Pharmacodynamics

Pharmacokinetis
Introduction to drug absorption,
disposition, elimination using
pharmacokinetics important pharmacokinetic parameters in defining
drug disposition and in therapeut
ics. Mention of uses of
pharmacokinetics in drug development process

Unit II
Parmacodynamics
Introduction elementary treatment
of enzyme stimulation, enzyme
inhibition, sulphonamides, member
s active drugs, drug metabolism
xenobiotics, biotransformation signific
ance of drug medicinal chemistry.

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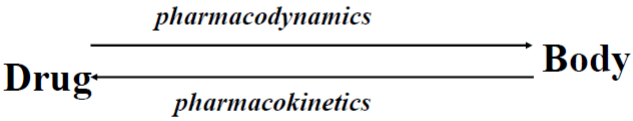
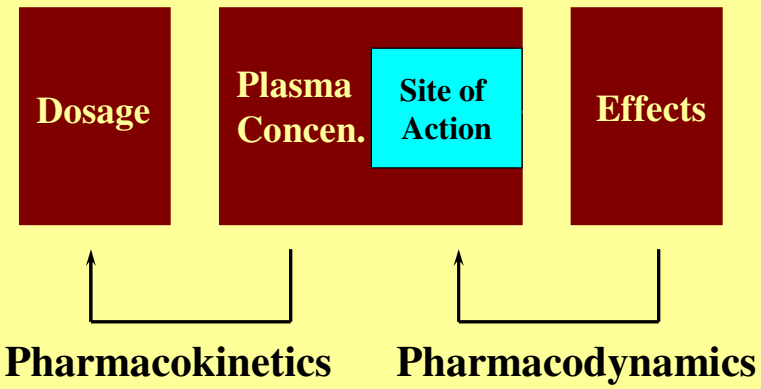
Pharmacology

what the drug does to the body
drug action/effect



what the body does to the drug
metabolism



Role of enzymes

Drugs may bind to the desired enzyme at the site of action and elicit biological response.

Enzymes and receptors most often show high specificities for their sub-strates and agonists, respectively.

Exceptions are some metabolic enzymes, e.g., the cytochromes that oxidize a large number of different drugs,

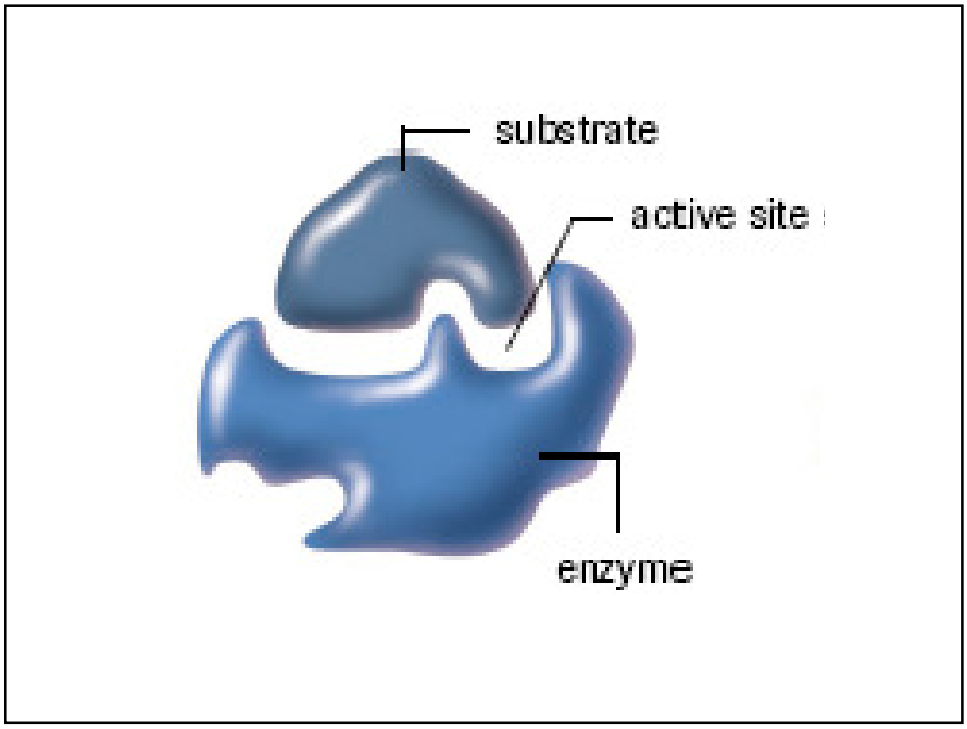
Importance

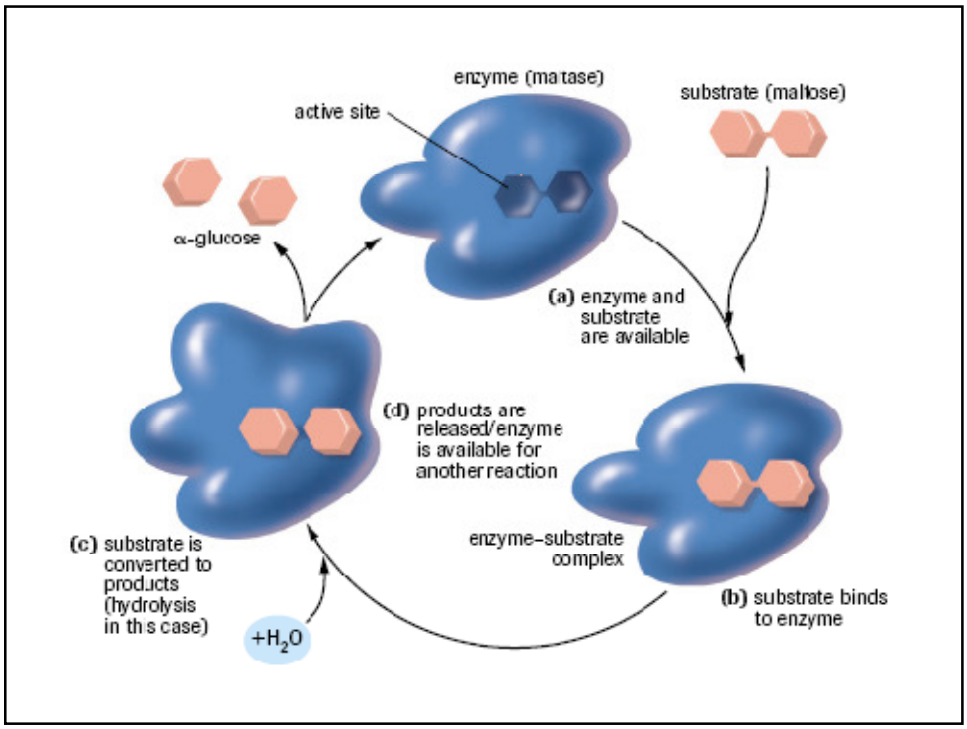
- Thus, enzymes play an important role in Metabolism, Diagnosis, and Therapeutics.
- All biochemical reactions are enzyme catalyzed in the living organism.
- Level of enzyme in blood are of diagnostic importance e.g. it is a good indicator in disease such as myocardial infarction.
- Enzyme can be used therapeutically such as digestive enzymes (when digestive organs fail).

Define enzymes
(Enzymes as Biological Catalysts)

- **Enzymes** are proteins that increase the rate of reaction by lowering the energy of activation
- Not altered or consumed during reaction.
- Reusable

- Enzymes are NOT reactants or products
- Enzymes are NOT used up in a reaction
- Enzymes may be used again over and over again (so long has they have not been denatured)
- Enzymes are specific to a particular substrate (or group of substrates)


substrate
active site
enzyme

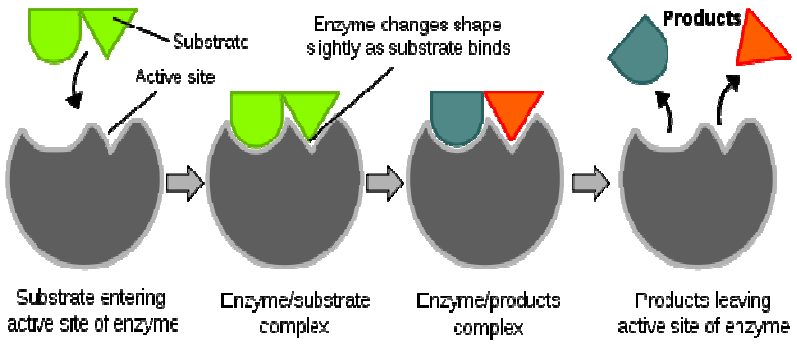

enzyme (maltase)
active site
substrate (maltose)
α-glucose
(a) enzyme and substrate are available
(d) products are released/enzyme is available for another reaction
enzyme–substrate complex
(b) substrate binds to enzyme
(c) substrate is converted to products (hydrolysis in this case)
+H2O

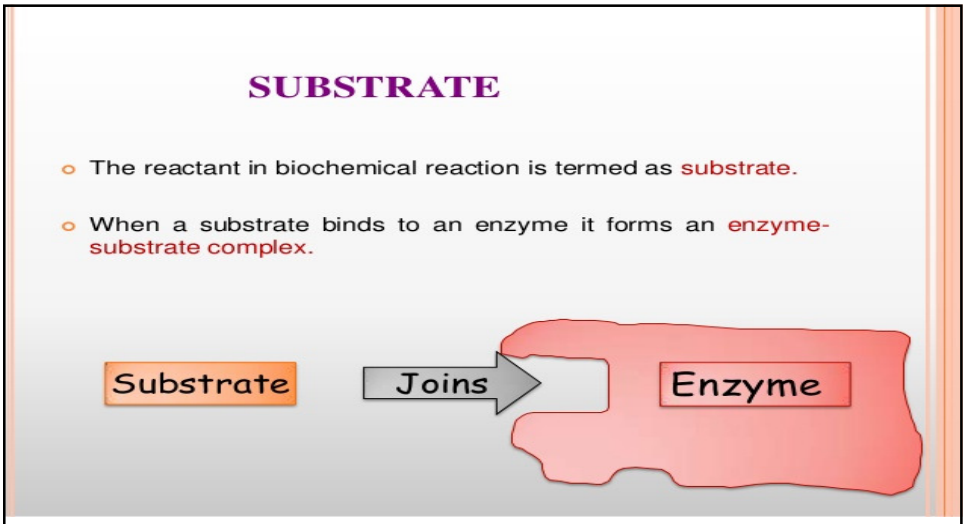
Important Terms to Understand Biochemical Nature And Activity of Enzymes

- **Active site:**
  The area on the enzyme where the substrate or substrates attach to is called the active site.
- **Enzymes are usually very large proteins and the active site is just a small region of the enzyme molecule.**

ACTIVE SITES

- Enzyme molecules contain a special pocket or cleft called the active sites.



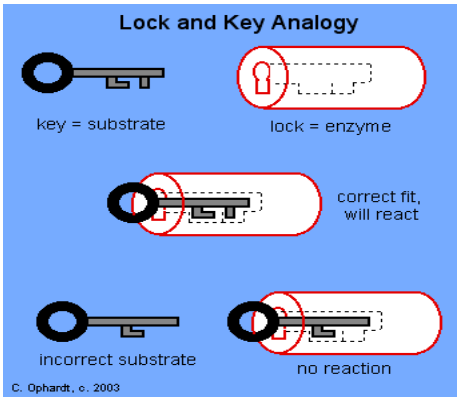


In enzymatic reactions, the substance at the beginning of the process, on which an enzyme begins it's action is called substrate.

Lock-and-Key Model

- **In the lock-and-key model of enzyme action:**
  - **the active site has a rigid shape**
  - **only substrates with the matching shape can fit**
  - **the substrate is a key that fits the lock of the active site**

This explains enzyme specificity
This explains the loss of activity when enzymes denature



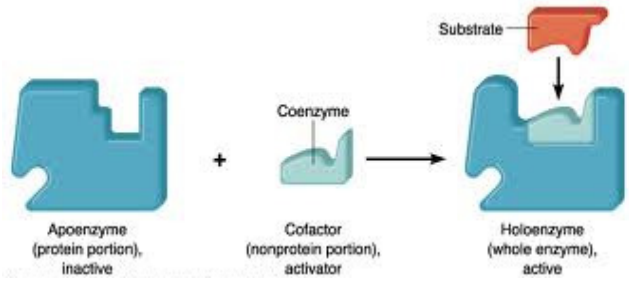
Important Terms to Understand Biochemical Nature And Activity of Enzymes

- **Cofactor:** a substance (other than the substrate) or metallic ion whose presence is essential for the activity of an enzyme.
  - **A cofactor is a non-protein chemical compound that is bound (either tightly or loosely) to an enzyme and is required for the functioning of an enzyme..**
  - **Types of Cofactors:**
    - **Coenzymes.**
    - **Prosthetic groups (Binds tightly).**

APOENZYME and HOLOENZYME

- The enzyme without its non protein moiety/metal ion is termed as apoenzyme and it is inactive and is ready to catalyze a reaction.

- Holoenzyme is an active enzyme with its non protein component.

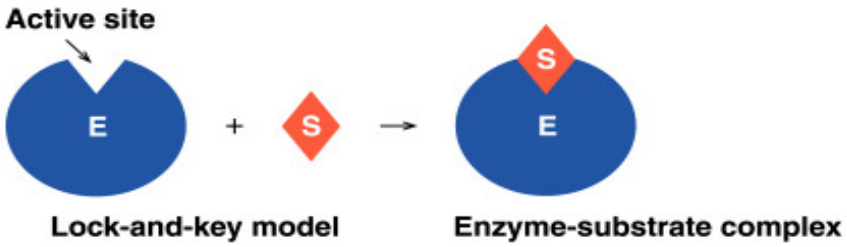


Mechanism of Action of Enzymes

- **Enzyme-Substrate Interactions:**
  - **Formation of Enzyme substrate complex by:**
  - Lock-and-Key Model
  - Induced Fit Model

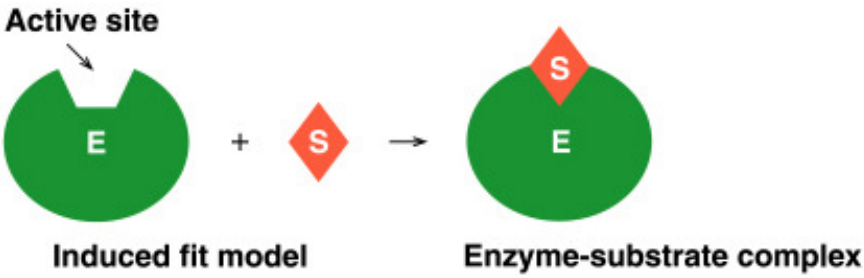
Lock-and-Key Model

- In the **lock-and-key model** of enzyme action:
  - the active site has a rigid shape
  - only substrates with the matching shape can fit
  - the substrate is a key that fits the lock of the active site
- This is an older model, however, and does not work for all enzymes



Induced Fit Model

- In the **induced-fit model** of enzyme action:
  - the active site is flexible, not rigid
  - the shapes of the enzyme, active site, and substrate adjust to maximumize the fit, which improves catalysis
  - there is a greater range of substrate specificity
- This model is more consistent with a wider range of enzymes



Enzyme Catalyzed Reactions

- When a substrate (S) fits properly in an active site, an **enzyme-substrate (ES) complex** is formed:

  $E + S \leftrightarrows ES$

- Within the active site of the ES complex, the reaction occurs to convert substrate to product (P):

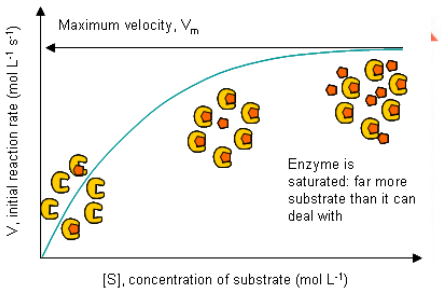
  $ES \rightarrow E + P$

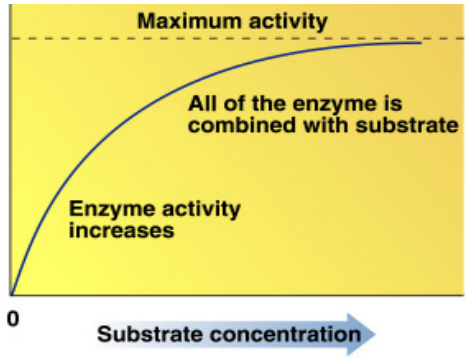
- The products are then released, allowing another substrate molecule to bind the enzyme
  - this cycle can be repeated millions (or even more) times per minute
- The overall reaction for the conversion of substrate to product can be written as follows:

  $E + S \leftrightarrows ES \rightarrow E + P$

Substrate Concentration and Reaction Rate

- **The rate of reaction increases as substrate concentration increases (at constant enzyme concentration)**
- **Maximum activity occurs when the enzyme is saturated (when all enzymes are binding substrate)**





ENZYME INHBITION

- Cells must control enzyme activity to coordinate cellular activities
- This can be done by:

1) Restricting the production of a particular enzyme (enzymes are proteins, so your body can control how much you make of them) called inhibitors

2) Inhibiting the action of an enzyme

This may involve

a) reversible competitive inhibition

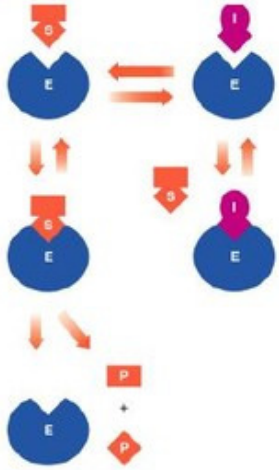
b) noncompetitive inhibition

c) feedback inhibition

Reversible Competitive Inhibition

A **competitive inhibitor**:

- Has a structure like the substrate.
- Competes with the substrate for the active site.
- Has its effect reversed by increasing substrate concentration.



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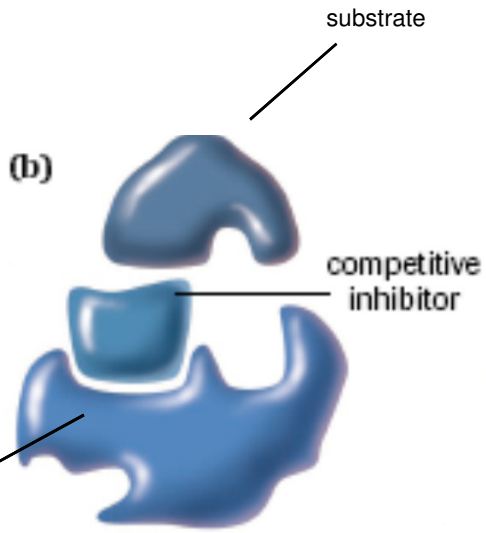
Competitive Inhibitor

Now the substrate cannot get to the active site because it is blocked by the inhibitor.



Competitive Inhibitors

- These are molecules that are similar in shape to the substrate
- They bind to the enzyme's active site preventing the real substrate from binding
- The molecule "competes" with the substrate for the active site
- This can be overcome by increasing the substrate concentration

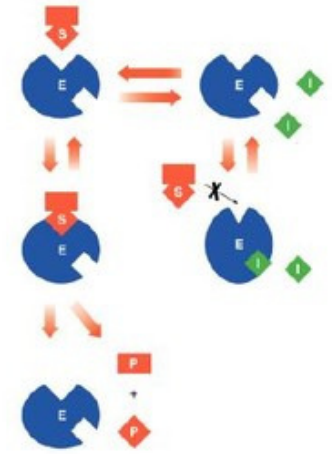
Ex: PRONTOSIL (competitive inhibitor)

- PRONTOSIL – a sulfonamide is an antibacterial drug
- Bacteria require folic acid for replication of genetic material
- Prontosil binds to the enzyme that makes folic acid preventing other substrates from binding
- As a result, folic acid is not longer made and the bacterial cell dies
- Since animal cells don't make folic acid themselves, they do not have this enzyme and so Prontosil has no effect on them

Noncompetitive Inhibition

A **noncompetitive inhibitor**:

- Has a structure different than the substrate.
- Distorts the shape of the enzyme, which alters the shape of the active site.
- Prevents the binding of the substrate.
- Cannot have its effect reversed by adding more substrate.



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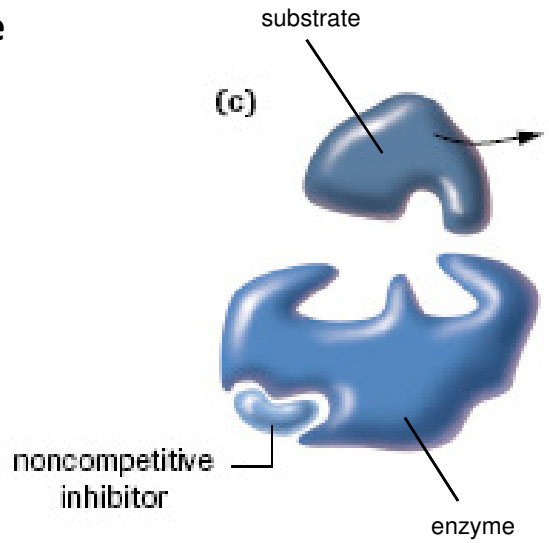
Non competitive inhibitor

The shape of the active site has changed

Therefore, the substrate no longer fits in



b) Noncompetitive Inhibition

- A molecule binds to the enzyme at a location other than the active site
- This binding alters the shape of the enzyme, changing the shape of the active site
- The enzyme is now dysfunctional because the substrate now cannot bind to the active site.
- Adding more substrate will not affect the reaction because the active site is unavailable.

Ex: Cytochrome C Oxidase

(noncompetitive inhibition)

<u>Regular Function</u>

- Speeds up the reduction of oxygen to water in cellular respiration
- Without it, the reaction will not occur fast enough and the organism will die because not enough energy is released.

Ex: Cytochrome C Oxidase

(noncompetitive inhibition)

Inhibition by cyanides (or CO)

- CN^- attach to the –SH groups in the enzyme
- This destroys the disulfide bridges and thus changing the tertiary structure of the enzyme
- Change in the shape results in the change in the active site thus the substrate cannot bind and cytochrome c oxidase is nonfuctional.

Role of enzymes in metabolism

Drug metabolism and xenobiotics

- Adsorption- drug gets into bloodstream
- Distribution - gets to site of action
- Metabolism - is "changed" so that it can be excreted
- Elimination - leaves the body

Metabolism

- Metabolism is responsible for the in vivo formation of biologically active molecules.
- The metabolism of drugs and other xenobiotics into more hydrophilic metabolites **(Biotansformation)** is essential for the elimination of these compounds from the body and termination of their biological activity
- Pharmacological effects for the prodrugs
- Toxic effects:

Xenobiotics: is a foreign chemical substance found within an organism that is not naturally produced by or expected to be present within.

It can also cover substances that are present in much higher concentrations than are usual.

Sites of drug biotransformation.

The Liver

- Is the most important organ in metabolism and detoxification of endo- and exogenous compounds.
- It is well perfused.
- Very rich in metabolising enzymes (most of them!)
- Orally administered drugs are usually susceptible to the ***first pass metabolism***.
- May be significant and result in reduced oral bioavailability.

First-Pass Metabolism

- Following nonparenteral administration of a drug, a significant portion of the dose may be metabolically inactivated in either the intestinal endothelium or the liver before it reaches the systemic circulation
- Limits oral availability of highly metabolized drugs

Sites of drug biotransformation.

The Intestines

- Important site for extra hepatic metabolism of orally administered drugs.
- Contain CYP 3A4 isozyme → drug metabolism.
- Also contains p-glycoprotein → Drug extrusion to GIT
- Esterases are important for metabolism of ester prodrugs.
- Bacteria micro flora also produce azo and nitro reductases for activation of prodrugs. E.g. sulfasalazine
- Intestinal β-*glucuronidase* enzyme is also responsible for hydrolysis of glucuronide conjugates that are circulated in bile e.g. digoxin.

Sites of drug biotransformation. *Other Organs*

- Kidneys
- Lungs
- Adrenal Glands
- Brains
- Placenta
- Brain
- Skin

Application of Biotransformation

- Generates more polar (water soluble), inactive metabolites that are readily excreted from body
- Metabolites may still have potent biological activity (or may have toxic properties)
- Generally applicable to metabolism of all xenobiotics as well as endogenous compounds such as steroids, vitamins and fatty acids

Renal Excretion

- Renal excretion of unchanged drug plays small role in elimination of drug
- In the kidney, lipophilic compounds are largely reabsorbed back into systemic circulation during passage through renal tubules
- Needs to be water soluble (hydrophilic)

What causes Biotransformation?

- Predominantly it is Enzyme-based, called drug metabolizing enzymes.
- They are called (CYPs) mixed-function oxidase or monooxygenase and contain many enzymes: including cytochrome P450, cytochrome b5, NADPH-cytochrome P450 etc.
- CYPs are a superfamily of haemoprotein enzymes.

How many different CYPs are there?

There are over a thousand different CYPs in living system, although the number in man is only about fifty (49 genes and 15 pseudogenes have been sequenced). It is likely that most of the human CYPs have already been discovered.

Among the diverse human genes, several have been identified as particularly important in
oxidative metabolism. They are:
CYP3A4 (by far the most important)
CYP2D6
CYP2C9
CYP2C19

CYP3A4 is the most prevalent CYP in the body, and metabolises many substrates.

Drug metabolizing enzymes; CYP

'CYP' is a host of enzymes that use iron to oxidise things, often as part of the body's strategy to dispose of potentially harmful substances by making them more water-soluble.

Adding something like a hydroxyl group to a xenobiotic is just part of the body's strategy to get rid of the 'drug' - this is often followed by conjugugation to groups such as glucuronide to increase the solubility even further.

The initial P450-mediated oxidation is often referred to as "*Phase I metabolism" and the subsequent* conjugation (which has nothing to do with P450) as "Phase II".

Within cells Cytochrome P450 are located in

1. **Endoplamic reticulum**: involved in protein processing and transport.

- ✓ Usually metabolizes external substances such as drug and environmental pollutants.
- ✓ The phase I oxidative enzymes are almost exclusively localized here.

2. **Cytosol**: Intracellular fluid present inside cells.

- ✓ Involved in the synthesis and metabolism of internal substances.
- ✓ Phase II enzymes are located predominantly in the cytosol.

Drug interaction with CYPs

Many drugs may increase or decrease the activity of various CYP isozymes either by **inducing the biosynthesis of an isozyme** (enzyme induction) or **by directly inhibiting the activity of the CYP** (enzyme inhibition).

This is a major source of adverse drug interactions, since changes in CYP enzyme activity may affect the metabolism and clearance of various drugs.

For example, if one drug inhibits the CYP-mediated metabolism of another drug, the second drug may accumulate within the body to toxic levels. Hence, these drug interactions may necessitate dosage adjustments or choosing drugs that do not interact with the CYP system.

Cytochrome P-450 enzyme induction and inhibition

The cytochrome P-450 enzyme system has several curious properties.

It will metabolise a chemically and pharmacologically diverse range of substrates (both endogenous and exogenous) including steroids, barbiturates, aromatic hydrocarbons, pesticides, fungal toxins, and carbon tetrachloride.

The phenomenon of increased drug metabolizing ability of the enzymes by several drugs and chemicals is called as enzyme induction.

The phenomenon of decreased drug metabolising ability of the enzymes by several drugs and chemicals is called as enzyme inhibition.

Types of metabolism

One of the most widely studied inducible enzyme systems in mammals is the hepatic enzyme system responsible for the detoxication of a large number of foreign chemicals (drugs)-that is, substances which do not occur naturally in the body.

The liver provides a major route for the metabolism of many of these compounds.

Two types of reaction may take place:

phase 1 detoxication or functionalization reactions, a degradative reaction;

phase 2 detoxication, a synthetic conjugation reaction.

Phase I Metabolism

Includes oxidation, reduction, hydrolysis, and hydration and isomerization (plus rarer misc.)

- Many drugs undergo a number of these reactions
- Main function of Phase I metabolism is to prepare the compound for phase II metabolism

Phase I

- Converts the parent drug to a more polar metabolite by introducing or unmasking a functional group (-OH, -NH2, -SH).
- Usually results in loss of pharmacological activity
- Sometimes may be equally or more active than parent

Phase I/ Functionalization Reactions

- Include oxidative, reductive and hydrolytic biotransformation rxns.
- Introduce polar groups into the xenobiotic to produce a more water soluble molecule.
  - COOH
  - OH
  - NH2
  - SH
- The products may not be sufficiently hydrophilic but are suitable precursors for phase II/ Conjugation.

Oxidation.

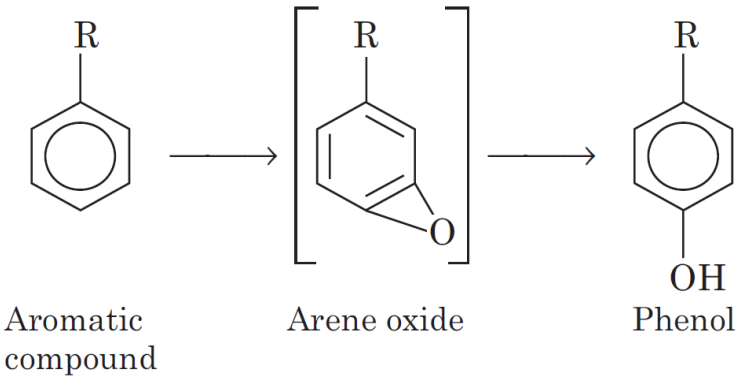
- Are by far, the most common and important in drug metabolism.
- Normally the first step of drug metabolism
- Mainly carried out by mixed function oxidases.

Oxidation of Aromatic Moieties

- Aromatic hydroxylation refers to mixed function oxidation of aromatic cpds (*arenes*).
- They corresponding end products being *arenols.*
- Proceed initially through an epoxide intermediate known as an “arene oxide”
- This undergoes spontaneous rearrangement to form an arenol in most cases.

Oxidation of Aromatic Moieties

- Arene oxides are important in metabolic, toxicological and formation of arenols.



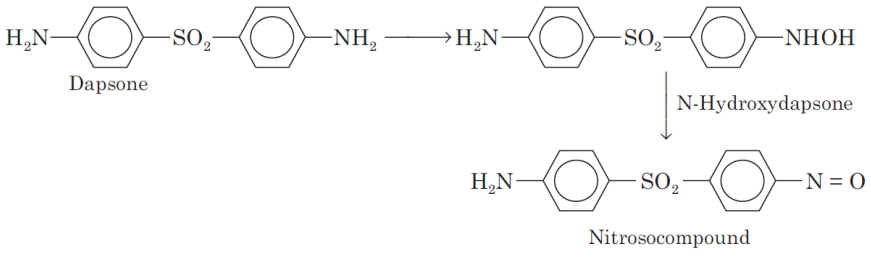
Oxidation of carbon-heteroatom systems.

- Carbon-heteroatom systems (N, O, S) are commonly present in many drugs.
- They are metabolized by any of the following oxidation processes :

1. **Oxidation or hydroxylation of heteroatom**: Ex: N-oxidation, N-hydroxylation, S – oxidation.
2. Hydroxylation of carbon atom attached to the heteroatom followed by **cleavage of carbon-heteroatom bond**. Ex: N-dealkylation, S- dealkylation, O-dealkylation.

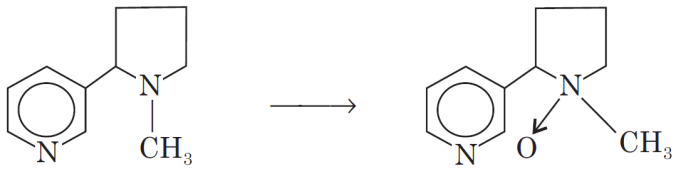
N-Hydroxylation

- Drugs containing non-basic nitrogen atom (amides), non-basic aromatic amines and basic amines are metabolized by N-hydroxylation.



N-Oxidation

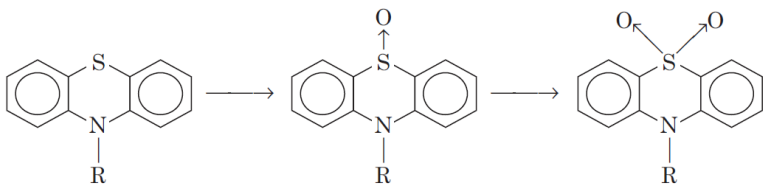
- Compounds possessing of basic nitrogen are metabolized by N-oxidation process



Nicotine Nicotine-1′-N-oxide

S-Oxidation

- Compounds possessing of carbon-sulfur bonds are metabolized to sulfoxides by S-oxidation.
- The sulfoxides may be excreted as urinary metabolites or oxidized to sulfones (—SO2—).

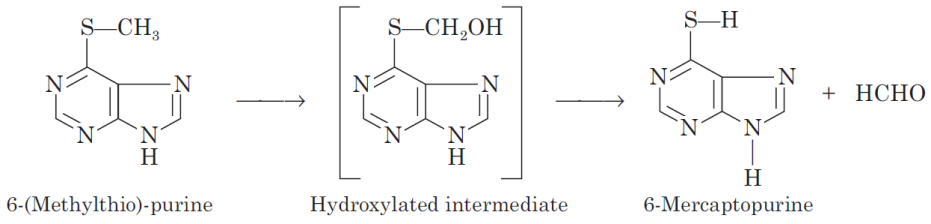


Phenothiazine Phenothiazine sulfoxide Phenothiazine sulfone

DEALKYLATIONS

S-Dealkylation

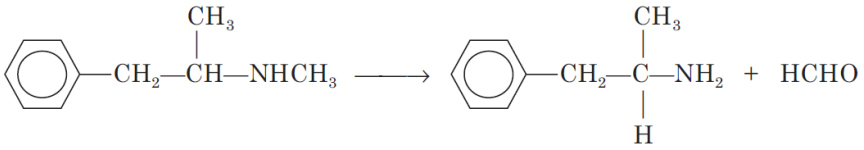
- S-Dealkylation involves oxidative cleavage of alkyl carbon-sulfur bonds.



6-(Methylthio)-purine — Hydroxylated intermediate — 6-Mercaptopurine

N-Dealkylation
(1^O / 2^O Amines)

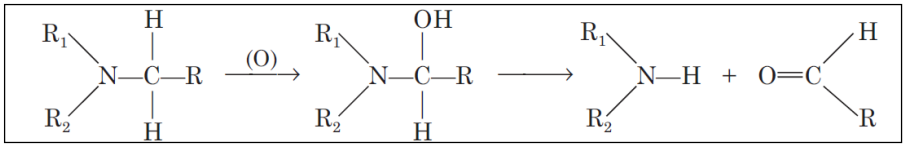
In the case of primary or secondary amines, dealkylation of an alkyl group **starts at the carbon adjacent** to the nitrogen;



Mephenteramine Amphetamine

N-Dealkylation.
(3^O Amines)

- In the case of tertiary amines, with **hydroxylation** of the nitrogen.



- The intermediate products are labile and break up into the dealkylated amine and aldehyde.

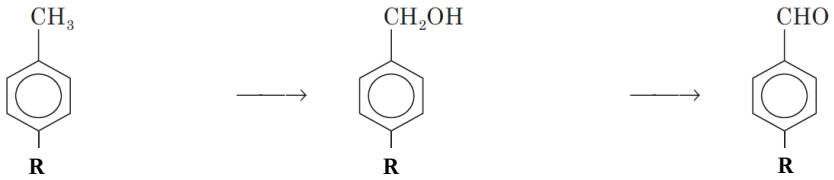
O-Dealkylation

- O-Dealkylation of drugs possessing C—O bond involves hydroxylation of α-carbon to form an unstable hemiacetal or hemiketal intermediates.
- These intermediates spontaneously cleave to form alcohol and carbonyl compound.

Phenacetin Paracetamol

Oxidation of benzylic carbons

- The carbons directly attached to aromatic rings are oxidized to aldehydes and carboxylic acids via alcohols.



Reductive Reactions

- Drugs containing carbonyl, nitro, and azo groups are metabolized by reduction to alcohols and amines respectively.
- The reduced compounds are conjugated and eliminated from the body.

Read about the reduction of chloral hydrate

Phase II (conjugation reactions)

- Subsequent reaction in which a covalent linkage is formed between a functional group on the parent compound or Phase I metabolite and an endogenous substrate such as glucuronic acid, sulfate, acetate, or an amino acid
- Highly polar – rapidly excreted in urine and feces
- Usually inactive - notable exception is morphine 6-glucuronide

Phase II Metabolism

- Phase II is usually the true detoxification of drugs
- Occurs mostly in cytosol
- Gives products that are generally water soluble and easily excreted.
- Includes sugar conjugation, sulfation, methylation, acetylation, amino acid conjugation, glutathione conjugation

Phase II/ Conjugation Reactions
Overview

- Attach ***small***, ***polar*** and **ionisable**, **endogenous**, functionalities to
  - handles of phase metabolites.
  - Parent compounds with existing suitable functional groups.

- Functionalities include:
  - Glucuronic acid
  - Sulphate
  - Glycine
  - Amino Acids

Glucuronidation

- Glucuronidation involves conjugation of metabolite or drug molecule with glucuronic acid. Sugars, glucose, xylose or ribose may be conjugated
- In these reactions glucuronic acid molecule is transferred to the substrate from a cofactor.
- Glucuronides are generally inactive and are rapidly excreted into the urine and bile.
- Molecules that undergo glu. Rxns are associated with:
  - phenolic hydroxyl,
  - Alcoholic hydroxyl, and
  - carboxylic acid groups.

Sulfation

- Sulfate conjugation involves transfer of a sulphate molecule from a cofactor (to the substrate (metabolite or drug moiety) by the enzymes (sulfotransferases).
- Substrate molecules include:
  - Alcoholic hydroxyl,
  - phenolic hydroxyl and
  - aromatic amine groups.

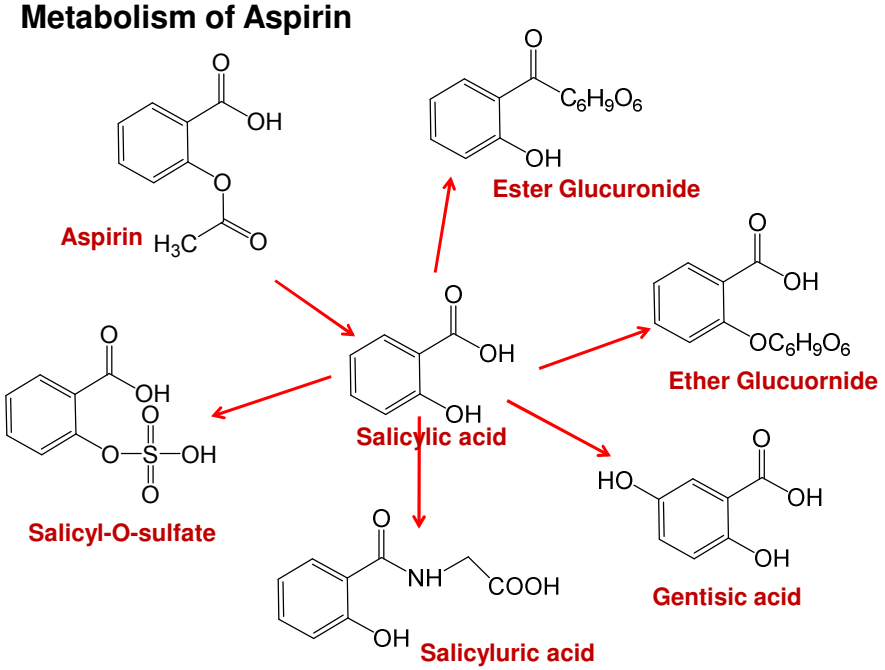
Hydrolysis.

- Hydrolysis is also observed for a wide variety of drugs.
- The enzymes involved in hydrolysis are **esterases**, **amidases**, and **proteases**.
- These reactions generate hydroxyl or amine groups, which are suitable for phase II conjugation.

Glutathione Conjugation

- Glutathione is a protective compound (tripeptide, Gly-Cys-Glu) within the body for removal of potentially toxic electrophilic compounds
- Many drugs are, or are metabolized in phase I to, strong electrophiles
- React with glutathione to form non- toxic conjugates
- Glutathione conjugates may be excreted directly in urine or bile, but are usually metabolized further.

Metabolism of Aspirin



Metabolism of Diazepam

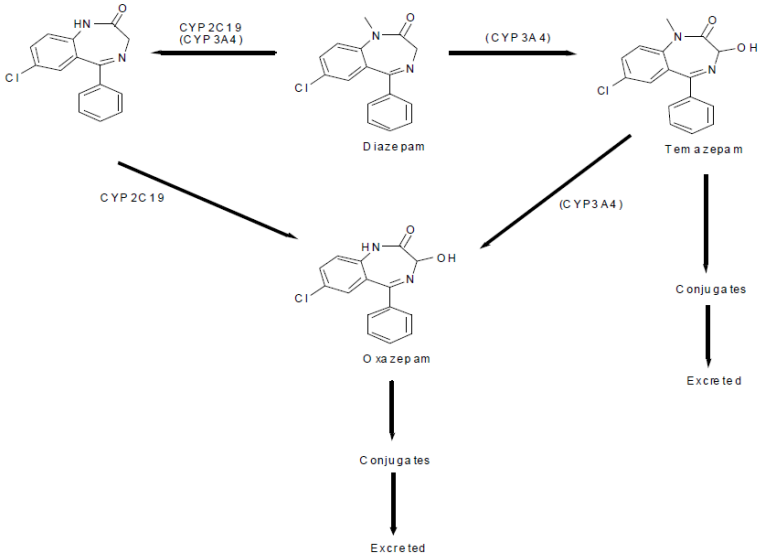
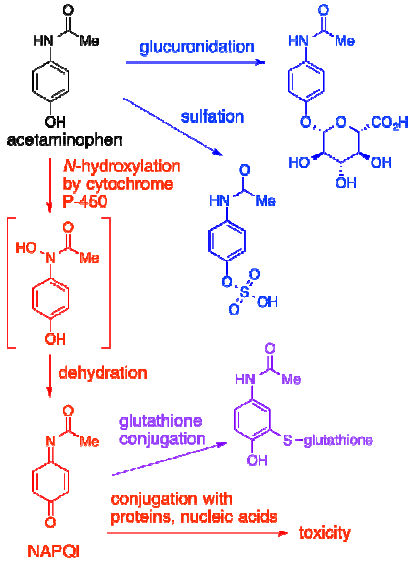


Figure 5. Diazepam metabolism pathway by CYP450.

Metabolism of Acetaminophen



. Pathways shown in blue and purple lead to non-toxic metabolites; the pathway in red leads to NAPQI, which is toxic if not conjugated to glutathione.

Biotransformation and medicinal chemistry

II-Improving metabolism:

I-Making drugs more resistant to chemical and enzymatic degradation

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- There are various strategies that can be used to make drug more resistant to hydrolysis and drug metabolism and thus prolonged their activity (more duration of action) such as:
- 1)- Steric shields
- Some functional groups are more susceptible to chemical and enzymatic degradation than other.
- For example: esters and amides are prone to hydrolysis. A common strategy that is used to protect these groups is to add steric shields.

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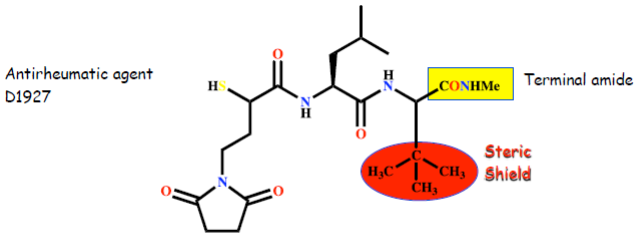
Metabolic Drug Stability by Steric Shields:

- Used to increase chemical and metabolic stability
- Introduce bulky group as a shield
- Protects a susceptible functional group (e.g. ester) from hydrolysis
- Hinders attack by nucleophiles or enzymes



Blocks hydrolysis of terminal amide

'**Electronic shielding**' **of NH_2**

- Used to stabilize labile functional groups (e.g. esters)
- Replace labile ester with more stable urethane or amide
- Nitrogen feeds electrons into carbonyl group and makes it less reactive
- Increases chemical and metabolic stability

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