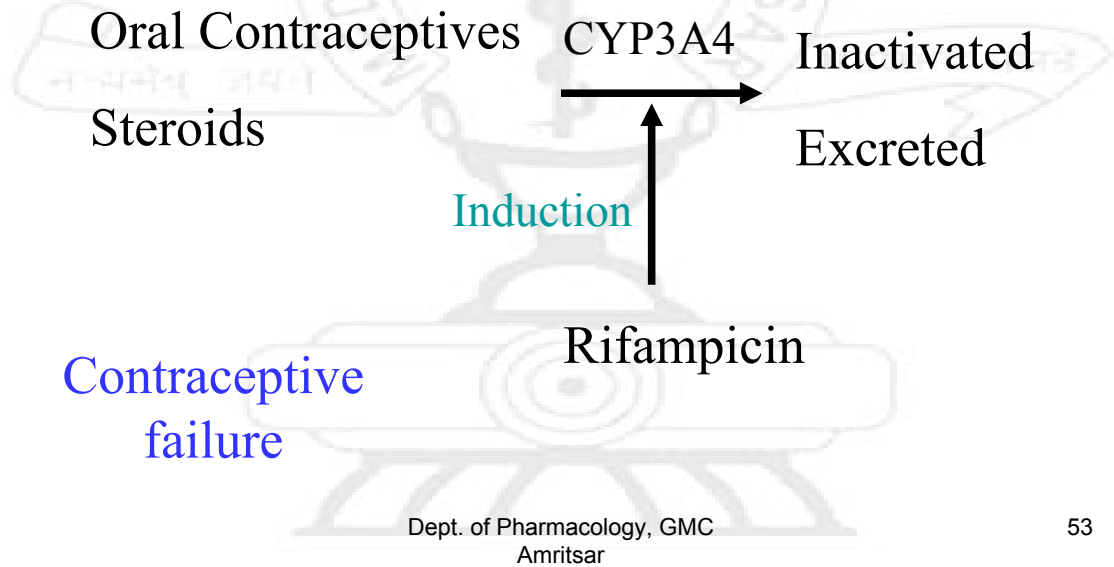
## ...Disease...

- Cardiac disease leads to decreased blood flow to liver and delayed metabolism.
- Pulmonary disease may impair metabolism of certain drugs.
- Thyroid disorders may lead to fast metabolism-hyperthyroidism or vice versa.

## Drug-drug interactions

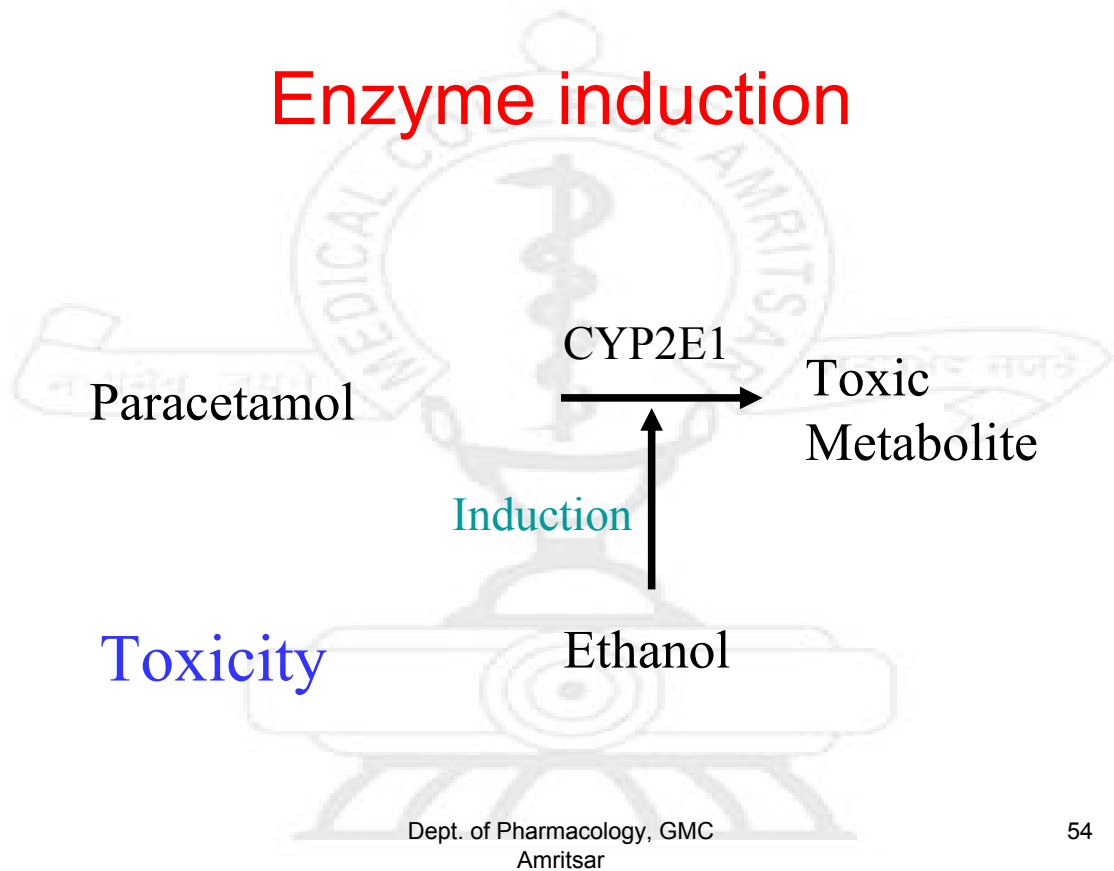
- When two or more drugs are co-administered
  - Enzyme inducers may lead to increased metabolism of other drugs.
  - There is impaired elimination of slowly metabolized drug and thus prolonged effect and toxicity.

# Enzyme induction



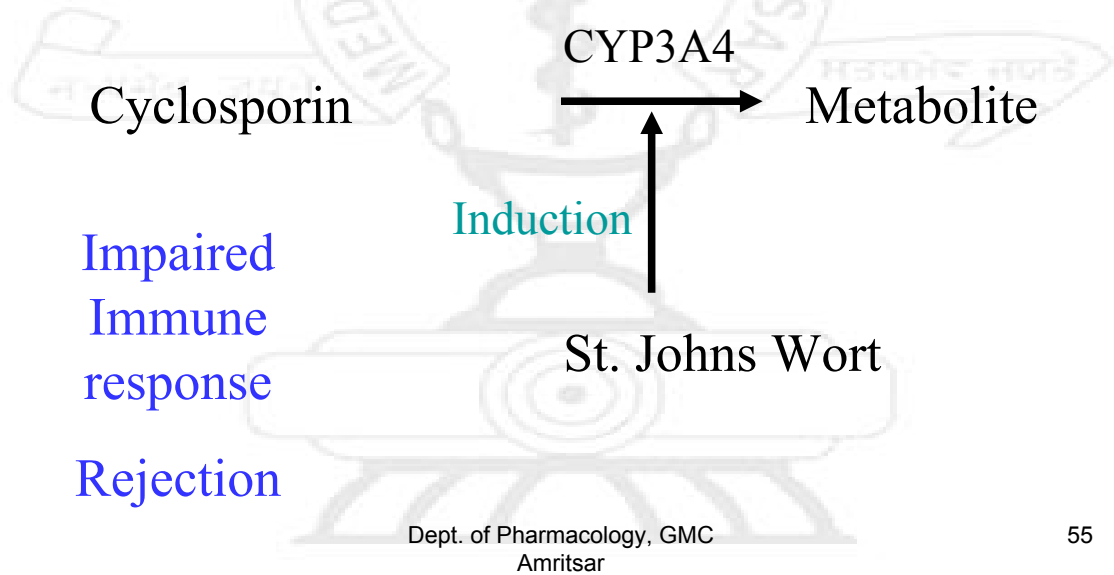
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# Enzyme induction

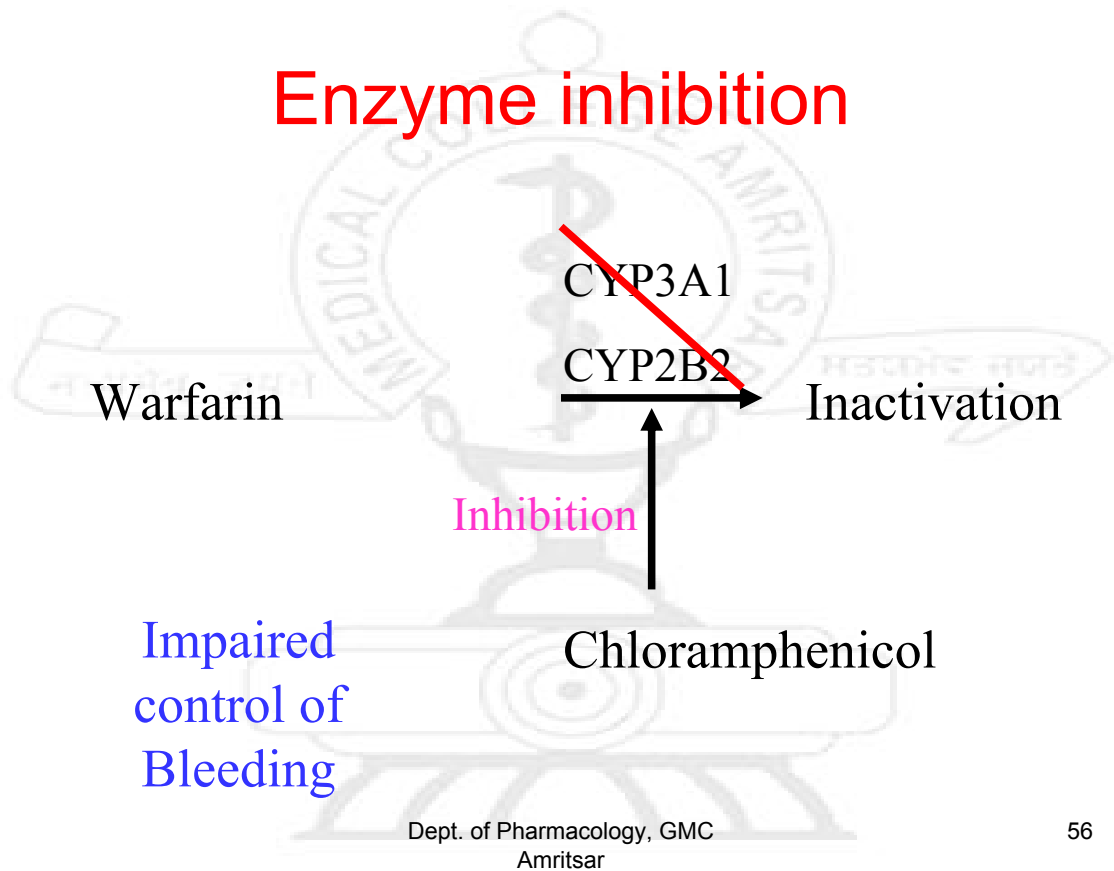


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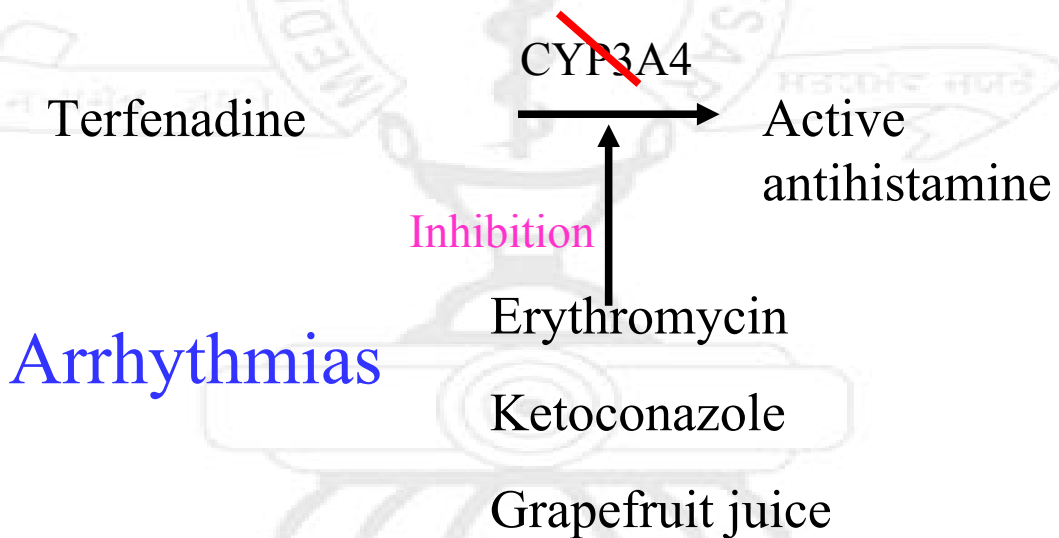
# Enzyme induction/ Herbal Drug



# Enzyme inhibition



# Enzyme Inhibition



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## Genetic factors

- Pharmacogenetics as a discipline
- Explains why one patient's response to drug therapy is different from another patient's when both are being treated with the same drug for the same problem
- Provides an understanding of the outcomes of therapy.

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# Pharmaco-genetics/ genomics

- Pharmacogenetics is to use a patient's genetic profile to optimize drug therapy and minimize drug toxicity
- Pharmacogenomics: identifying innovative drug targets and accounting for the effect that DNA-sequence variations have on a drug's effectiveness.
- Pharmacogenomics may ultimately result in the development of new medications for unmet medical needs

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- Pharmacogenetics studies gene variations, which can exist either as
  - rare defects (eg, mutations seen in diseases like cystic fibrosis) or
  - as polymorphisms-a genetic variation that occurs in 1% or more of humans.
- Many of the known polymorphisms involve cytochrome P-450 (CYP450) isoenzymes.

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# Examples

Drug	Genetic Variation	Result
Codeine	Defective <i>CYP2D6</i> gene; conversion to morphine cannot occur	Decreased analgesia
Phenytoin	Defective <i>CYP2C9</i> gene, resulting in overdosage	Ataxia Confusion
Warfarin	Defective <i>CYP2C9</i> gene; decreased warfarin clearance	Bleeding

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- Ethnicity has a role in determining how well a patient metabolizes drugs
- Categorized as: Poor/ intermediate/ extensive and ultrarapid metabolisers.
- The incidence of toxicity or decreased efficacy depends on how the specific variant of the gene affects an enzyme, causing
  - poor metabolism (resulting in toxicity) or
  - extensive metabolism (resulting in decreased efficacy) of the drug.

# Isoniazid

- **Slow acetylator phenotype** leads to accumulation of the drug and enhances toxicity manifested as **peripheral neuropathy**
- **Fast acetylators** tend to metabolise the drug rapidly leading to low therapeutic levels and **hepatotoxicity**.

# Species Differences

- Inter- and intraspecies variation.
- Cats form sulfates (lack UDP-GT) but Pigs form glucuronides (lack of AST).
- Animal models for predicting metabolism.
- Humans have ONE CYP2D isoform (CYP2D6).
- Rats have SIX CYP2D isoforms.