Biological Chemistry Department

Biological Chemistry

Pharmaceutical Biochemistry

Biotransformation of Xenobiotics Including Medicinal Preparations

Speciality: Pharmacy for foreign students (Language of instructions – English)

Lecturer: ass. prof. Kravchenko G.B.

Lecture Plan

1. General Features of Adaptability.
2. Biochemical Transformation of Drugs.
4. Biotransformation Reactions: phase I.
5. Biotransformation Reactions: phase II.
6. The Role of Metabolic Activation.

Information Resources

1. Biological Chemistry: Textbook / A.L. Zagayko, L.M. Voronina, G.B. Kravchenko, K.V. Strel`chenko. – Kharkiv: NUPh; Original, 2011. – 183-194 p.

2. Training Journal for Licensed Exam "KROK-1": Study Material in Biological Chemistry. – Kharkiv: NUPh, 2017. – 173-180 p.

4. Ethanol Metabolism: The Medical Biochemistry Page. Available on: **https://themedicalbiochemistrypage.org/ethanol-metabolism.php.**

5. Introduction to Cytochrome P450 (CYP) Enzymes: The Medical Biochemistry Page. Available on: **https://themedicalbiochemistrypage.org/cytochromep450.php.**

6. Nuclear Receptors in Metabolism: The Medical Biochemistry Page. Available on: **https://themedicalbiochemistrypage.org/nuclear.php.**

One of the most remarkable features of living organisms is their adaptability. Throughout its immense incomprehensibly rich history, life on earth has constantly been challenged by an ever changing and frequently hostile environment.
An even more interesting adaptive feature of living process is the capacity to convert noxious foreign molecules to less dangerous products.

In the modern world, living organisms have been exposed to new threats. Since the beginning of the Industrial Revolution, hundreds of thousands of new compounds produced in enormous number of industrial processes have been distributed throughout most ecosystems.

It is now estimated that toxic chemicals and other environmental threats now cause the extinction of between several dozen to hundreds of species every year.

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"Everyone has Problems - Chemists have Solutions"

(Albert Einstein 1941)

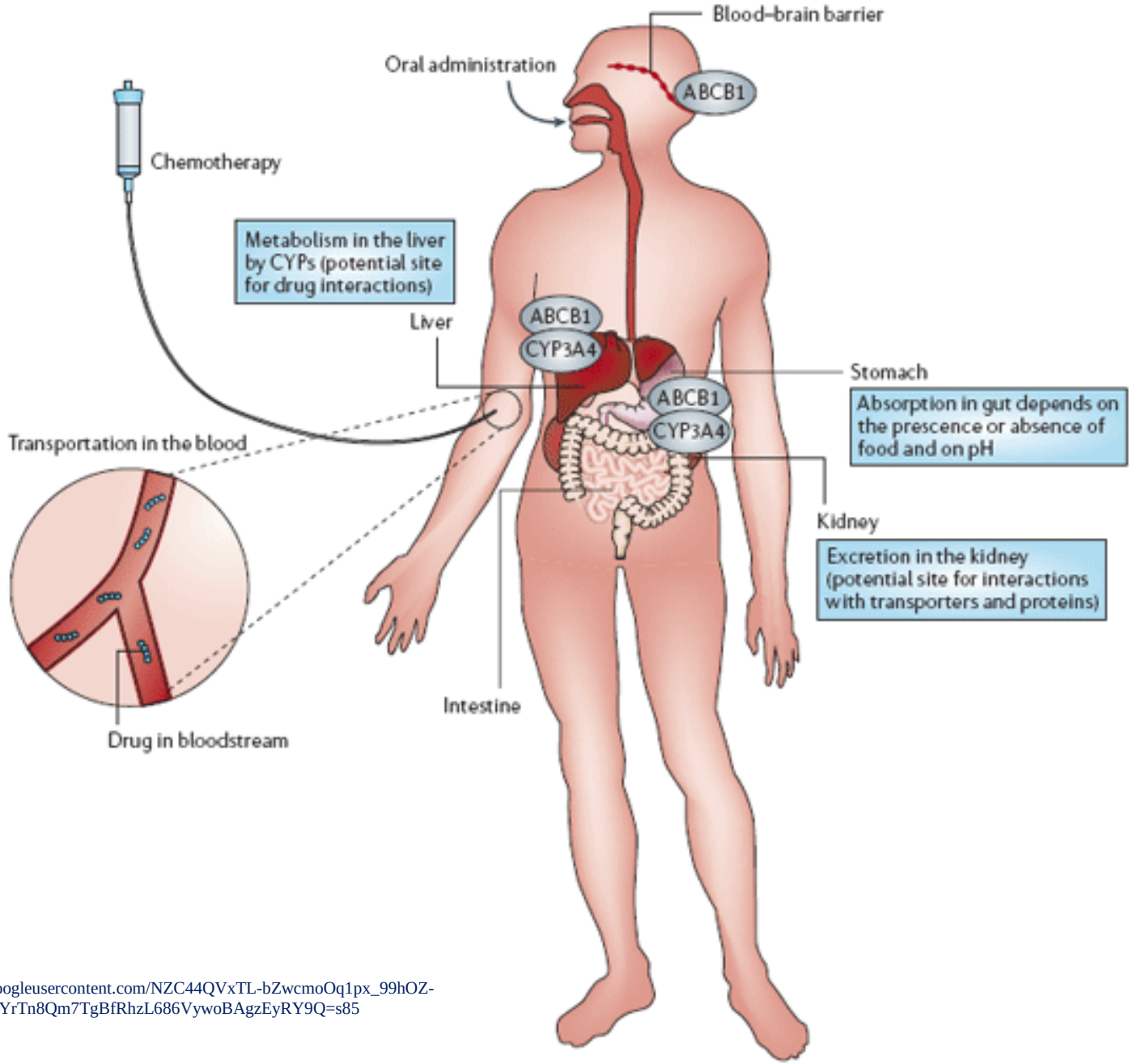
Xenobiotics are chemicals found in organisms, but not expected to be produced or present in them; or they are chemicals found in much higher concentrations than usual.

The term detoxication is used to describe the process by which the potentially toxic molecule is converted to a more soluble (and usually less toxic) product.

The more familiar term detoxification implies the correction of a state of toxicity, that is, the chemical reactions that produce sobriety in an inebriated person.

There are several possible consequences of the biochemical transformation of drugs:

1) inactivation, during which an active drug is converted to inactive metabolite(s);
2) activation, during which an inactive drug (or pro-drug) is converted to a pharmacologically active primary metabolite;
3) modification of activity after the conversion of an active drug to a metabolite that also has pharmacologic activity;
4) lethal synthesis (or intoxication), in which a drug is incorporated into a normal cellular metabolic pathway that ultimately leads to failure of the reaction sequence because of the presence of spurious substrate (cell death then occurs).



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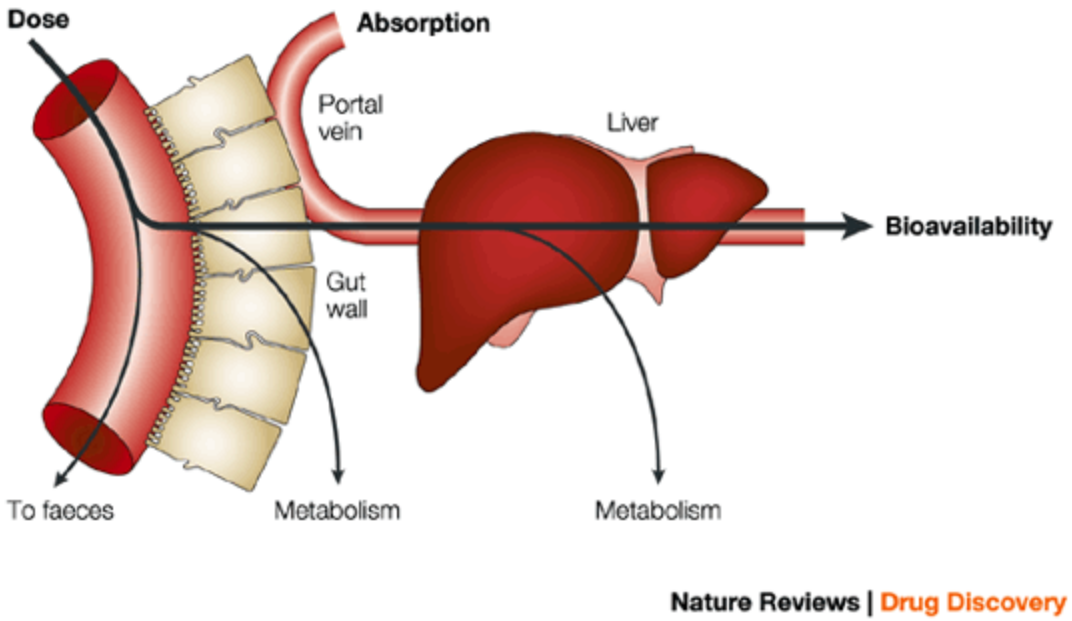
The body will try to eliminate xenobiotics, including drugs. For many drugs, this first requires metabolism or biotransformation, which takes place partly in the gut wall during uptake, but primarily in the liver.



The figure shows where metabolism occurs during the absorption process. The fraction of the initial dose appearing in the portal vein is the fraction absorbed, and the fraction reaching the blood circulation after the first-pass through the liver defines the bioavailability of the drug.

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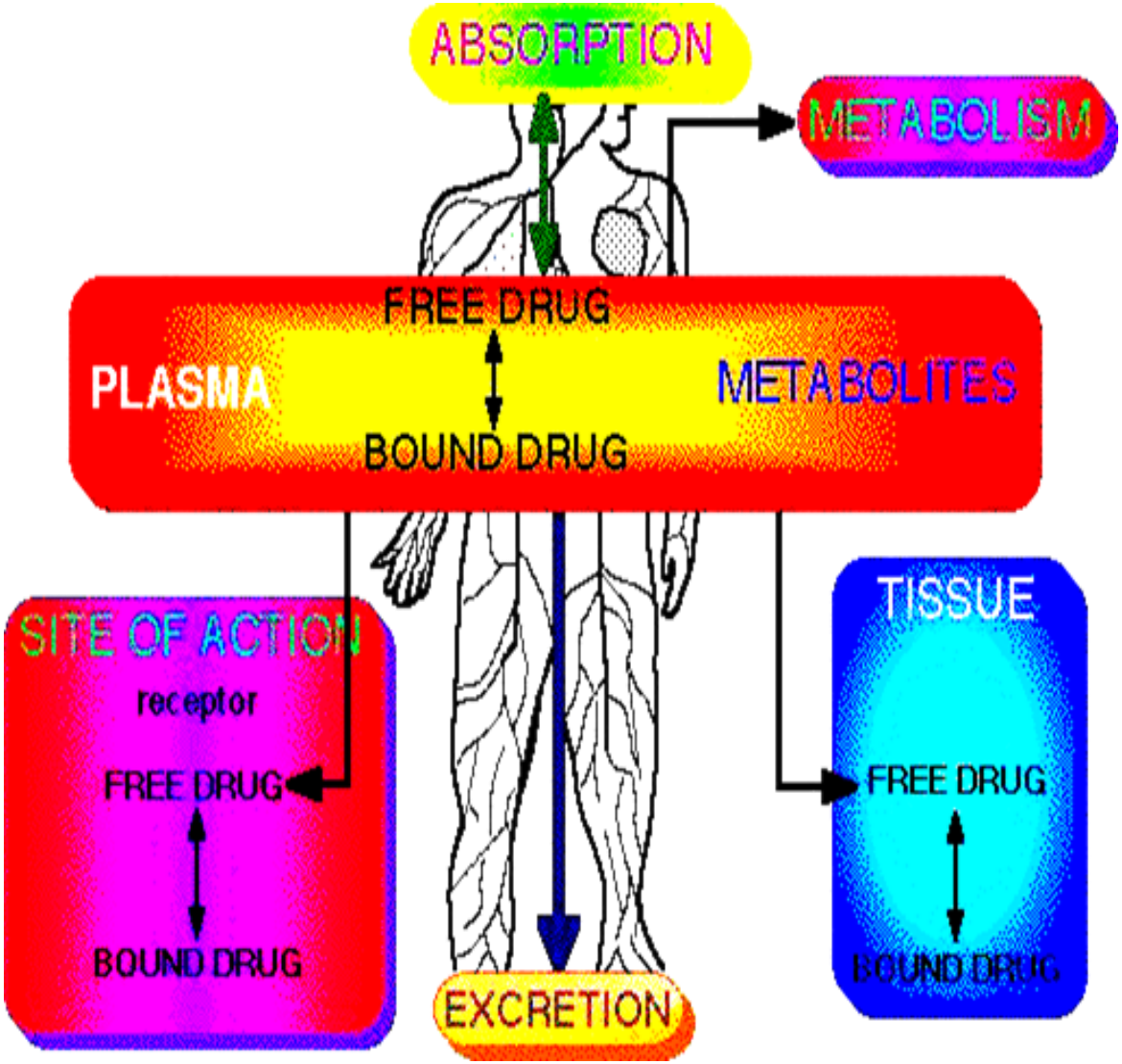
Several aspects of the biotransformation of drugs have direct clinical significance. These include microsomal enzyme induction and inhibition, nutritional state, age, disease conditions, and species differences.



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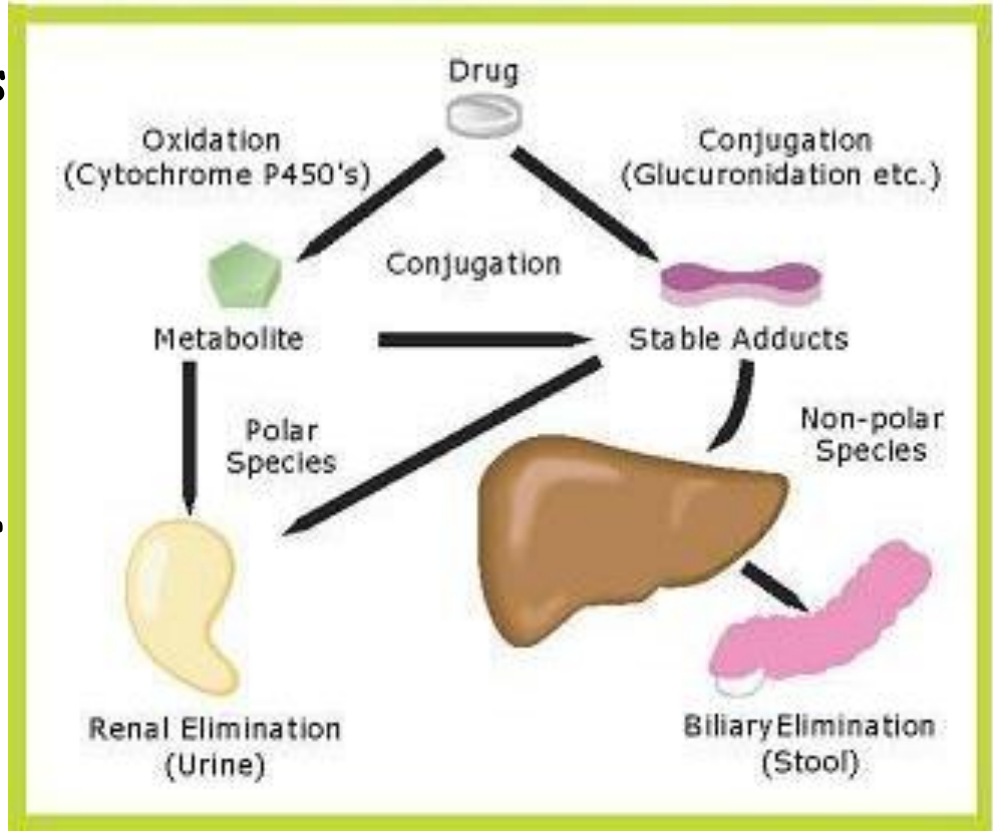
Drugs and foreign chemicals that are lipid soluble are converted by enzymatic processes to compounds of ever-increasing water-solubility until they can be excreted via one or several of the routes available.

Metabolism or biotransformation and the subsequent excretion of drugs is known as "elimination."

Metabolism generally occurs in 2 phases: **Phase I** induces a chemical change (most frequently oxidation, but also reduction) that renders the drug more conducive to phase II. **Phase II** is a conjugative or synthetic addition of a large, polar molecule that renders the drug water soluble and amenable to renal excretion.



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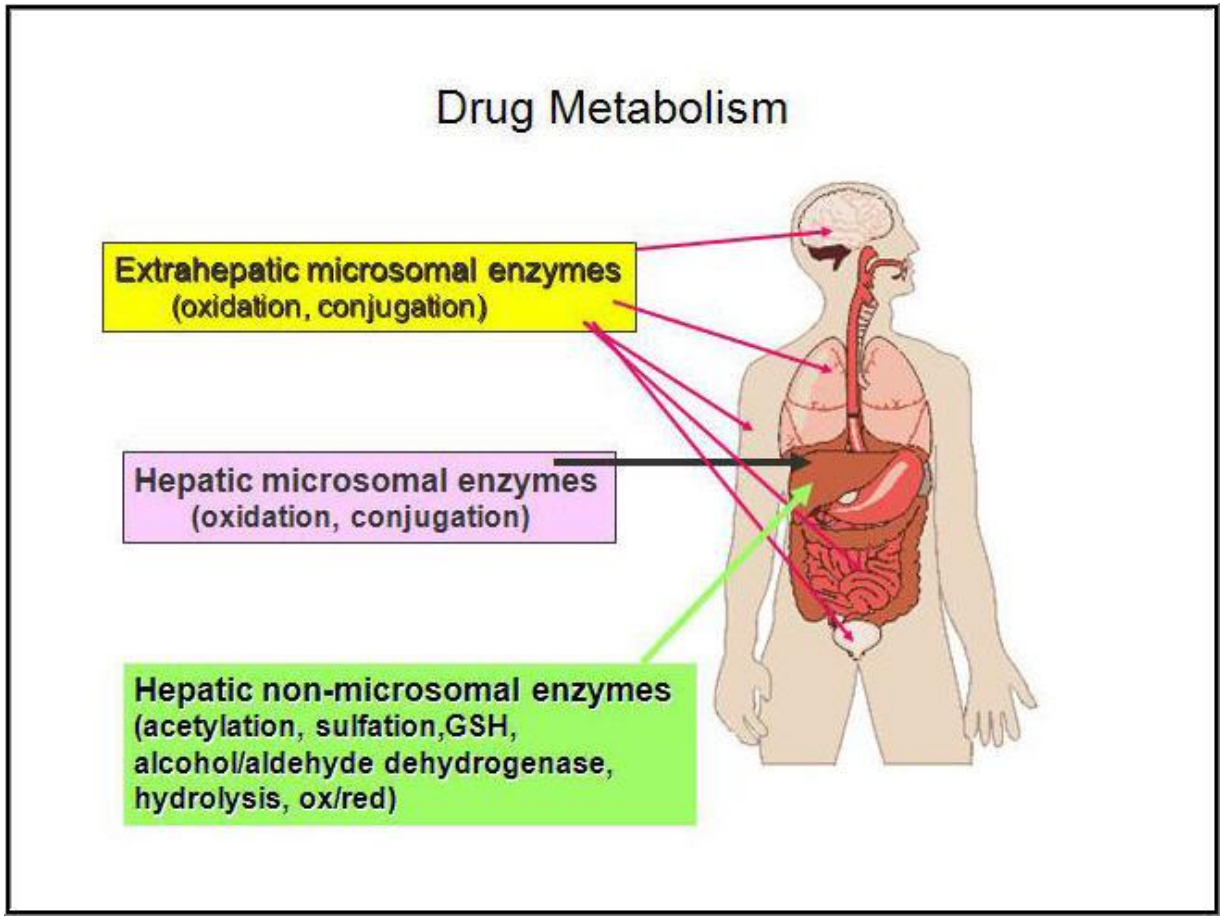
Biotransformation reactions

Biotransformation reactions occur in several locations within the cell (e.g., the cytoplasm and mitochondria), most occur within the endoplasmic reticulum.

Cell types also differ in biotransforming potential. Cells that are located near the major points of xenobiotic entry into the body – liver, lung, and intestine – possess greater concentrations of biotransforming enzymes than others.



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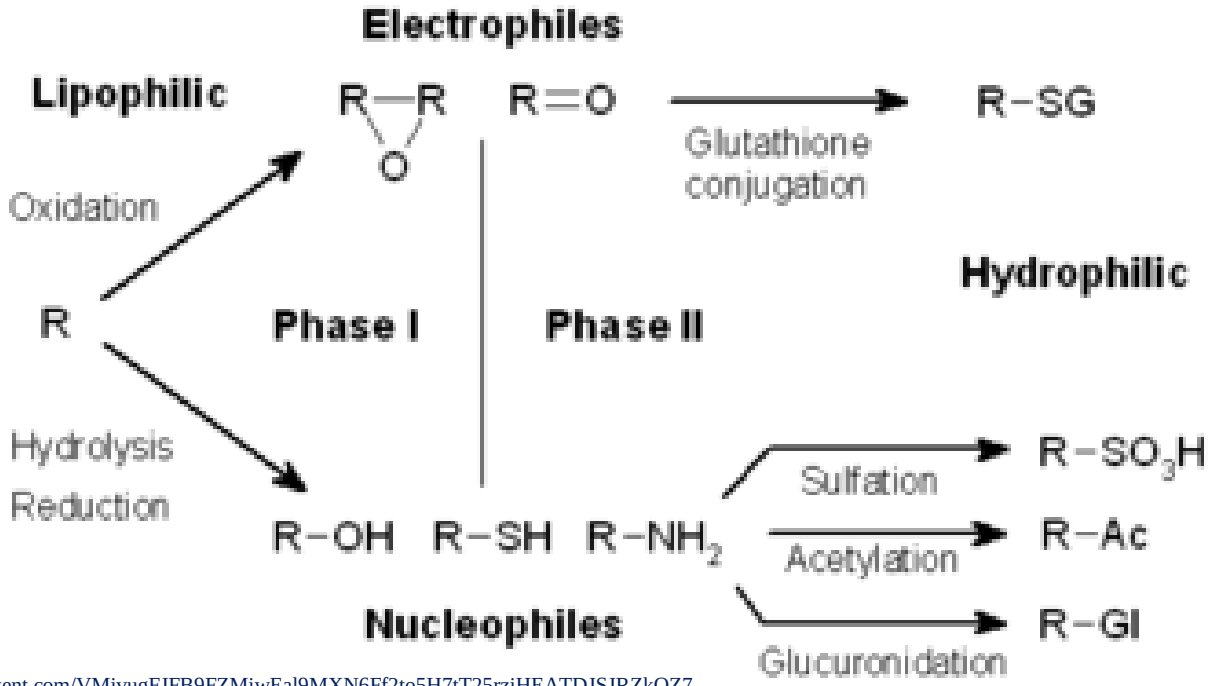
Biotransformation processes have been differentiated into two major types.

During **phase I**, various reactions involving oxidoreductases and hydrolases convert hydrophobic substances into more polar molecules.

Phase II consists of reactions in which metabolites containing appropriate functional groups are conjugated with substances such as gluconate, glutamate, sulfate, or glutathione.



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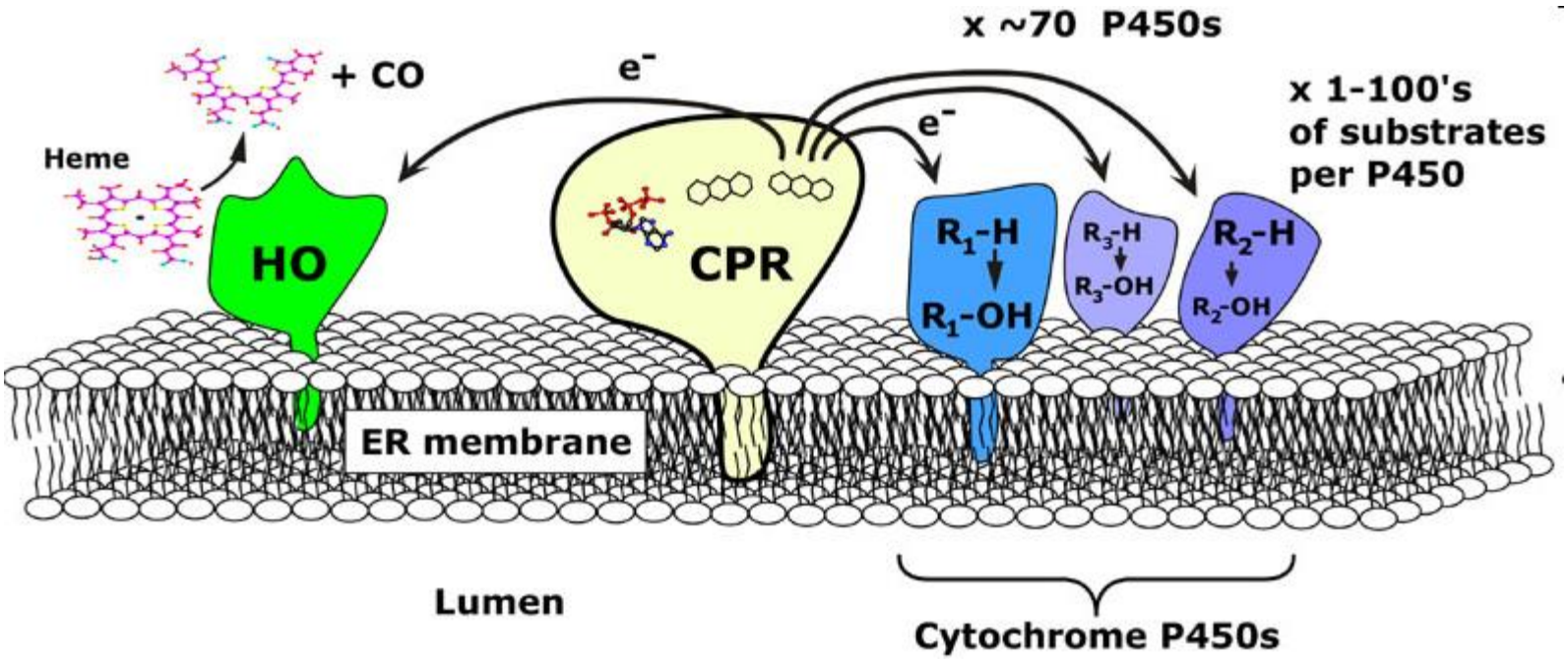
Phase I Reactions

Various reactions involving oxidoreductases and hydrolases covert hydrophobic substrates to more polar forms by introducing or unmasking a functional group (e.g., -OH, -NH_2, or –SH).

Many phase I enzymes are located in the ER membrane, but others such as the dehydrogenases occur in cytoplasm, while steel others are localized in mitochondria.



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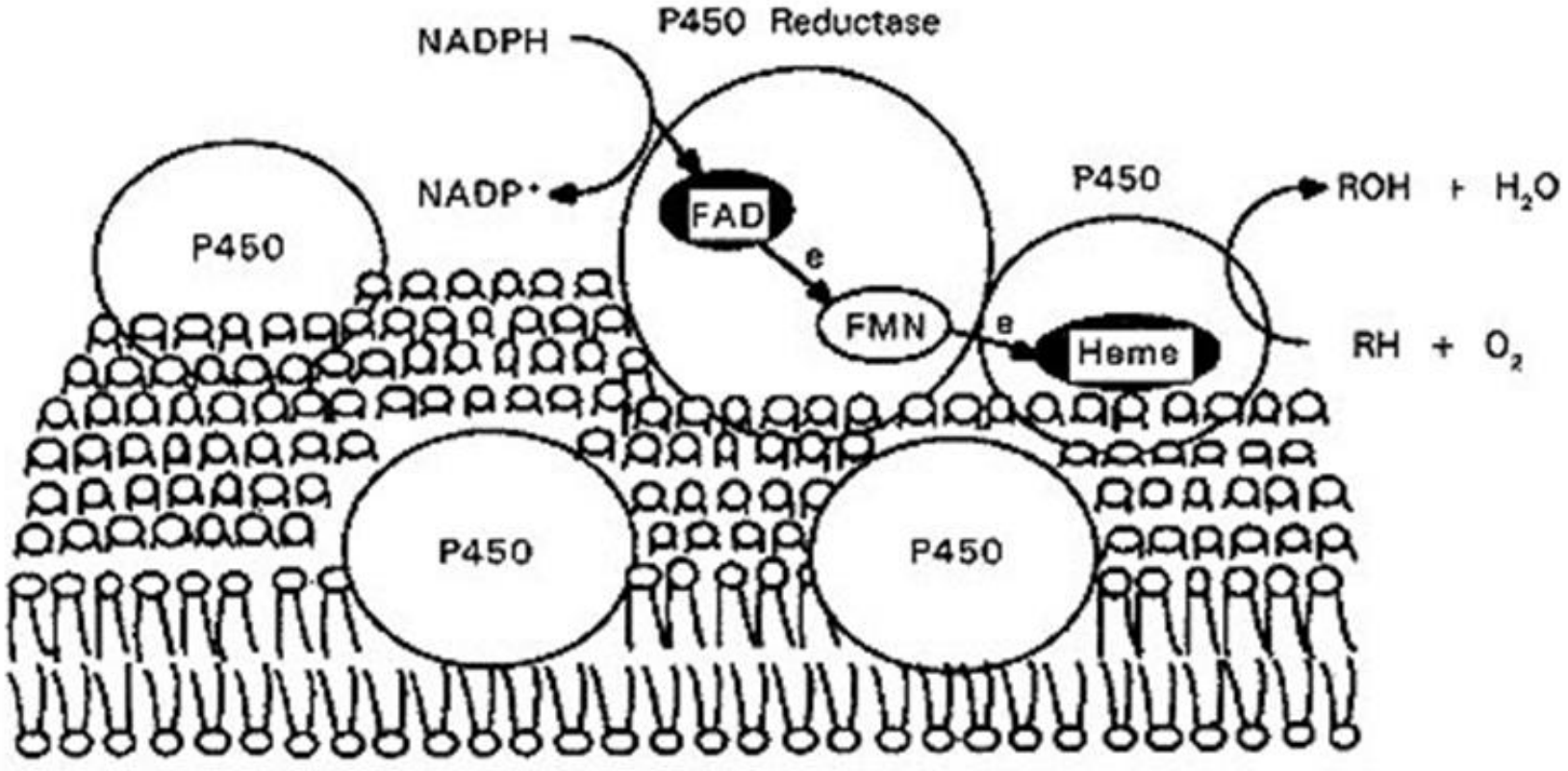
The predominant enzyme of microsomal oxidative metabolism are monooxygenases. There are two major types of microsomal monooxygenases, both of which require NADPH as an external reductant: the cytochrom P450 system and flavin-containing monooxygenases.



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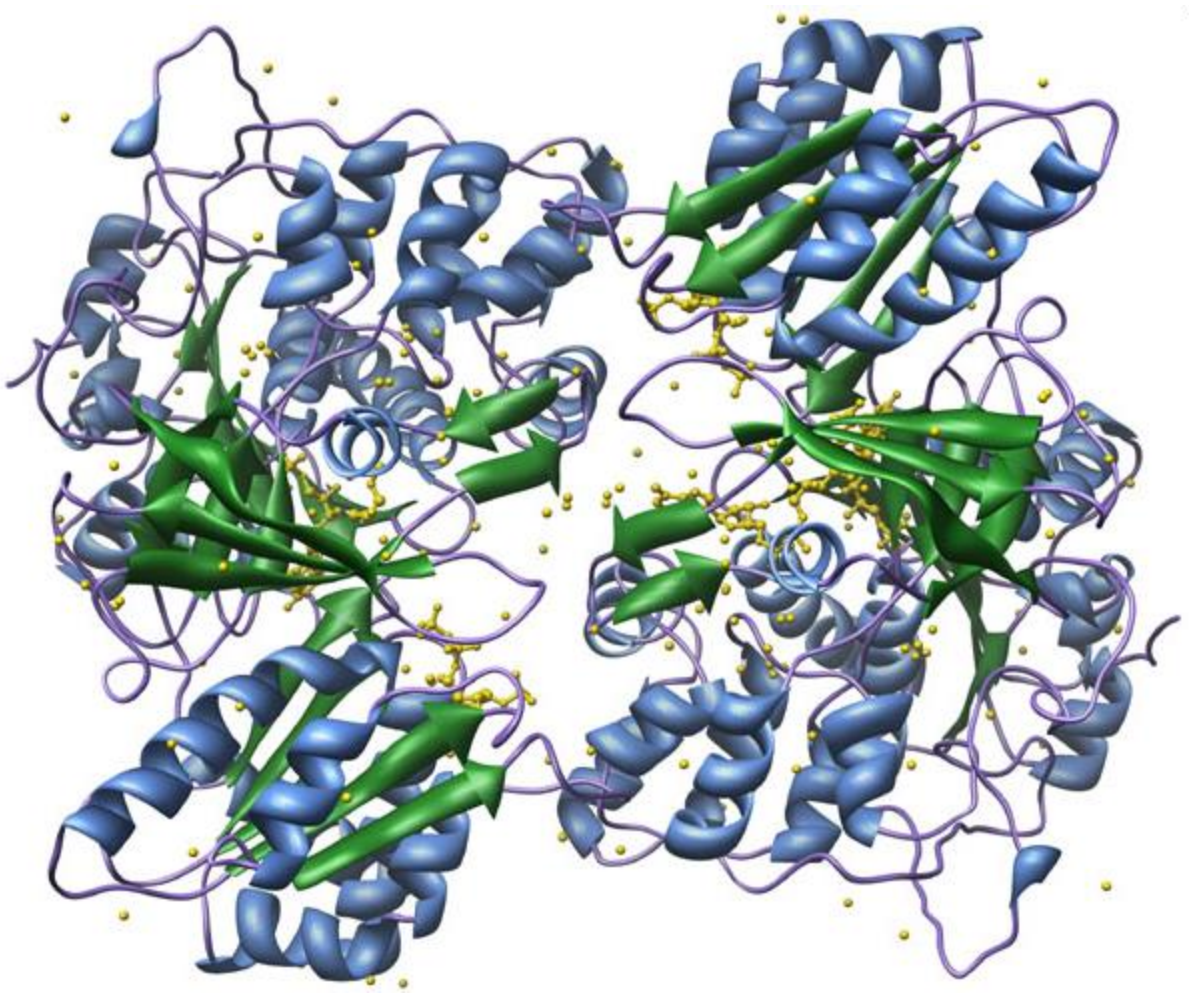
Cytochrome P450



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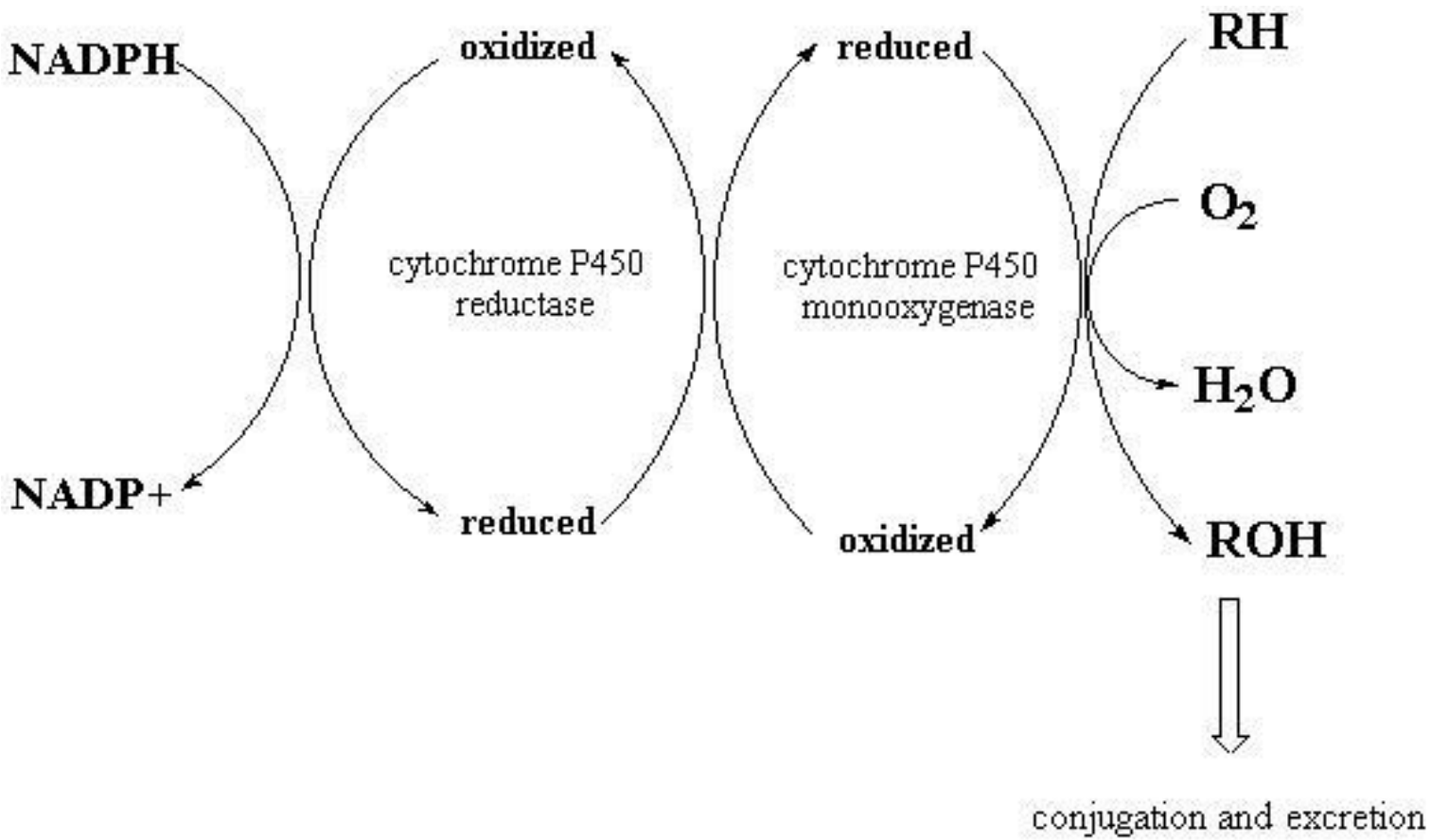

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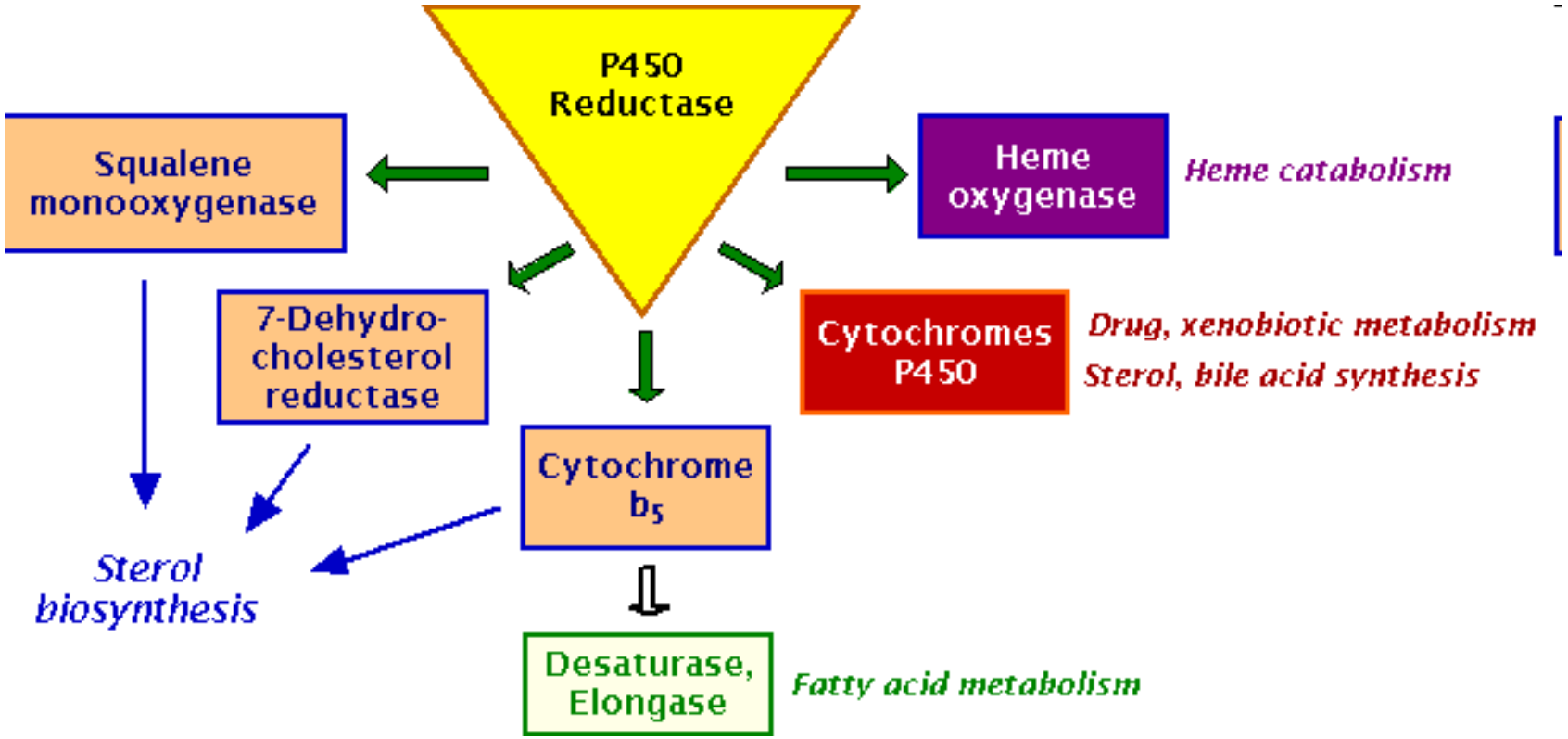
The cytochrome P450 system, which consist of two enzymes (NADH-cytochrome P450 and cytochrome P450) is involved in the oxidative metabolism of many endogenious substances as well as detoxication of wide variety of xenobiotocs.



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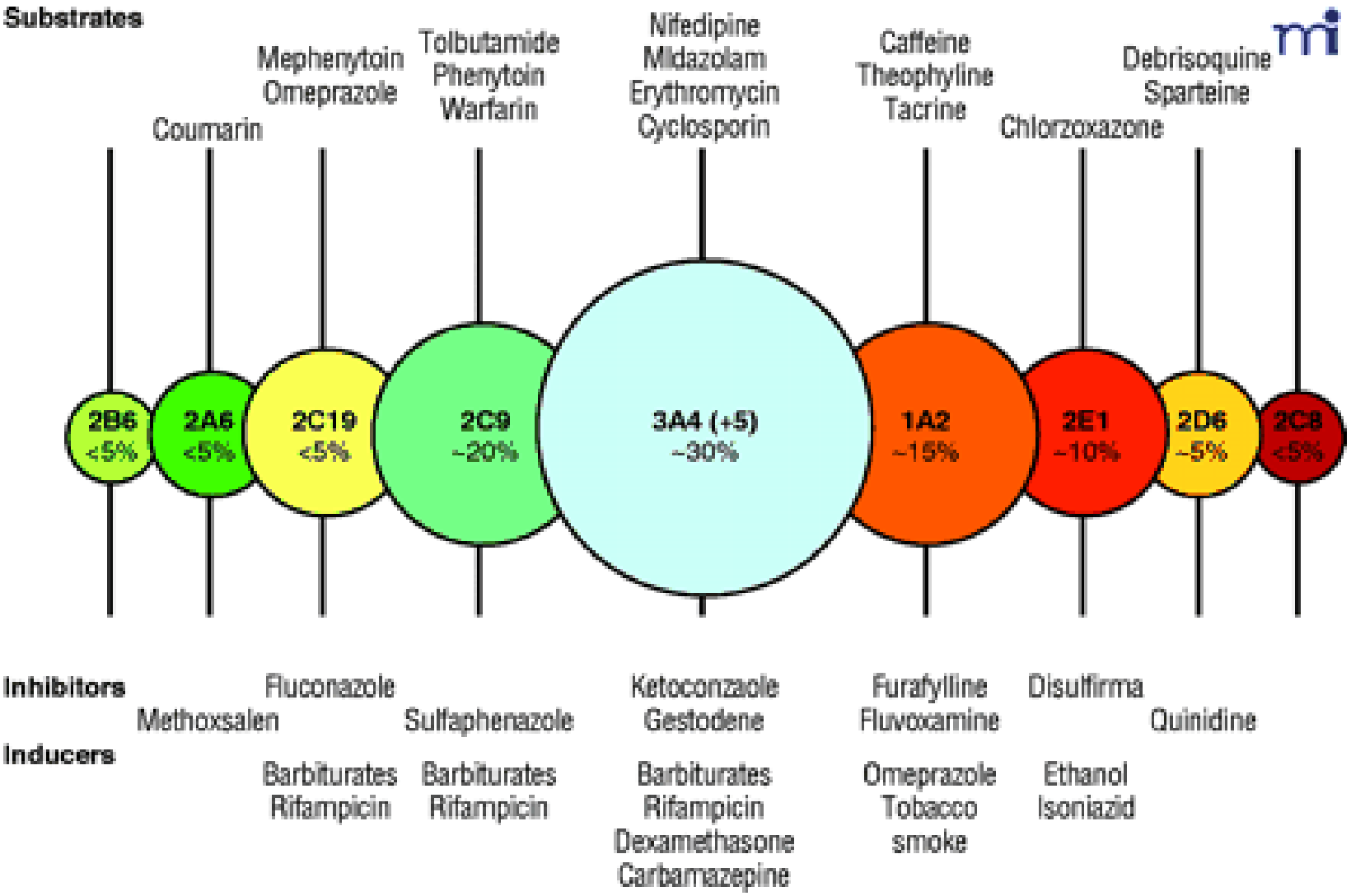
Major Hepatic P450 Enzymes Involved in Drug Metabolism



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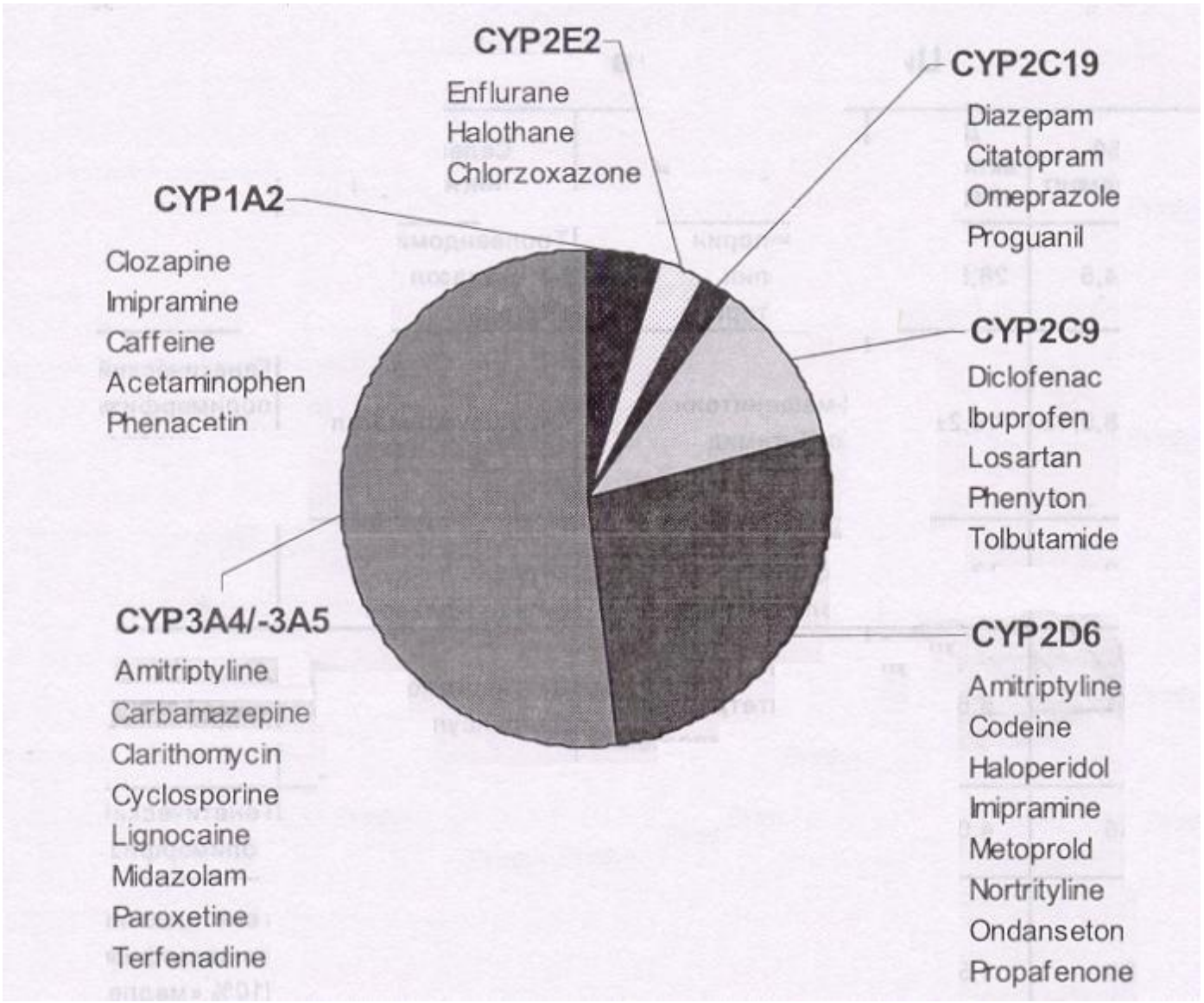

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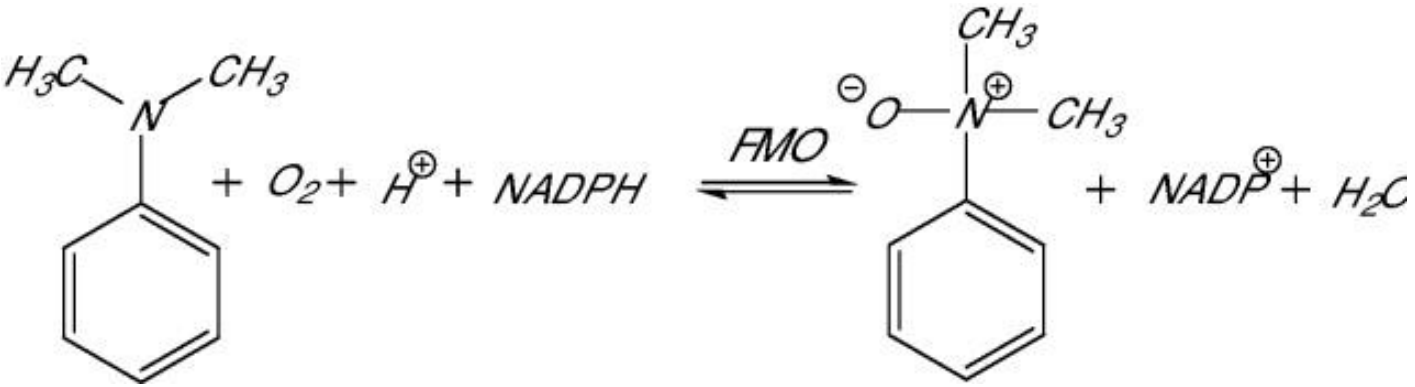
The flavin-containing monooxygenases catalyze an NADPH- and oxygen-requiring oxidation of substances (primarily xenoboitics) bearing functional groups containing nitrogen, sulfur, or phosphorus.

The catalytic cycle begins with the binding of NADPH and transfer of two electrons to the flavin moiety. Oxygen then adds to the reduced flavoprotein to produce peroxyflavin. Subsequent substrate binding is followed by direct oxygenation. One atom of oxygen is transferred to the substrate, while the second is released as water. In the last step, NADP+ is released from the enzyme.



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Phase II Reactions

Conjugation

Conjugation reactions involve the addition of molecules naturally present in the body to the natural metabolites xenobiotics or drug molecule. This occurs in the liver.

Glucuronidation

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 (NH_2CH_2COOH) for example nicotinic acid.

Sulfate

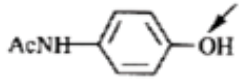
Sulfate ($-SO_4$) for example morphine, paracetamol.

Glucuronidation

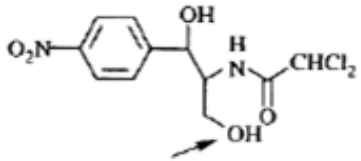
O-Glucuronide

Hydroxyl

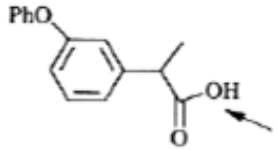
phenol — **acetaminophen**



alcohol — **chloramphenicol**



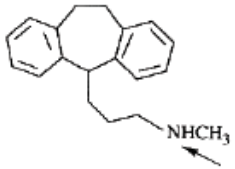
Carboxyl — **fenoprofen**



N-Glucuronidation

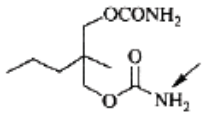
N-Glucuronide

Amine — **desipramine**

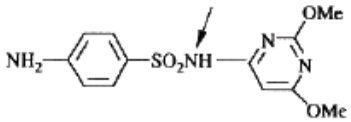


Amide

carbamate — **meprobamate**



Sulfonamide — **sulfadimethoxine**



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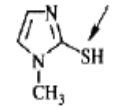
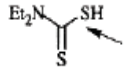
S- and C-Glucuronidation

***S*-Glucuronide**

Sulfhydryl **methimazole**



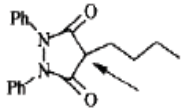
Carbodithioic acid **disulfiram**



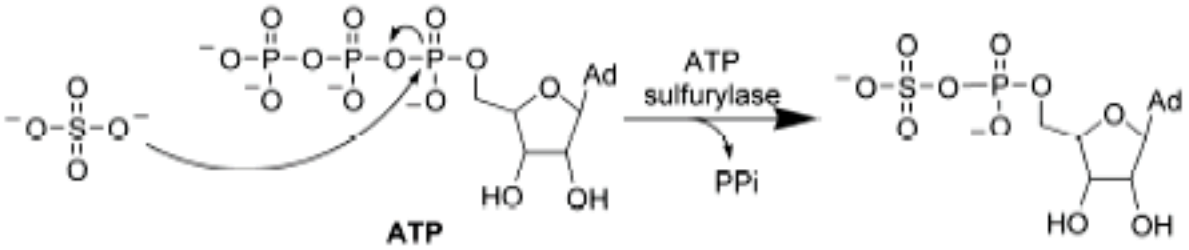
(reduced metabolite)

***C*-Glucuronide**

phenylbutazone



Sulfate Conjugation



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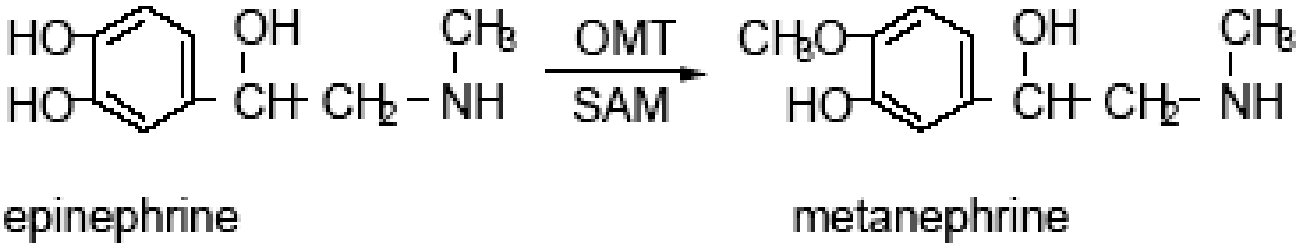
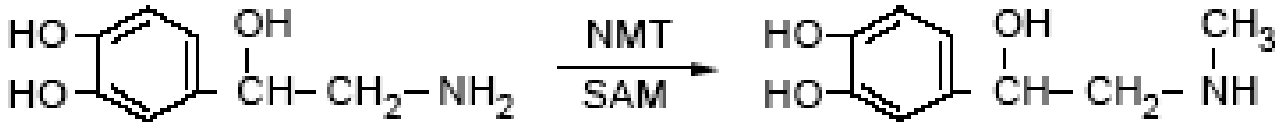
Methilation



epinephrine metanephrine



norepinephrine epinephrine

O-, N-methyltransferase (OMT & NMT)
S-Adenosylmethionine (SAM)

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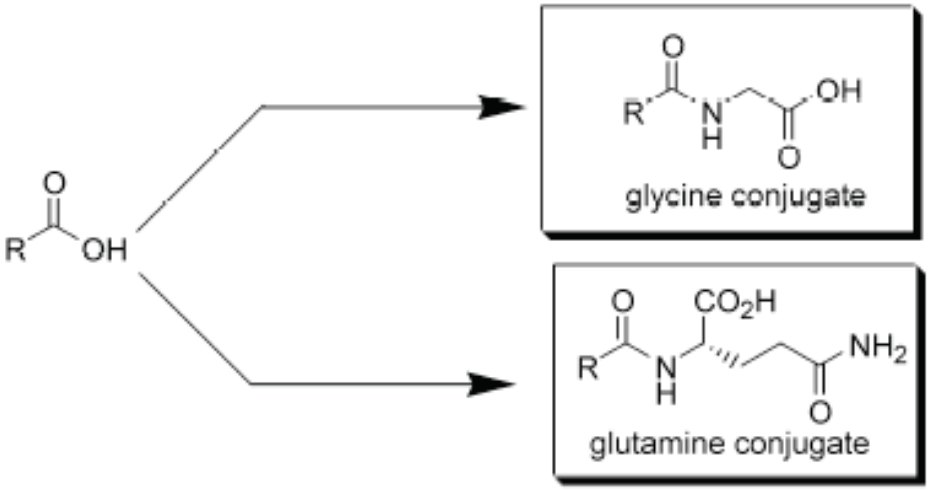
Conjugation with Amino Acids



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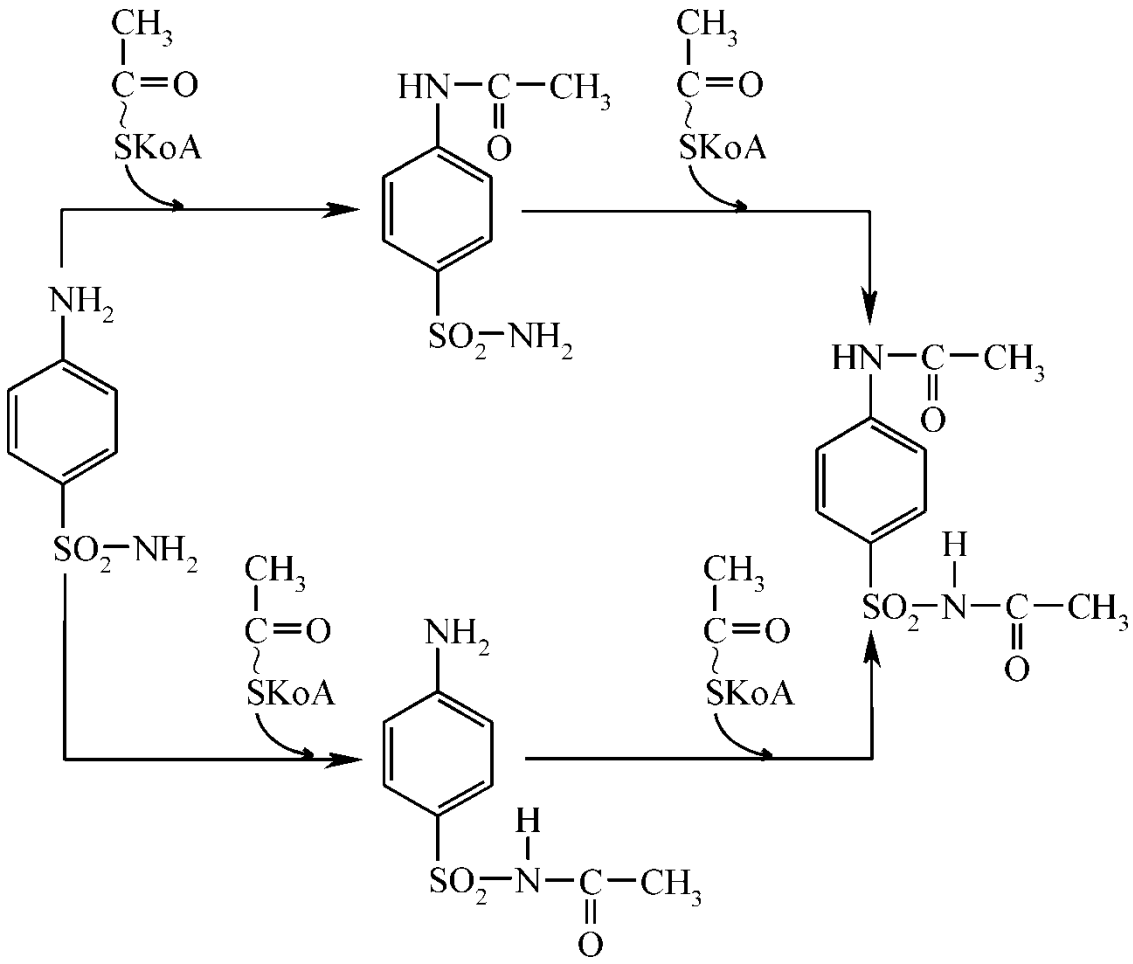
Sulfanilamides are acylated and excreted in urine



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Drug as a Pro-drug - Active Metabolite

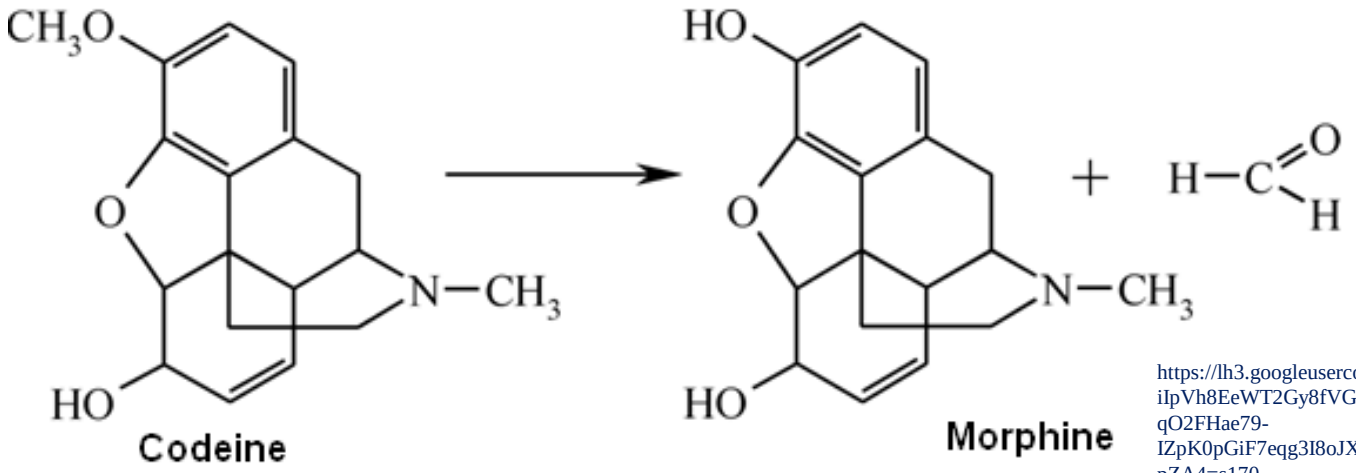
In most cases the metabolites are inactive, however, occasionally the metabolite is also active, even to the extent that the metabolite may be the preferred compound to be administered. The original drug may take on the role of a pro-drug. For example:-

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.



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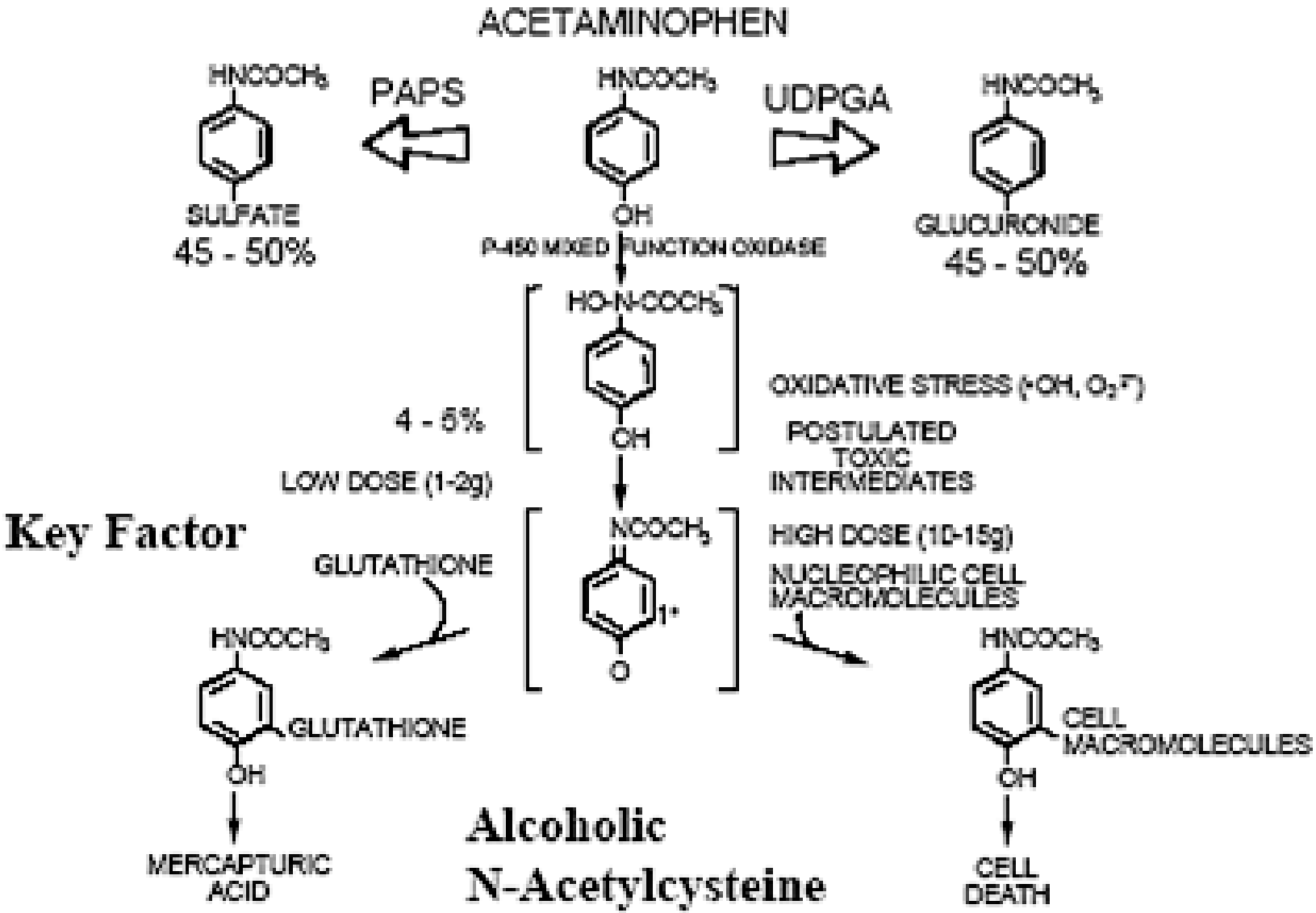

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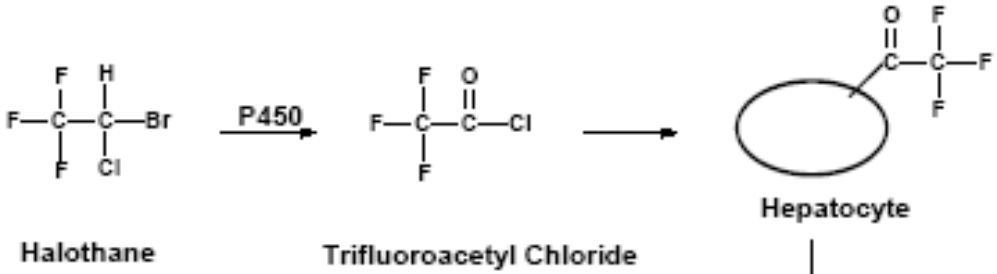
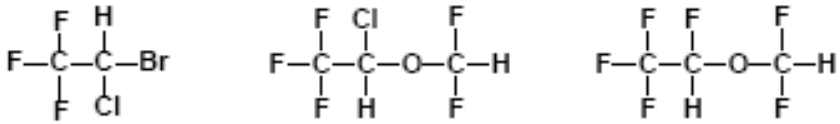
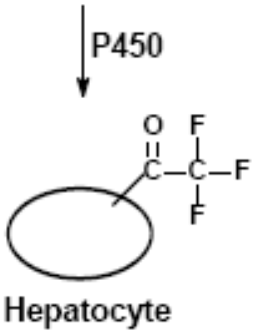


Humoral and Cellular Immune Responses

Toxicity





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The Role of Metabolic Activation

Chemical carcinogens promote tumorigenesis through the interaction with biomolecules and interference of expression patterns that are involved in surveillance of cellular growth, proliferation, and death. Several decades ago it became clear that a diverse set of compounds such as mustard gas, *N*-nitrosodimethylamine, benzo[*a*]pyrene, 2-acetylaminofluorene, and others bind to DNA in vitro or in vivo.

In the 1960s it had been suggested that the level of DNA binding of a particular chemical and its carcinogenic potency were roughly correlating. In order to react with cellular nucleophiles such as DNA, however, most carcinogens require enzymatic conversion. Thus numerous chemical carcinogens are rather precarcinogens to be bioactivated into their ultimate (DNA-reactive) forms.

Biotransformation of inert chemicals is catalyzed by cytochrome P450-dependent monooxygenases (CYPs) and a great variety of additional "xenobiotic metabolizing enzymes" such as hydrolases, peroxidases, transferases, and so forth. Members of the CYP families 1 to 3 are most important in oxidation of a large number of carcinogenic chemicals in mammalian species. From these, CYP1A1 is the major form in human lung, CYP1A2, 3A4 or 2E1 are mainly expressed in liver, and CYP1B1 is the main form in other organs such as prostate and uterus.

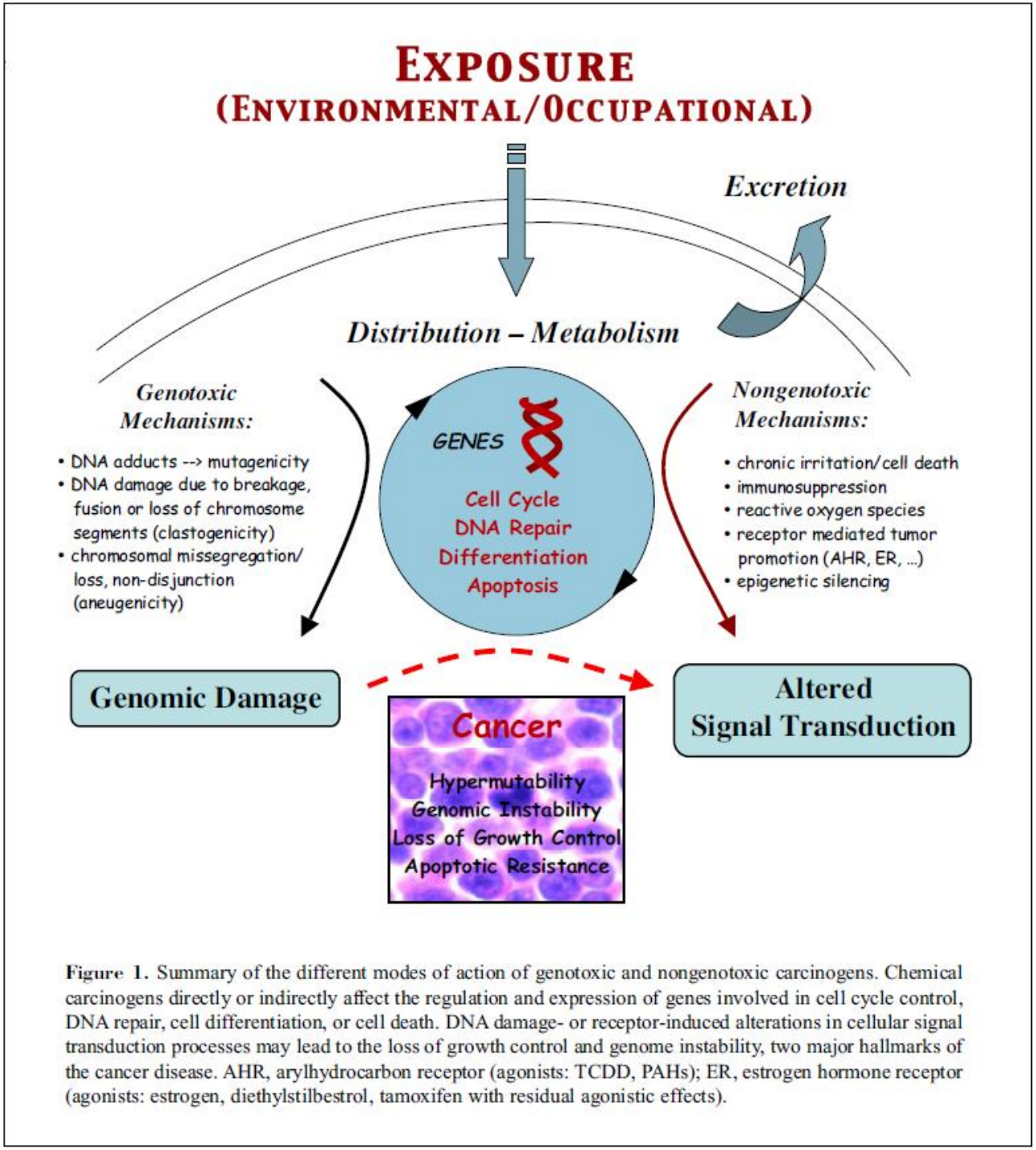

Figure 1. Summary of the different modes of action of genotoxic and nongenotoxic carcinogens. Chemical carcinogens directly or indirectly affect the regulation and expression of genes involved in cell cycle control, DNA repair, cell differentiation, or cell death. DNA damage- or receptor-induced alterations in cellular signal transduction processes may lead to the loss of growth control and genome instability, two major hallmarks of the cancer disease. AHR, arylhydrocarbon receptor (agonists: TCDD, PAHs); ER, estrogen hormone receptor (agonists: estrogen, diethylstilbestrol, tamoxifen with residual agonistic effects).

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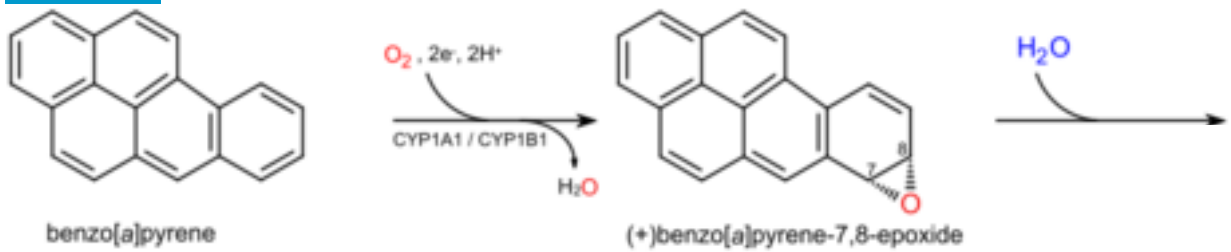
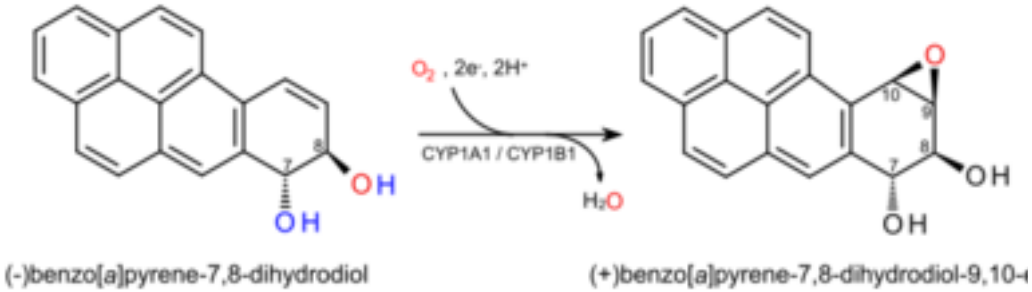
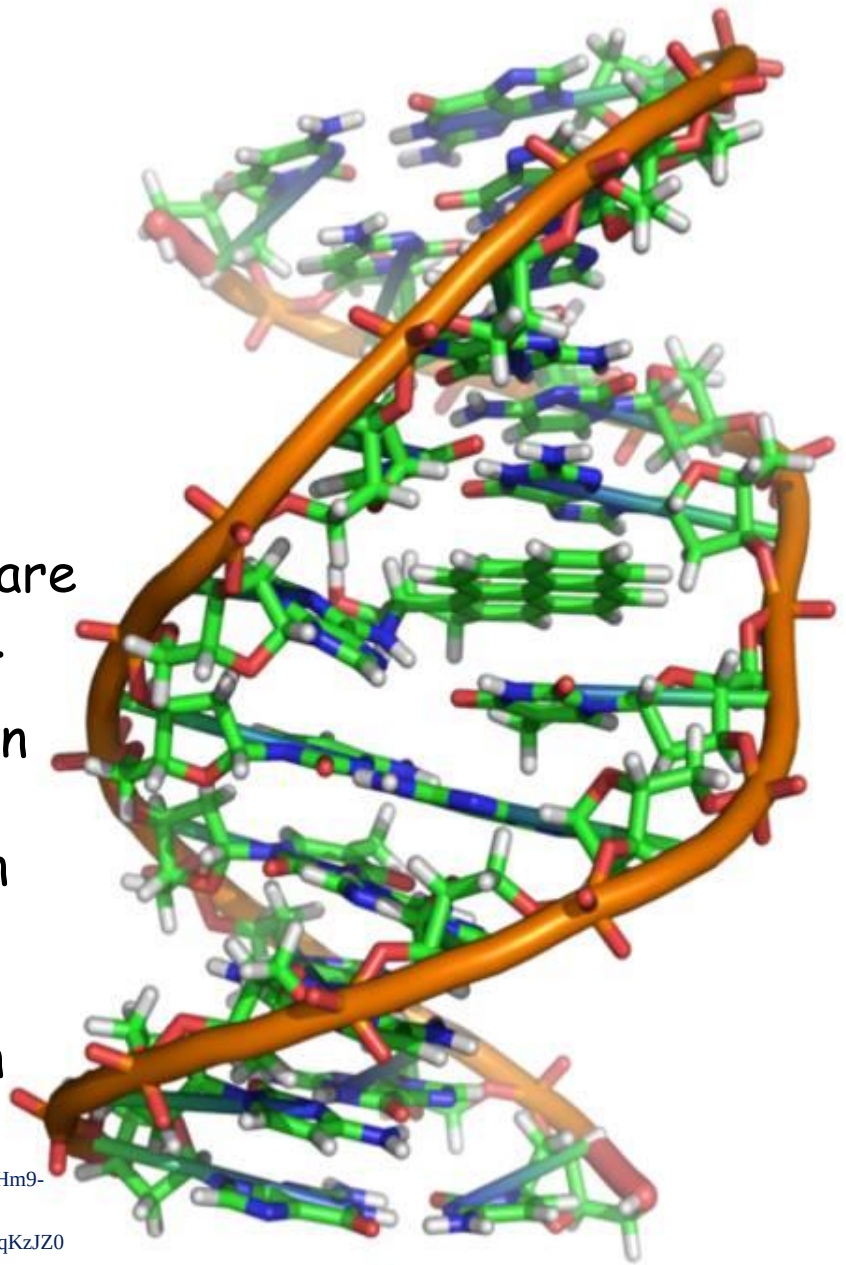




Benzopyrene metabolites are mutagenic and highly carcinogenic.

Benzopyrene is found in coal tar, in automobile exhaust fumes (especially from diesel engines), in all smoke resulting from the combustion of organic material (including cigarette smoke), and in charbroiled food.



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Pharmacogenomics

Pharmacogenomics and the older term pharmacogenetics describe the interaction between drug pharmacokinetics or activity and genetic or genomic parameters. While pharmacogenetics deals with genetic difference between individuals, pharmacogenomics deals with the more specific interaction with genes and single nucleotide polymorphisms (SNPs). Genetic polymorphism will cause differences in enzymes, proteins, transporters and receptors.

Responses to Pharmacogenomic Variation

- Alteration in enzyme activity may produce clinically significant differences in drug metabolism.
- Altered protein structure can cause altered drug protein binding.
- Changes in drug transporters can alter drug absorption or distribution.
-Drug receptor formation can be controlled genetically.
-Alterations in drug receptors may significantly change drug response.

Examples

The muscle relaxant succinylcholine is usually rapidly deactivated by plasma butyrylcholinesterase within a few minutes. However, in some individuals genetic variation in the expression of this enzyme results in reduced enzyme activity, reduced metabolism and prolong drug activity. Drug activity may last up to an hour in these individuals .

During World War II it was observed that some African-American soldiers suffered hemolytic toxicities after usual doses of the anti-malarial primaquine. This was later identified as a higher frequency of genetically controlled lack of the enzyme glucose-6-phosphate dehydrogenase (G6PD) .

Fast and slow acetylators (N-acetyltransferase, NAT) of isoniazid have been identified in varying frequencies in different populations. Normal doses given to slow (unidentified) slow acetylators results in toxicities such as numbness, pain and tingling .

Drug transporters, MDR1 .

Codeine metabolism to morphine - CYP 2D6.

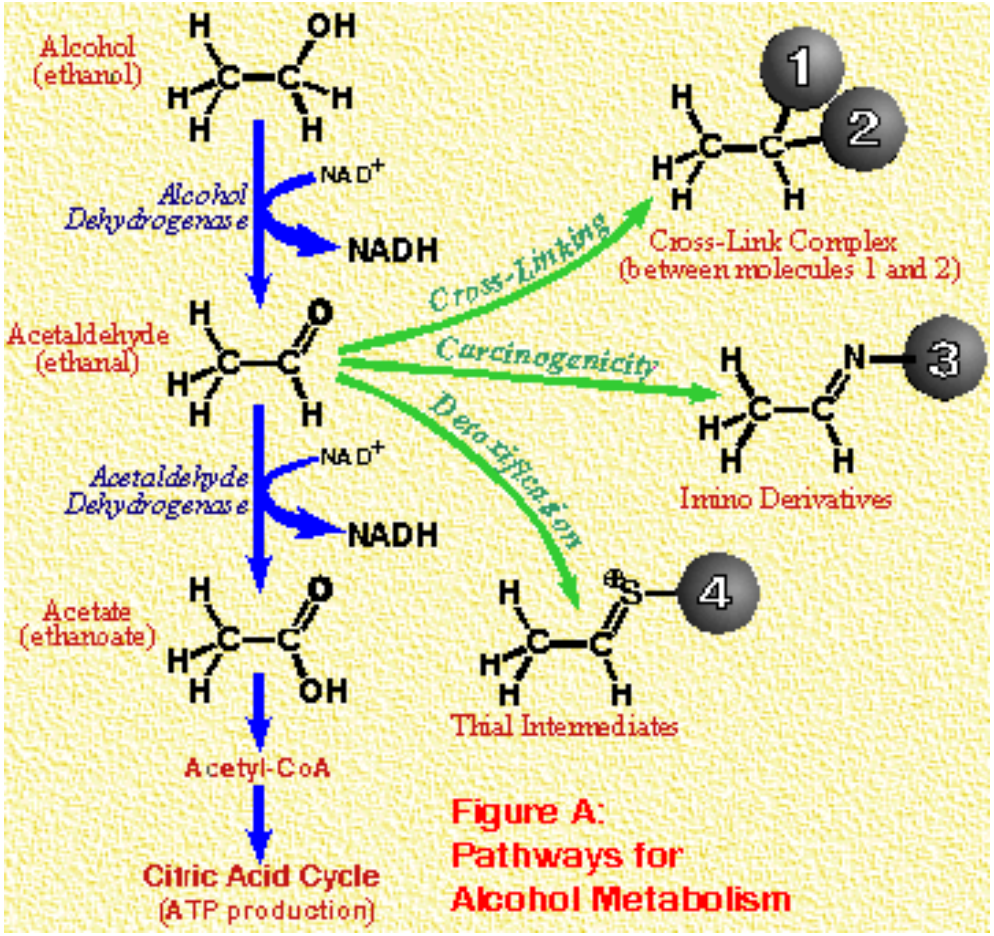
Ethanol Detoxification

The major mechanism by which ethanol is detoxified involves cytoplasmic enzymatic activity of alcohol dehydrogenase (ADH).

ADH catalyzes the oxidation of ethanol to form acetaldehyde.

Acetaldehyde is converted to acetate by aldehyde dehydrogenase located within the mitochondrial matrix.

Most of acetate produced in this reaction is metabolized further in other tissues, such as cardiac and skeletal muscles.



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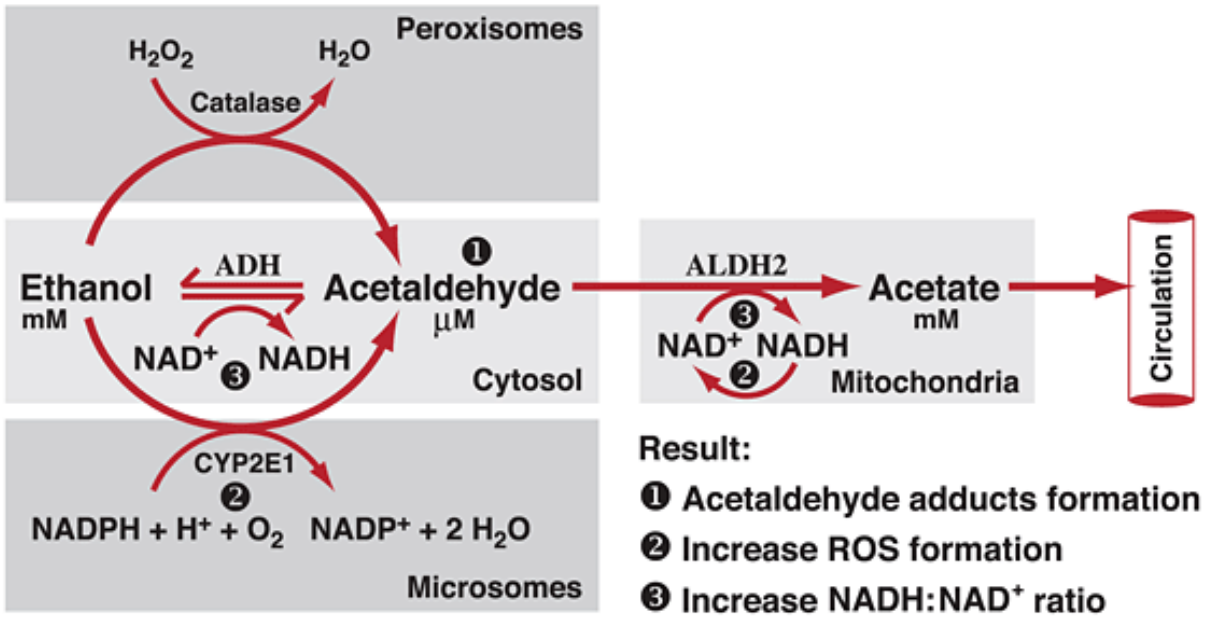
When the concentration of ethanol in hepatocytes becomes sufficiently high, it is also detoxified by the microsomal ethanol-oxidizing system (MEOS). MEOS cosists of an ethanol-inducible cytochrome P_{450} isozyme and NADPH cytocpome P_{450} reductase.

Significantly less important mechanism for ethanol detoxification involves catalase, an enzyme found predominantly within peroxisomes.



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Hepatotoxic Effects of Ethanol

Ethanol`s toxic effects are principally mediated by acetaldehyde, free radicals, and altered cellular redox conditions.

Acetaldehyde -covalently bond to proteins (because protein adduct formation is nonspecific, a wide variety of enzymes and structural proteins can be affected);
-promote lipid peroxidation (depleting cells of GSH).

Excessive consumption of ethanol results in the induction of MEOS.

Excessive tissue-damaging ROS is caused by

- increased concentration of ethanol-inducible cytochrome P_{450} ;
- increased activity of xanthine oxidase, which results from excessive increased purine degradation.

Excessive production of NADH that is created by increased activity of ADH and aldehyde dehydrogenase has effects

- depression of citric acid cycle activity;
- ethanol is preferred energy substrate.

Damage caused by alcohol

Hepatic steatosis – abnormal accumulation of fat withing the liver. The promotion of fat synthesis is principally the result of excessive NADH production. Fat also accumulates because of decreased fat oxidation and decreased lipoprotein secretion. As fat accumulates, hepatocytes are less and less able to perform their metabolic roles.

Hyperlactatacidemia results from decreased conversion of pyruvate to acetyl-CoA, a consequence of depressed citric acid cycle activity. High blood levels of lactate contribute to a reduction of the kidney`s capacity to excrete uric acid, thereby promoting hyperuricemia.

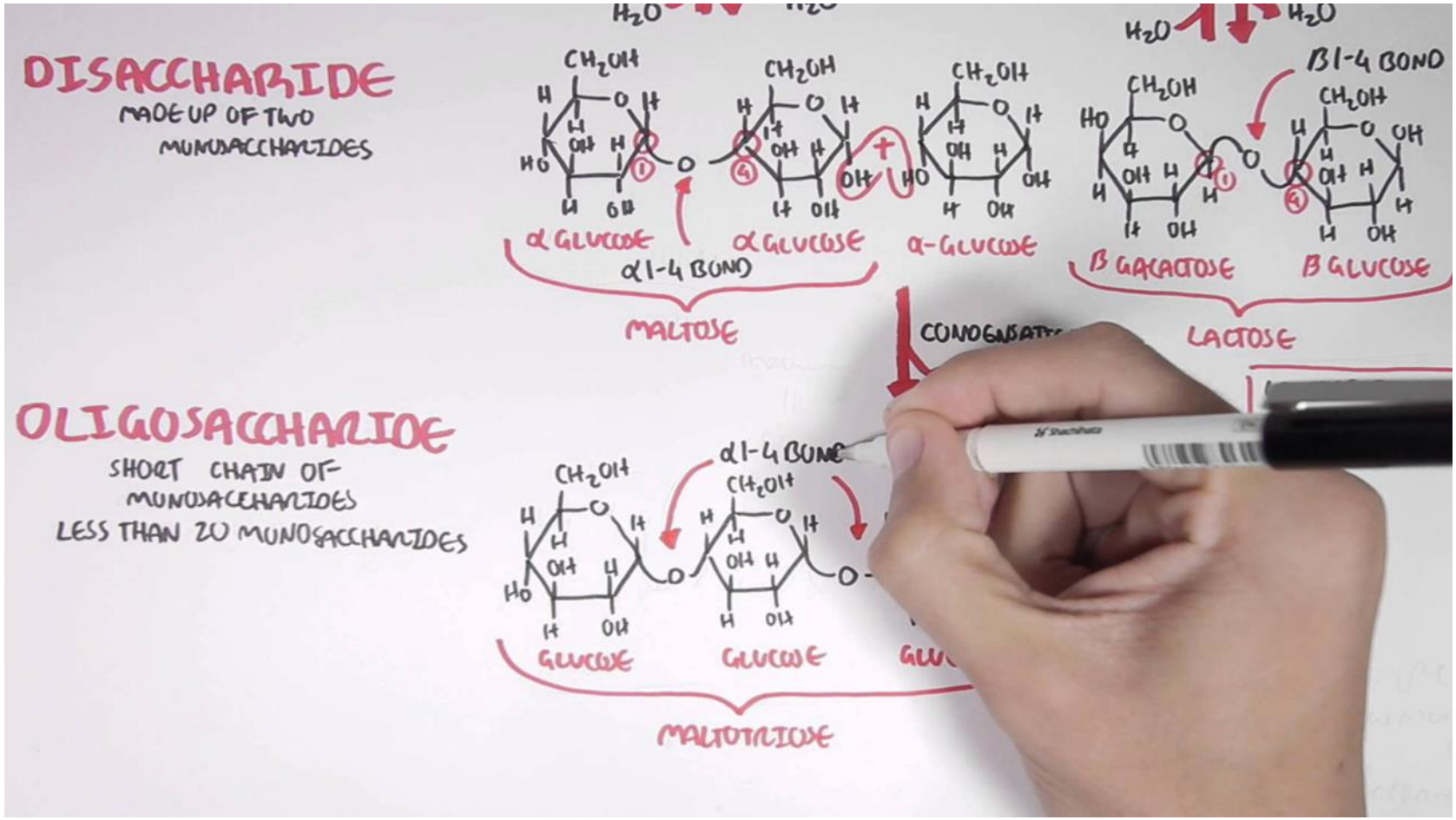
Malnutrition. Because of ethanol`s high caloric value (29 kj/g), alcoholic beverages often displace other foods. Chronic excessive alcohol consumption so damages the gastrointestinal tract that the digestion and absorption of food is compromised. Ethanol metabolism promotes the depletion of the body`s stores of vitamins and minerals.

Conclusions

1. One of the most remarkable features of living organisms is there adaptability.
2. Xenobiotics are chemicals found in organisms, but not expected to be produced or present in them.
4. Metabolism generally occurs in 2 phases:
5. Phase I induces a chemical change (most frequently oxidation, but also reduction) that renders the drug more conductive to phase II;
6. Phase II is a conjugative or synthetic addition of a large, polar molecule that renders the drug water soluble and amenable to renal excretion.

Do you have any questions?

Thank you for your attention!



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