C. 7 & 8

Drug

metabolism

- While absorption is of prime importance in the growth and maintenance of a constant concentration of a pharmacon in the plasma, thus determining the intensity and duration of action of the drug,

- other processes operate to reduce this concentration.

- The influence of distribution, in the dilution of the drug is reinforced by the removal of the free active drug through the process of elimination.

- This includes the:

  - metabolic inactivation of the pharmacon and

  - excretion, of both, the intact molecules of the pharmacon as well as the modified one's.

- These changes often reduce or even block the activity of the pharmacon.

- Most drugs are metabolized in the liver and excreted by the kidneys.
- The microsomal enzyme system in the liver has a role in metabolizing fat soluble drugs
- Drug metabolism may also occur in blood plasma and in the intestinal lumen where hydrolytic and reduction reactions occur.
- After the metabolism phase, the appearance of metabolites with favourable physico-chemical properties for excretion, is observed.

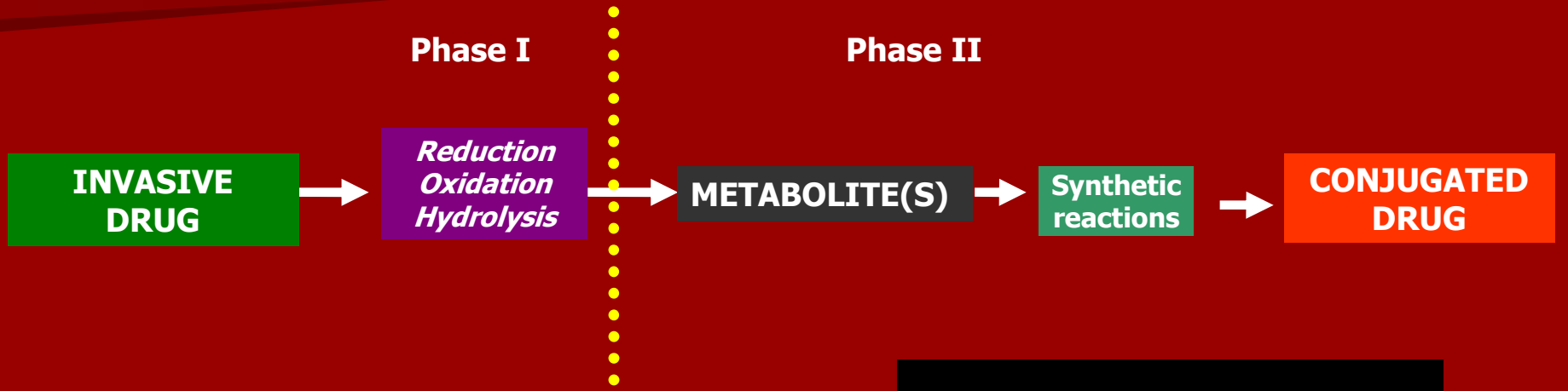
Ionized (polarized) drugs and their metabolites are excreted by the kidneys.

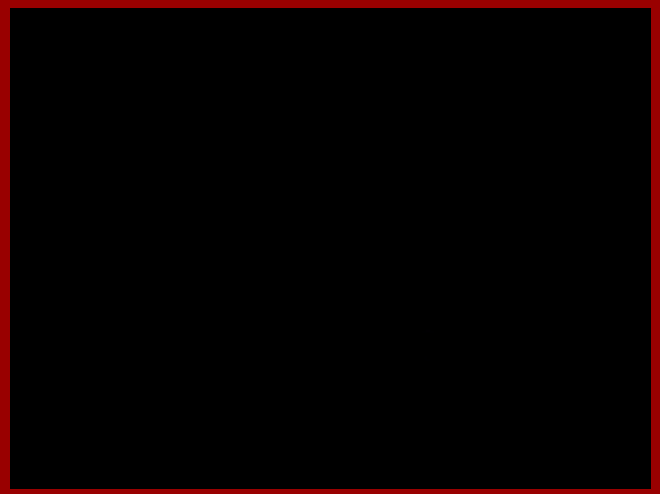
This includes :

- **metabolic inactivation of the pharmacon and**
- **excretion, of both the intact molecules of the pharmacon as well as the modified ones.**

These changes often reduce or even block the activity of the pharmacon.

Simplified scheme of the metabolism





Factors

that influence drug metabolism

- The factors that influence drug metabolism and elimination are pharmacokinetic and pharmacodynamic whose interweaving causes the direct activity of the pharmacon after the invasive phase.

Physiological (pharmacokinetic)

factors

Renal blood flow

effective hemodynamic is essential for renal function, this function influences the rate of drug excretion the most, through the fact that:

► glomerular ultra-filtration is dependent on: pressure of filtration.

- In healthy animals, the kidney receives about 25% of the cardiac output, converts about 1/5 of this in glomerular ultrafiltrate, then reabsorbs about 99% of the filtrate.

- For a drug that is eliminated massively by excretion, the rate of blood flow through the kidneys is an important determinant of its existence in the body, (ex. Digoxin and gentamicin).

Solubility in the ultrafiltrate

Drugs with hydrophilic character are most commonly excreted in urine, in their unaltered state, while fat soluble pharmacons may be subject to metabolism (to form water-soluble compounds before being excreted).

- Occasionally (eg, quinolones and old acetilsulfamides) a metabolite is less soluble than the parenteral pharmacon in the concentrate acid ultrafiltrate from the proximal convoluted tubule.
- In this case, there is a risk of precipitation of the drug in the convoluted tubules preventing renal function.

This situation may be avoided by urine alkalinization, by unrestricted administration of water and by using sulphonamidic mixtures.

Corresponding with the multitude of chemical compounds that are administered as pharmacons (or toxics), there are numerous possibilities for biotransformation, which lead to the formation of active or inactive metabolites.

- if a change occurs in a toxic, decreasing the intensity of the effect, one speaks of detoxification.

- If a substance is changed in the body, and turned into a toxic, then this process is called intoxication (eg: conversion of methanol to formaldehyde, of insecticide diethyl-p-nitrophenyl-thiophosphate in diethyl-p-nitrophenyl-phosphate etc.).

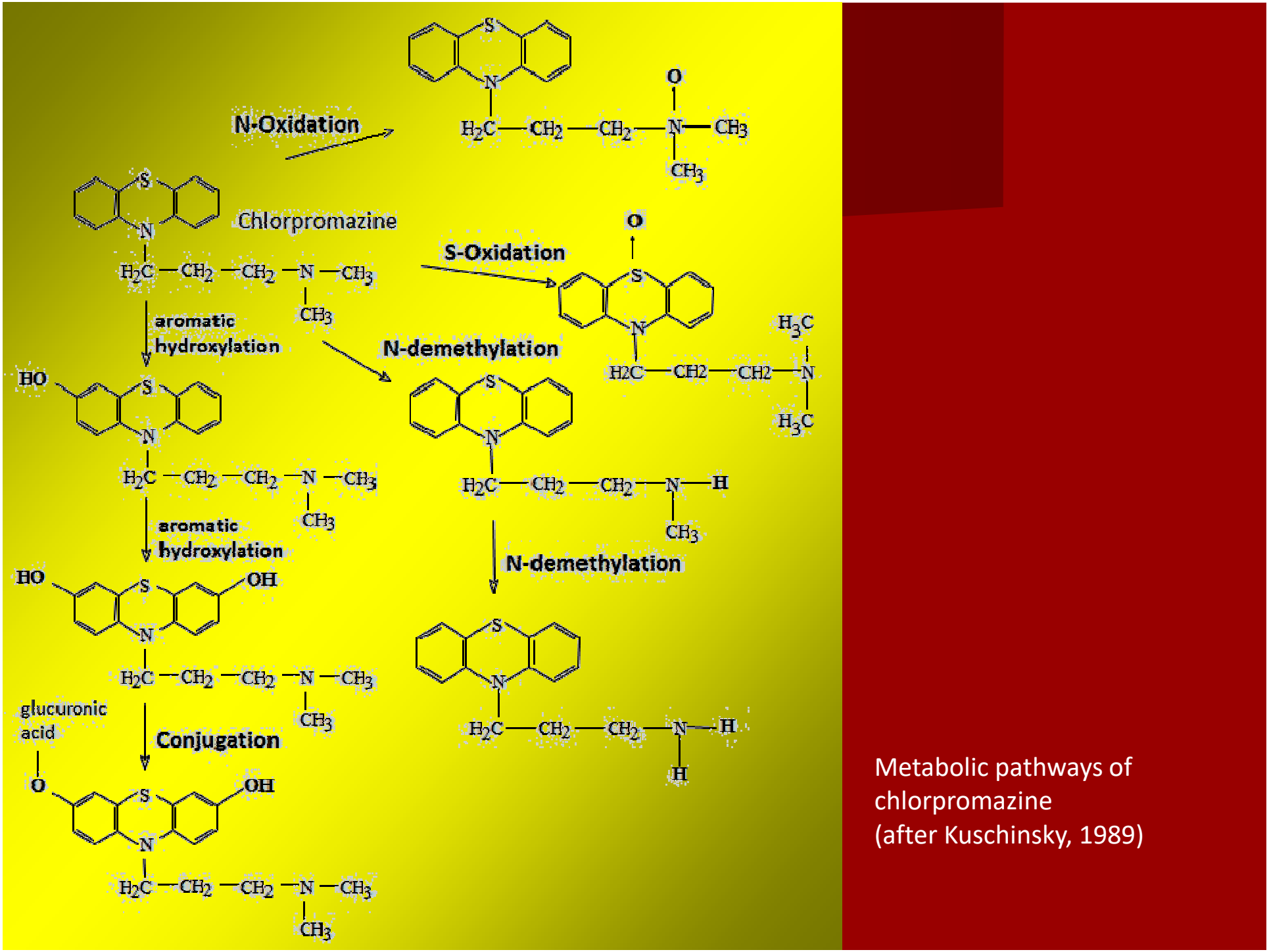
- The same thing happens in the case of drugs, which are primarily inactive, they become pharmacologically active only after their metabolic conversion or cyclization(eg: chlordiazepoxide, an antidepressant), opiates, levodopa, enalapril, pro-benzimidazoles etc.).
- The coupling with activated glucuronic acid is of great importance.
- Hydroxyl groups of alcohols and phenols, carboxyl, amino and amide groups are conjugated with glucuronic acid = increases hydrosolubility.

- In drug metabolism the reactions that occur are:
  - hydroxylation,
  - demethylation,
  - oxidation and finally, de
  - glucuronidation (in the phase of conjugation).

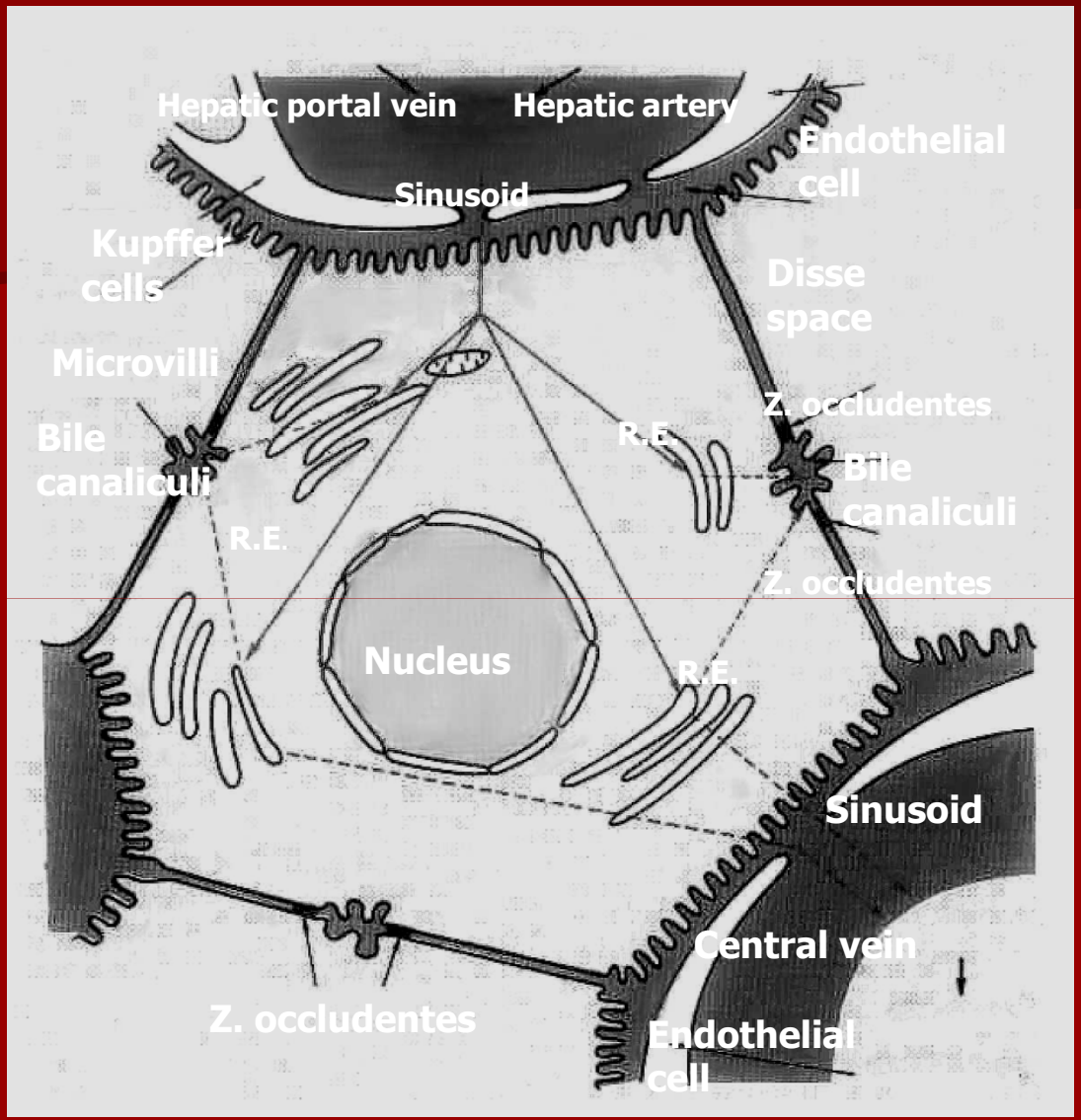
- This last step will increase the hydro solubility and will ease the removal. We know some principal pathways of decomposition :
  - scission and
  - burning up to CO2 and water (ex: ethanol);

Partial decomposition by:

- decarboxylation,
- deamination (catecholamine, α-methyldopa, histamine, serotonin)
- N-demethylation (chlorpromazine, morphine, pethidine).
- Oxidation (ex: chlorpromazine)
- Reduction (ex: nitrazepam);
- Hydrolysis (spontaneous or fermentative) (succinylcholine, ester-type local anesthetics);
- Coupling to acids (ex: acetylation of sulfonamides, coupling with glucuronic acid).



Metabolic pathways of chlorpromazine (after Kuschinsky, 1989)



Representation of coupling and scission of lipophilic drugs in the liver cell (after Kuschinsky, 1989)

Urinary pH.

Renal excretion of weak acidic or basic drugs is closely related to urinary pH.

So, weak acids are eliminated better when the urine is alkaline, while the weak bases in acidic urine.

When the elimination is reduced (due to unfavorable pH), it will activate the metabolic processes (to make substances more soluble), thereby increasing the rate of conjugated compounds.

Coupling with plasma proteins

Medicinal substances coupled to plasma proteins cannot be metabolized until they are severed from their links and transformed into free fraction.

As a result, their half-life is even longer as the medicine is of a higher percentage of coupling.

Enzymatic induction

- Enzymatic induction means the stimulation of the activity of liver enzymes, under the action of xenobiotic substances (non-biological), this includes drugs and pesticides, etc.
- These inductors speed up the metabolism, by increasing the rate of synthesis of the enzymes.

- So far, we know more than 200 substances that are considered enzyme inducers, having very different chemical structures
- A correlation cannot be established between the chemical structure and the inductive effect.
- The most studied enzyme inducer, phenobarbital, is considered to be the prototype of this action, given that it boosts the metabolic activity for numerous medicinal substances.

- Through enzyme self-inductance, some drugs after repeated administration, can stimulate their own metabolism.
- Most of the enzymes responsible for the biotransformation are in the liver, specifically in the endoplasmic reticulum (ER), in the microsomes.
- These enzymes can be multiplied by a number of different chemicals of pharmacons, even when a pharmacon interacts only with one enzyme of the ER.
- The consequence of this enzyme induction = faster and easier decomposition of that pharmacon.

The best known enzyme inducers are:

barbiturates, psihopharmacons, rifampicine, chlorphenotane, HCH, tolbutamide, some carcinogenic substances etc.

In the case of these, are known substances which cause a different enzyme induction, comparable in quality to the induction caused by barbiturates. So we can speak of two types of induction:

- a “phenobarbital type” and
- a “methylcholanthrene type”.

An important enzyme system, which is enabled by the Phenobarbital type mechanism of induction, is the pluri-functional oxidase which is responsible for the oxidation of organic links.

The terminal oxydase of the system is cytochrome P-450.

Enzymatic

Inhibition

There are some substances that inhibit the activity of hepatic microsomal enzymes, for e.g.:

- piperonyl-buthoxide,
- piperonyl-sulfoxide,
- sesamex,
- chloramphenicol,
- ketoconazole,
- cimetidine etc.

For example, the long-term administration of Chlortione will lead to a marked inhibition of microsomal rat liver enzymes.

In addition to the possibility of decomposition which is general and non-specific, there are a number of specific mechanisms for some pharmacons, within which a series of the body's own substances are involved.

So, for example,

- acetylcholine, is hydrolyzed by acetylcholinesterase,
- norepinephrine, under the action of O-methyltransferase is methylated, both substances being inactivated.

- The lung has the ability to inactivate the body's own substance (serotonin, noradrenaline) and the results from their synthesis (ex: angiotensin II, prostaglandins E and F).

- A series of amphiphilic pharmacons can accumulate in the lung (ex: neuroleptics, thymoleptic) and thus temporarily or permanently disappear from circulation (by presystemic elimination).

Animal

related factors

Species

Comparative studies on animal species revealed a wide variety of metabolic pathways.

The differences are mainly related to the development on the phylogenetic scale but are evident within the same group of species :

- in mammals there are large variations in the speed of metabolism and biotransformation or conjugation pathways.

Example:

- The rabbit has significant amounts of tropinesterase, which explains the great resistance of this species to atropine and atropinics.

- Cats have a low activity of hepatic glucuronyl transferase, resulting in a deficiency in the formation of the glucuronide conjugates.

- In dogs and foxes, acetylation of sulfonamides is done at N1 (amidic nitrogen) and not at N4 (amino nitrogen), as occurs in other species.

- The most important feature of the specie differences in drug metabolism is represented by the quantitative aspects.

- Thereby, in animals we can observe variations not only within the nature of the enzyme systems which they have, but especially in the quantitative distribution of their activity.
- As a consequence, there are variations in the metabolic pathways both in the biotransformation processes, as well as in the conjugation ones.
- Eg. specific differences in sheeps and goats in the metabolism of benzimidazole nematicides. Most of the times unawareness of these aspects can lead to the development of drug resistance.

A lack of the enzyme system can be regarded as a particular sensitivity of the cat to the phenolic products.

Amphetamine in rabbits, suffers oxidative deamination processes, while, many other species have the hydroxylation process.

Sulfadimethoxina (a retard sulfonamide) metabolized by acetylation in the proportion of :

- 80% in cattle,
- 20% in goats,
- 80% in rabbits and
- 10% in humans.

Individuality / breed

In veterinary practice, the type of nervous activity of an animal should be taken into account, because it can influence the rate of drug metabolism.

Example:

- The use of strychnine in therapeutic doses in animals may be followed by poisoning;
- apomorphine, in some breeds of pigs induces vomiting, while in others it doesn't.

Age

Newborns, especially premature ones, are put in danger by the administration of drugs, because the hepatic enzymes are still in small quantities, or have not yet been synthesized, and the renal elimination capacity is limited.

eg.: oxidative enzymes from hepatic microsomes are missing in fetuses. They are formed from the first day of life and they reach the adult limits after a month in rats and after 3 months in children.

Similarly, the ability for synthesis of conjugates is reduced, or it may even be missing in the case of: glucuronic acid, glycine and glutathione.

In case of old age, elimination of pharmacons is prevented by: reduced renal function and by decreased speed of hepatic metabolic processes = proper evaluation of required dose.

Acute toxicity of drugs in neonate and adult rats
(Yeary, Benish and Finkelstein, cit. Gherdan)

Drug	Oral LD50 (mg / kg)	
	Neonate (1-3 days)	Adult
d-Amphetamine	80	140
Aspirin	560	1500
Paracetamol	420	2400
Meprobamate	350	1500
Phenobarbital	120	320
Dicumarol	70	700

- While the process of conjugation takes place without any impediment, the dealkylation and hydrolysis processes will be slowed down.
- The elimination rate of drugs is dependent on the secretory and metabolic function of the: liver, kidney and lung.
- Any change in their function leads to high blood levels, with lower tendency to decrease.
- This results in a more prolonged effect, potential toxicity and tendency to accumulate.

- The hepatic metabolism increases progressively from birth to adulthood, then decreases gradually.
- In aged animals, oral drug absorption and distribution is slow: gastric pH increases and the intestinal transit, GI motility and the area of absorption are reduced.
- Metabolism and drug elimination are diminished due to reduced renal and hepatic clearance.

There are also exceptions:

- In dogs, the oxidation function peaks at 8 weeks after birth and disappears after weaning.
- In ruminants, metabolic changes occur when they change from pre-ruminants to ruminants, due to changes in feeding.
- Eg., ceftiofur will be metabolized into ***dis-fluoril-ceftiofur, a*** metabolite that is much higher in ruminants than in pre-ruminants.

Gender

- Females metabolize drugs more slowly and are more susceptible to poisoning.
- Studies in rats have shown that males have higher metabolic capacity for: alkaloids, piramidon, morphine, hexobarbital and pentobarbital.
- For example, rat females are more sensitive to strychnine sulphate than males:
  82% of females die taking a 2mg / kg.bw. dose of strychnine sulphate, compared with males ,only 30% died.

Gestation

Administration of drugs during gestation is contraindicated, because they will cross the placental barrier and reach the fetal circulation.

In gestating female rats and rabbits, glucuronidation (major route of metabolism) is reduced to 50%.

Cause: high levels of progesterone and pregnanediol (considered glucuronyl-transferase inhibitors).

Similar findings were made also over sulfonated conjugates (A reduction in oxidative biotransformation has been observed for phenacetin and amino phenazone, in gestating females).

Feeding

Undernourishment, reducing protein intake, deficiency states (lack of minerals and vitamins) decrease the ability of metabolism.

Microsomal enzymes are most commonly affected by dietary factors. Reducing the amount of drug substance after oral administration results in decreasing the efficacy, due to gastro-intestinal pH change, formation of chelates, etc.

Eg., in the case of oral administration of : penicillin, diazepam, codeine, the increased gastric acidity will diminish the absorption of these drugs.

Alimentation with lipids = stimulation of bile secretion and increased bioavailability of liposolubles, eg: griseofulvin, albendazole, mebendazole, etc.

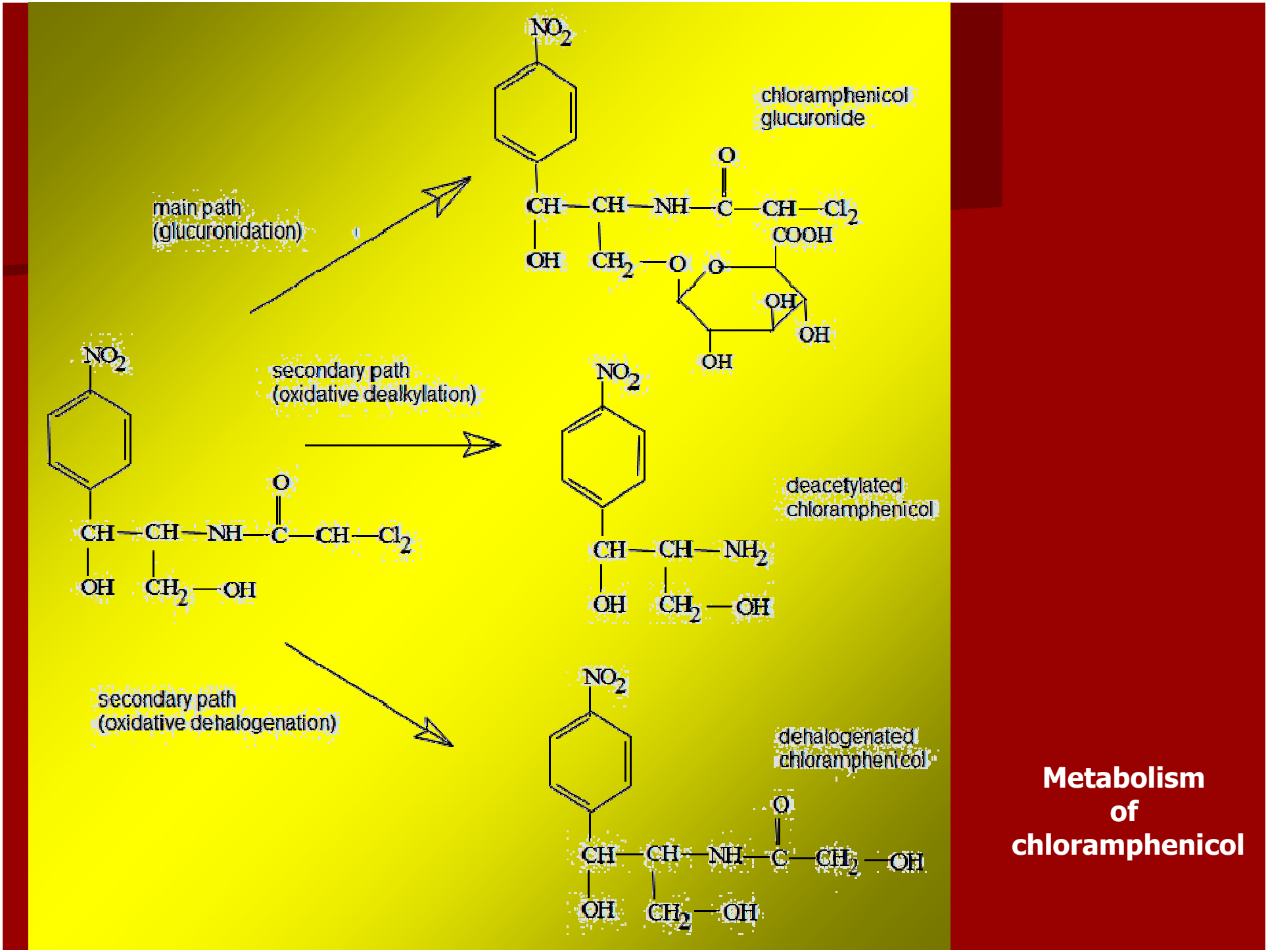
Health status

The normal functioning of the organs involved in metabolism, especially the liver, is an essential condition for carrying out this process. In animals with hepatic diseases, blood flow reduces = slow metabolism.

In this case, it is not recommended to administer:
lincosamides, β-lactams, macrolides and chloramphenicol.

Chloramphenicol for eg., metabolizes difficult especially in cirrhosis = hematopoietic accidents, result of reduced capacity for glucuronide conjugation.

Renal disease decreases the renal clearance for creatinine, frequently creating drug accumulations that can cause adverse reactions and intoxications. In the case of uremia, binding to plasma proteins and hepatic metabolism decrease significantly.



Metabolism of chloramphenicol

Genetic factors

Important differences in the metabolic capacity, conditioned by the specific enzymatic equipment for each individual: sensitizing genetic factors.

It is known that (due to enzyme polymorphism) there are individuals who genetically metabolize drugs more easily compared to others, the latter being more susceptible to drug poisoning.

The presence of sensitizing genetic factors has been demonstrated especially for improved breeds (ex: Arabian thoroughbred horses, Merinos sheeps, Landrace pigs, Supercuni rabbits, Cocker dogs etc.).

Exogenous

Factors

The circadian rhythm

Chrono pharmacology revealed differences in drug metabolism related to circadian rhythms in humans.

It has been found that the most active metabolism is reported around 2 AM, and the lowest at about 2 PM.

These metabolic phases have a maximum and a minimum specific value in animals too.

In sleep, the effect of the drug is closely related to the type of nervous activity of the animal.

This has been demonstrated by administering to animals of the same species, same gender, weight and age of CNS, depressants (hypnotic or narcotic), and it was found that the intensity and duration of the effect was different.

In most animals, narcotic sleep duration was average, but there were also identified limit situations (too long or too short).

Exogenous compounds

Chemical substances from the environment, (ex. insecticides, dyes, feed additives, auto oxidant substances etc.), ingested by animals through food and water, or entering the body in other ways, have a definite influence on the processes of metabolism.

Many of them have the effect of enzyme induction, especially after repeated contacts, when they produce a higher rate of metabolism (2 to 10 times).

Stress factors

Adverse conditions: cold, humidity, agglomeration, noise, increase the metabolic activity of microsomal enzymes, by stimulating the pituitary - adrenal reflex arc .

Stress increases the adrenal ascorbic acid, observed in treatments with phenobarbital (enzyme inducer).

Small amounts of radiation (ionizing, in particular) can act as stressors increasing the activity of microsomal enzyme systems.

Radiation reduces drug metabolisation by their effect on NADPH formation (nicotinamide adenine dinucleotide phosphate oxidase) and glucuronide conjugation.

Stages

of metabolism

Drugs entering the organism undergo transformation processes which lead to their elimination.

Metabolizing takes place, generally, after the pharmacodynamic action of the drug, but in some cases the active substance is produced from the metabolic transformation. In such cases we say that the substance was "activated".

For eg, acetanilide and phenacetin, two structurally close antipyretic analgesic substances, are metabolized in acetaminophen (which is actually the active ingredient that produces the pharmacodynamic effect.

The general rule is "deactivate" or "detoxify" by which drugs are inactivated, especially in the second phase of metabolism.

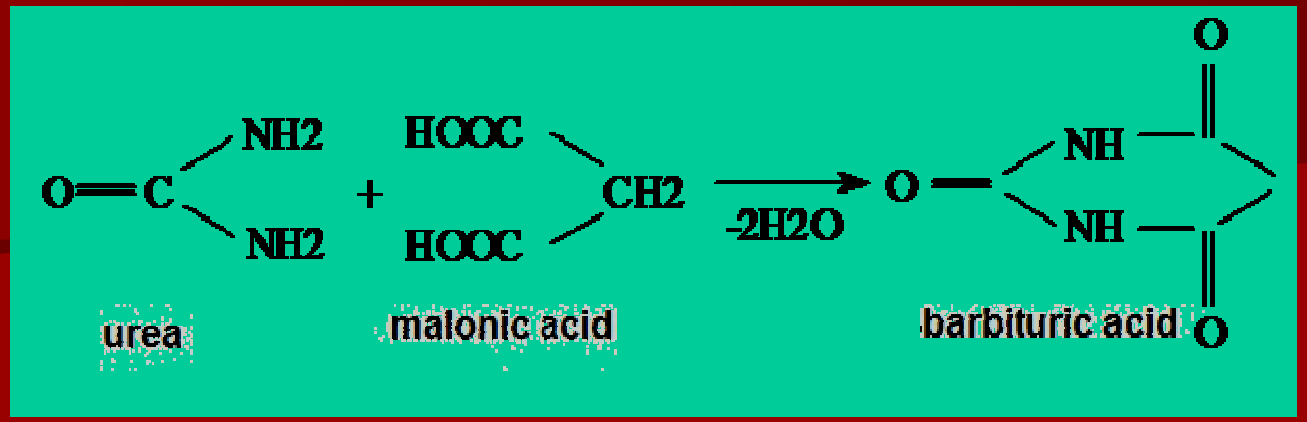
Some substances can be *removed* from the body without undergoing transformation processes.

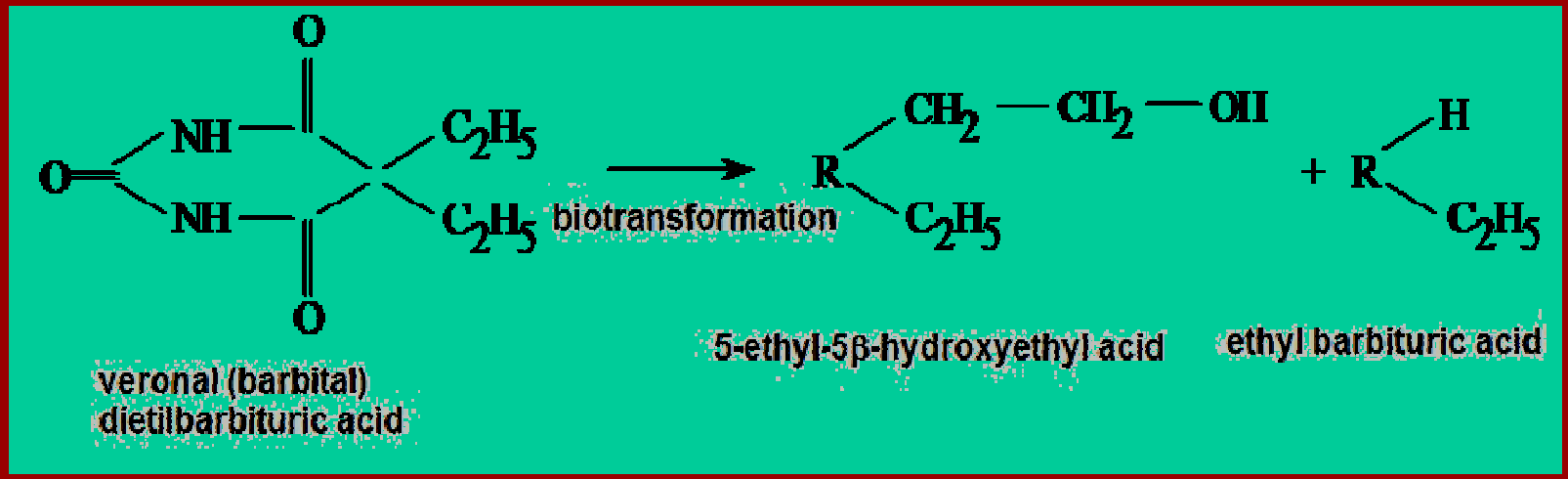
Such as: some inorganic substances, bromides, volatile narcotics, phenolphthalein, etc.

Others are metabolized in a smaller proportion, and can be recovered from urine as their active form (ex: penicillin, streptomycin).

- The purpose of the process of metabolism, is to increase the polarity and aqueous solubility of the substances, to increase renal elimination and to decrease tubular absorption.
- It is known that both the kidney, and the bile, excrete the polar compounds.
- Oxidation of CH3 in COOH (*biotransformation*) makes renal or biliar excretion of the drug, easier.

- Similar effects are observed by binding to the molecule of the radical sulfate (*conjugation*).
- Some drugs, that were initially considered not metabolized, were later found to be subject in a lower rate to the transformation process.
- For eg. veronal is eliminated in 95% of its unchanged form in urine, but 5% will undergo biotransformation, of which 3% by hydroxylation and 2% by oxidative dealkylation.





Biotransformation of barbital

Metabolization is achieved by two main processes:

- biotransformation and
- conjugation.

Biotransformation:

a process by which drugs are processed by oxidation, reduction or hydrolysis. During these processes the molecule will either maintain its size, or simplify it.

Conjugation:

a synthesis process in which the substance is amplified by binding a compound or a radical to the molecule.

Current drugs are generally more complex synthetic substances that are metabolized in several ways, resulting in a large number of metabolites.

Chlorpromazine, suffers many transformation processes, finally resulting in a total of more than 20 metabolites.

Drug

biotransformation

Biotransformation processes = activation / inactivation of the drug that renders non-polar compounds to polar, which will be removed as such or be subjected to processes of conjugation.

In general, biotransformation precedes conjugation reactions and sometimes medicinal substances undergo several successive biotransformation processes followed or not by conjugation.

Drug metabolism can begin:

- **after administration, before their resorption (in the digestive tract) or,**
- **in the internal medium of the body, immediately after resorption (in the blood) or,**
- **in the metabolization organs, the liver is the most important**

The metabolic processes from the digestive tract take place under the action of its own enzymes, but numerous transformations are produced by enzymes from the digestive microflora and micro fauna encountered in all species, especially in ruminants.

The presence of numerous esterase in the blood, leads to biotransformation by hydrolysis.

Thereby, intravenously administered procaine in horses with colic, is rapidly decomposed in: para-amino benzoic acid and diethyl-aminoethanol, (considered responsible for the calming, antispasmodic effect).

Most biotransformation processes take place in the main metabolic organ, the liver, but they may also occur in different proportions in the:

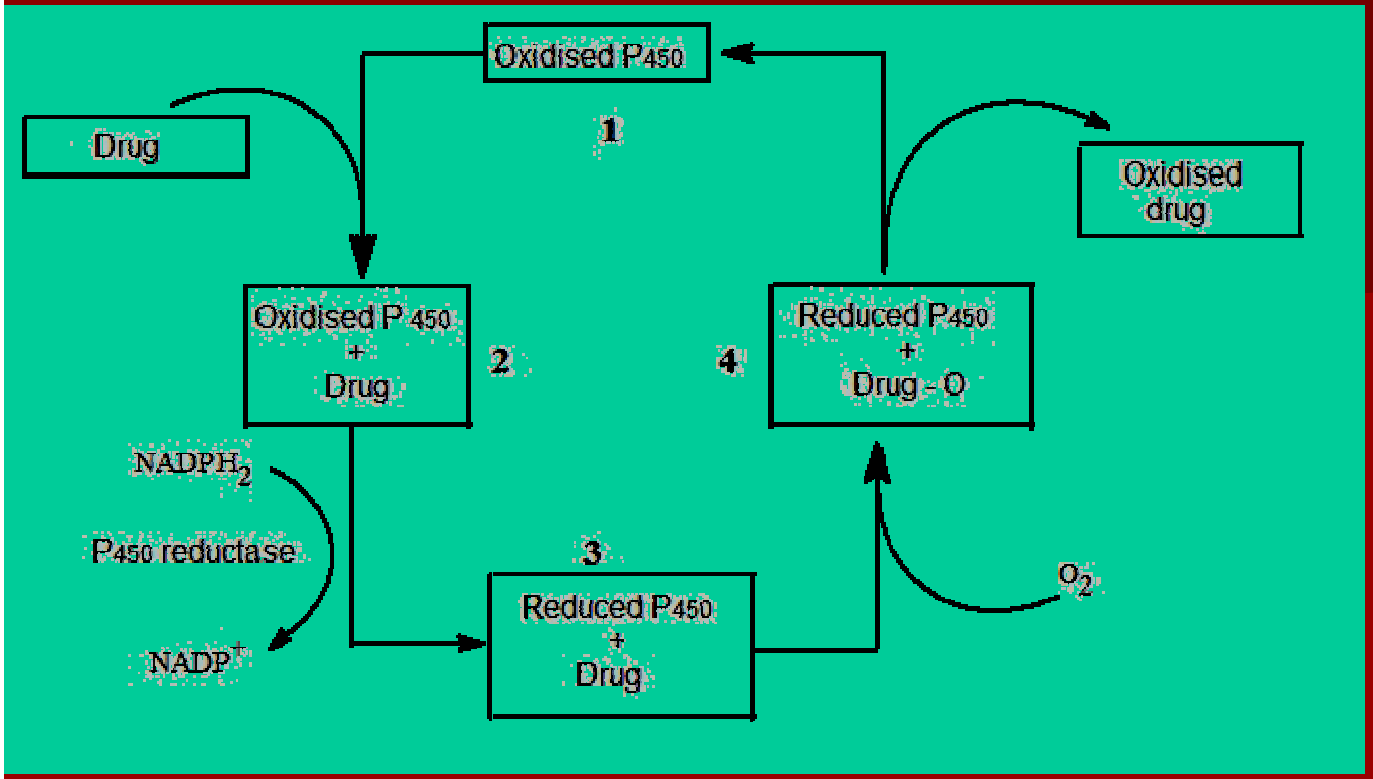
- kidney,
- spleen,
- lung,
- intestinal mucosa,
- blood and
- skin.

They occur under the action of metabolizing enzymes of xenobiotic substances (xenos = foreign; bios = life), but, as we have seen, also under the action of enzymes which metabolize nutrients.

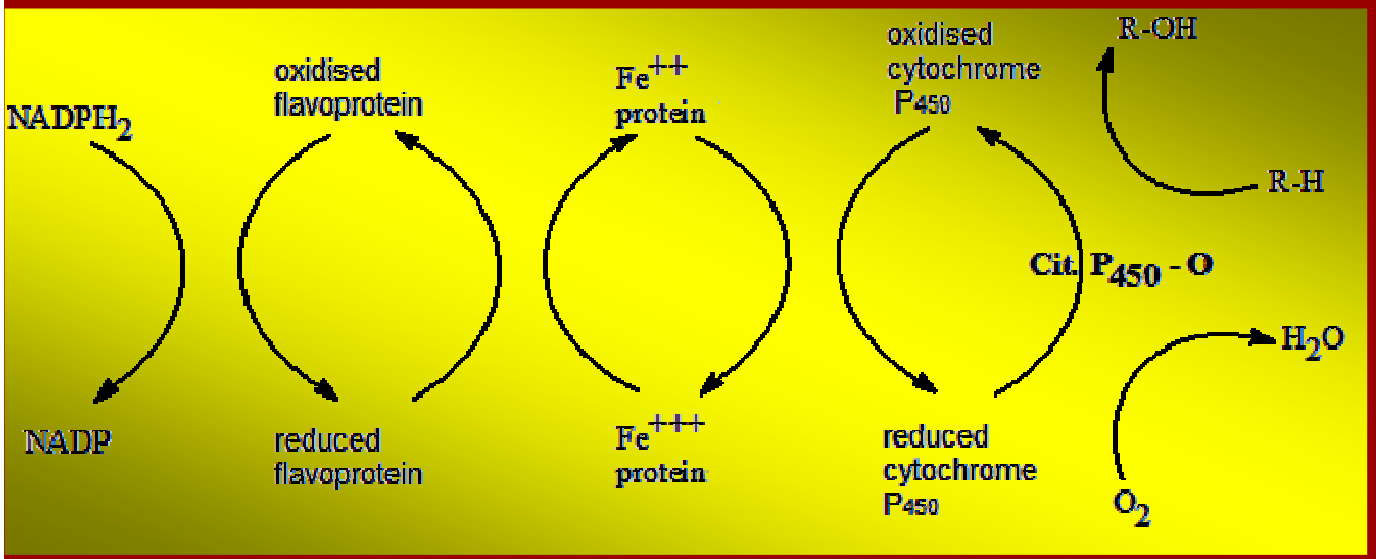
Microsomal oxidation reactions require cytochrome P-450 reduced nicotinamide adenine dinucleotide phosphate (NADPH2) and O2, being mediated by a coupled redox system consisting of : NADPH2, a flavoprotein, nonhemic Fe-protein and a hemoprotein known as cytochrome P-450.

NADPH2 acts as a hydrogen donor for the reduction of cytochrome P-450.

It oxidizes rapidly under the action of molecular oxygen (O2) and then gives O2 to the metabolized substance (after they previously coupled with the drug).



Microsomal
drug oxidation scheme



The coupled redox system
involved in the microsomal
oxidation of drugs

The processes of biotransformation are classified in processes of: *oxidation, reduction and hydroxylation*, these may be catalyzed by microsomal enzymes, no microsomal enzymes (mitochondria, cytoplasm, plasma blood) or enzymes from the intestinal flora.

Classification of drug biotransformation
(Parke, cit. Gherdan)

Type of reaction	Microsomal enzymes of metabolisation	Nonmicrosomal enzymes of metabolisation	Enzymes from intestinal flora
Oxidation	Aromatic hydroxylation Acyclic hydroxylation Alicyclic hydroxylation Epoxidation N-oxidation S-oxidation Desulphurisation Dealkylation Deamination	Oxidation of alcohols (cytoplasm) Oxidation of aldehydes (cytoplasm) Acyclic aromatization (mitochondria) Deamination (mitochondria & blood plasma)	-
Reduction	Nitro reduction Azo reduction Reductive dehalogenation	Reduction of sulfur-oxides and N-oxides (cytoplasm) Reduction of disulfide	Reduction of N-oxizides Azo reduction Dehydroxylation
Hydrolysis	Ester hydrolysis	Hydrolysis of esters and amides (blood plasma) Hydrolytic dissolution of cyclic compounds Hydrolytic dehalogenation	Hydrolysis of esters Hydrolytic dissolution of heterocyclic compounds

I. Microsomal

biotransformation

A. Microsomal oxidation

The microsomal oxidation process takes place under the action of some mixed-function oxidase of the smooth endoplasmic reticulum.

In "teaching terms" we are talking about : ten types of microsomal oxidation.

1. Aromatic hydroxylation

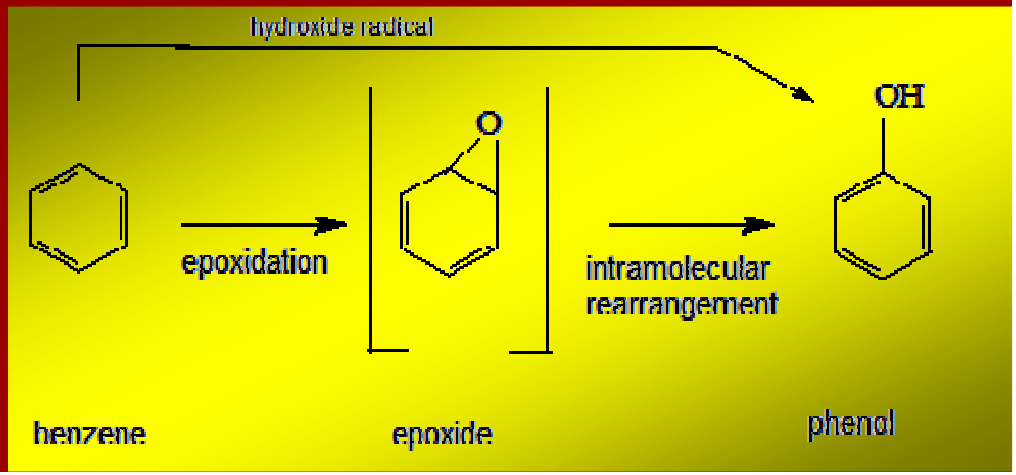
introducing a hydroxyl radical in an aromatic ring

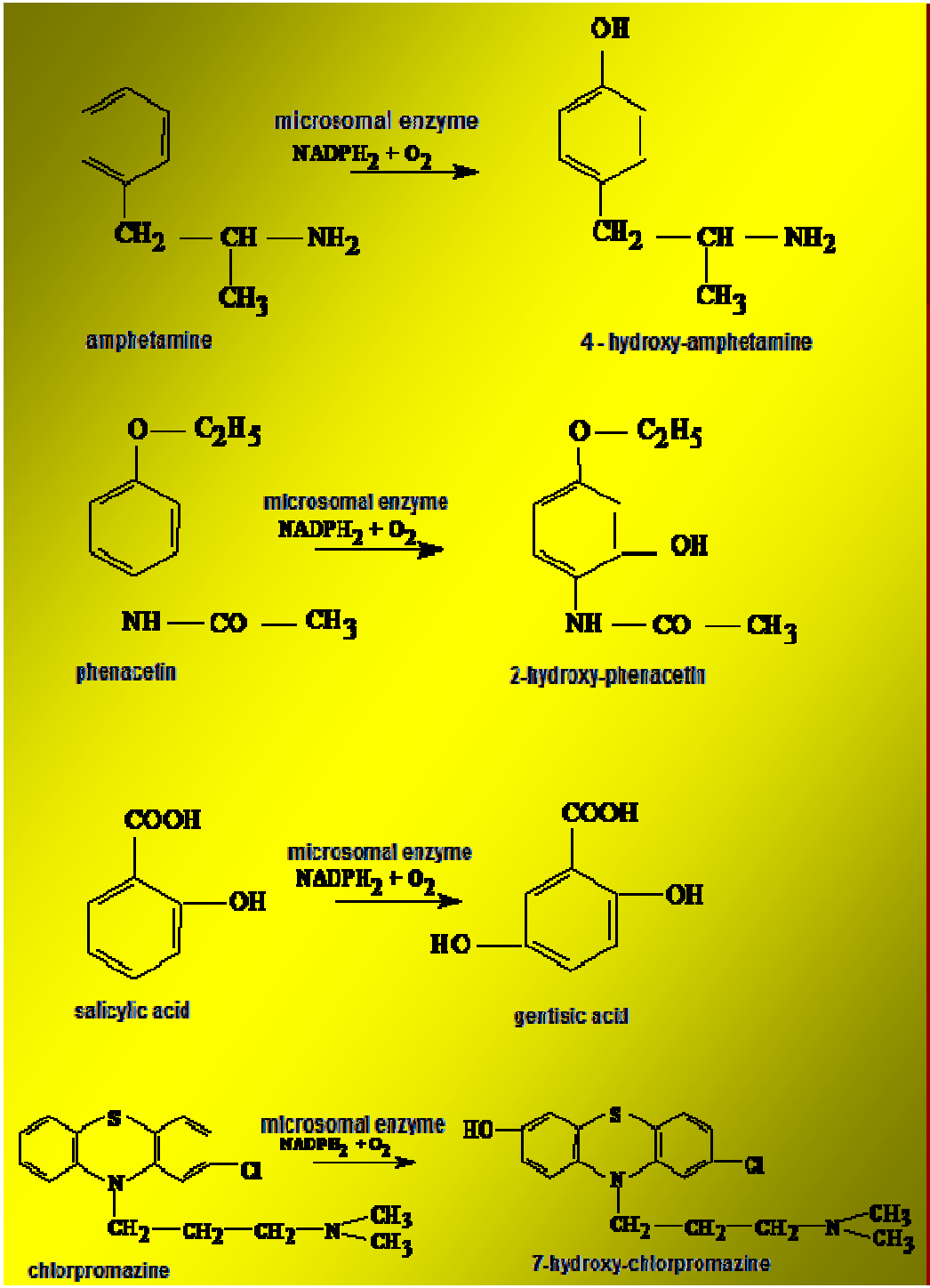
Hydroxylation of aromatic rings to give phenols is preceded by two mechanisms:

first, gives monophenols = hydroxylation via free radical transfer mechanism or passing through the phase of epoxide followed by intramolecular rearrangement;

second = epoxide formation which reacts with water and gives: **dihydrodiols, phenols and catechols (eg. case of naphthalene, amphetamine, phenacetin, ox. salicylic acid, chlorpromazine, etc.).**

Aromatic hydroxilation



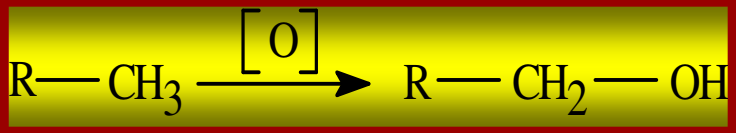


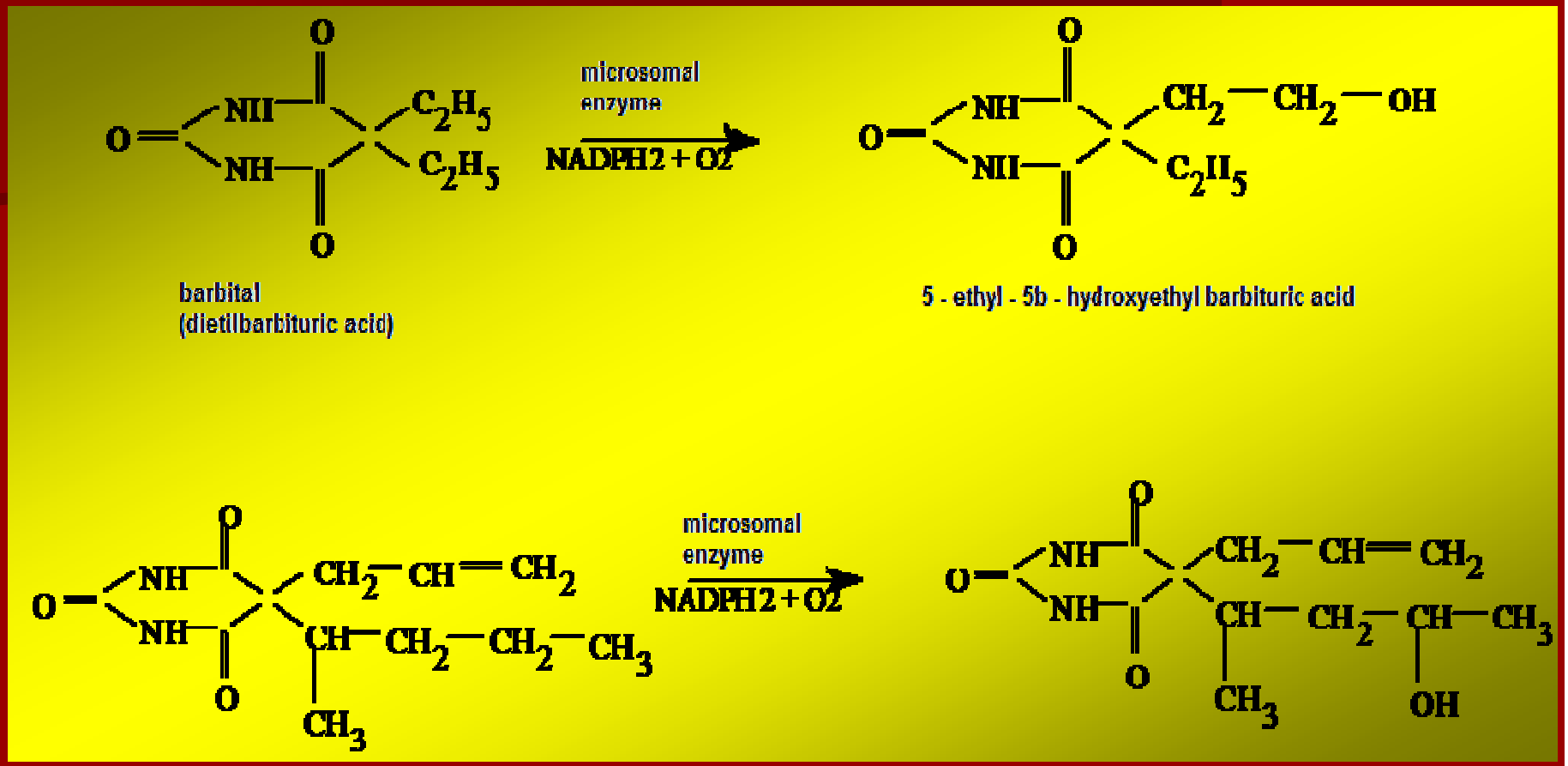
Aromatic hydroxylation of certain drugs

2. Acyclic (aliphatic) hydroxylation

In barbiturate medications (ex: barbital, pentobarbital, secobarbital, etc.), it refers to the oxidation of the side chains into corresponding alcohols, under the action of microsomal enzymes.

Hydroxylation can occur at any position of the alkyl chain, but usually occurs in the last carbon (ω-1) :



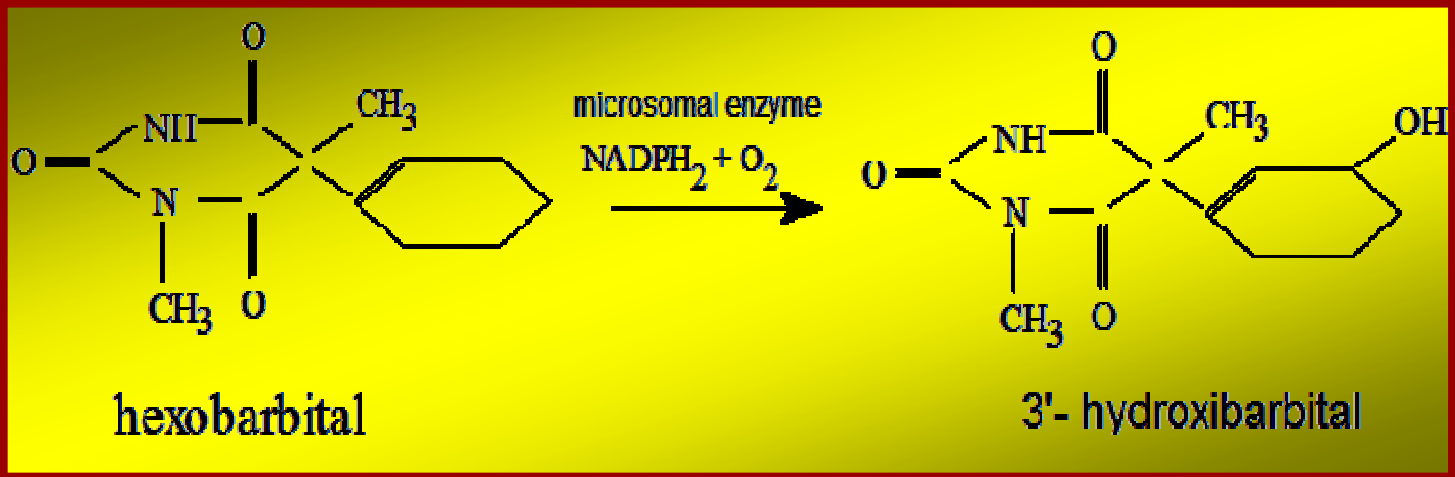


Types of acyclic (aliphatic) hidroxylation

3. Alicyclic hydroxylation

Cyclohexane derivatives are hydroxylated by the action of hepatic microsomal enzymes in cyclohexanol.

One of the metabolic pathway of hexobarbital is the alicyclic hydroxylation:

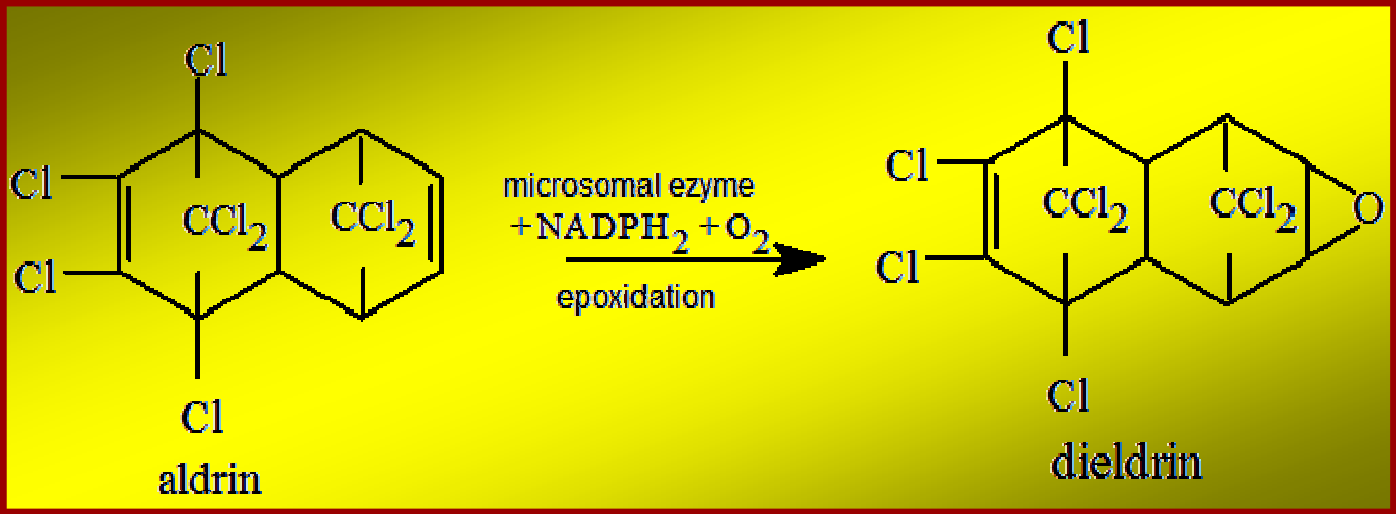


Alicyclic hydroxylation of hexobarbital

4. Epoxidation

Epoxides are intermediate compounds in the hydroxylation of aromatic compounds, but can be met in the form of stable products; in the case of insecticides from chlorinated cyclodienes group.

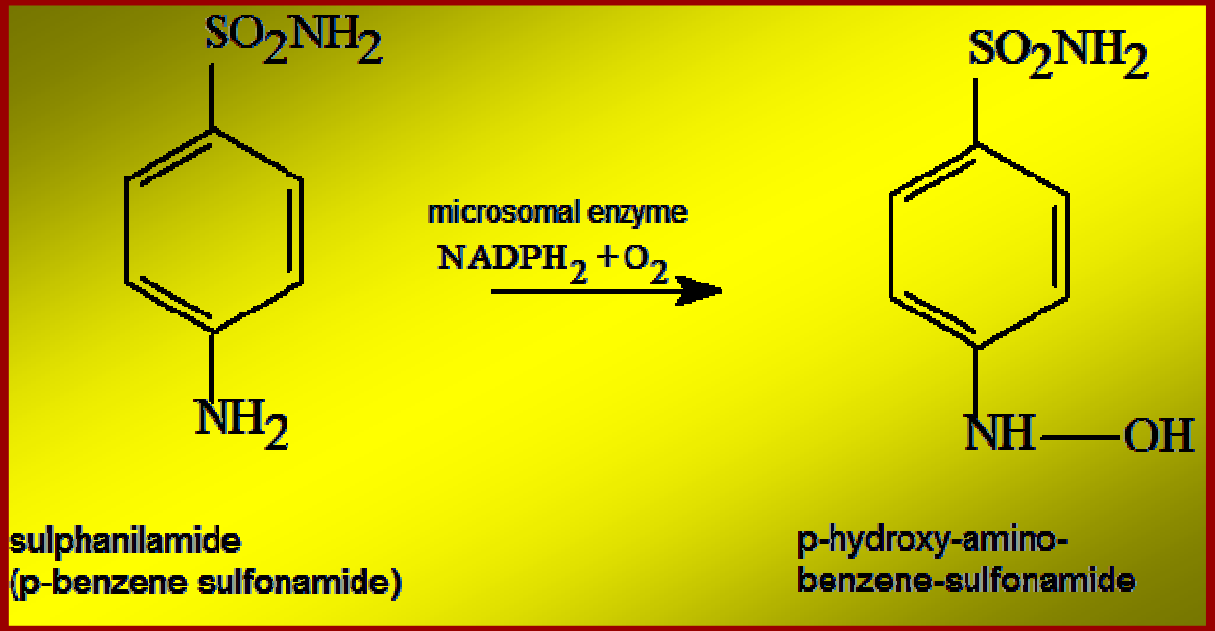
So, aldrin is metabolized to dieldrin (active compound, highly toxic).



Epoxidation of aldrin

5. N-hydroxylation

Aromatic amines undergo hydroxylations of the amino group forming hydroxyl-amino compounds.
The urethane, for example, is hydroxylated in N-hydroxy-urethane.
Sulphanilamide can be hydroxylated in the amine group (N4), p-hydroxy-amino-benzene sulfonamide being created.
Hydroxyl-amino metabolites are more toxic than the amine compounds which are produced.

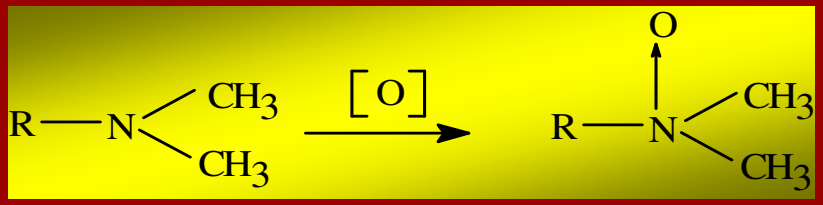


N–hidroxylation of sulfanilamide

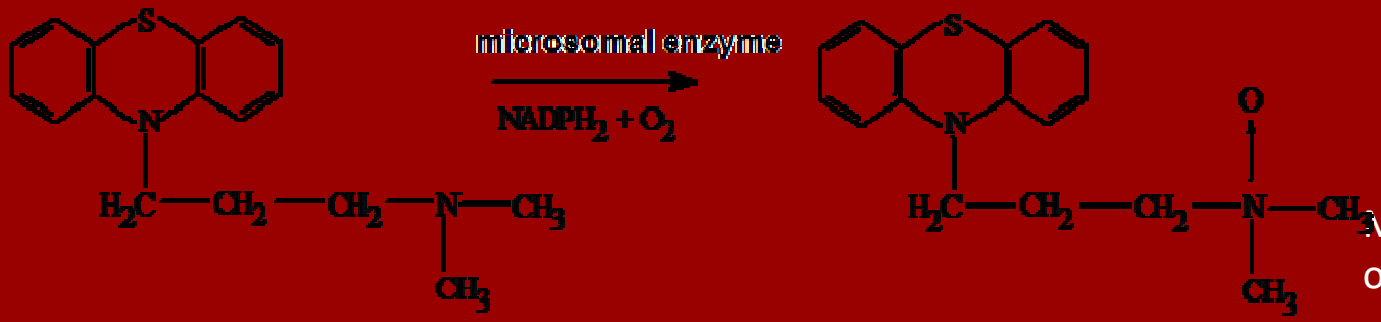
6. N-oxidation

Secondary and tertiary amines are metabolized to the corresponding N-oxides, which are intermediates in the N-dealkylation of these amines.

One of the metabolic pathways of chlorpromazine is N-oxidation :



Chlorpromazine's N-oxide is found in the urine of animals and humans, in some species being the major metabolite.



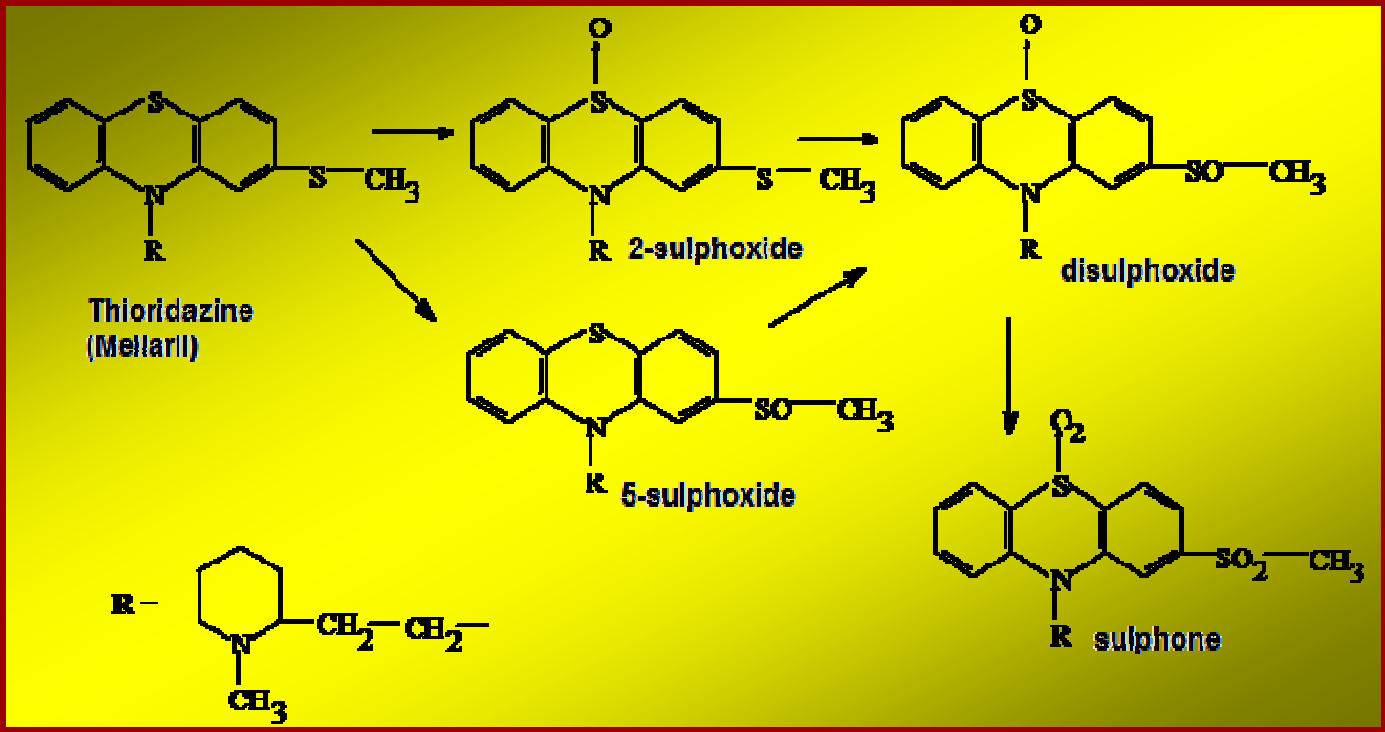
Chlorpromazine

Chlorpromazine N-oxide

Microsomal N-oxidation of chlorprpmazine

7. S-oxidation

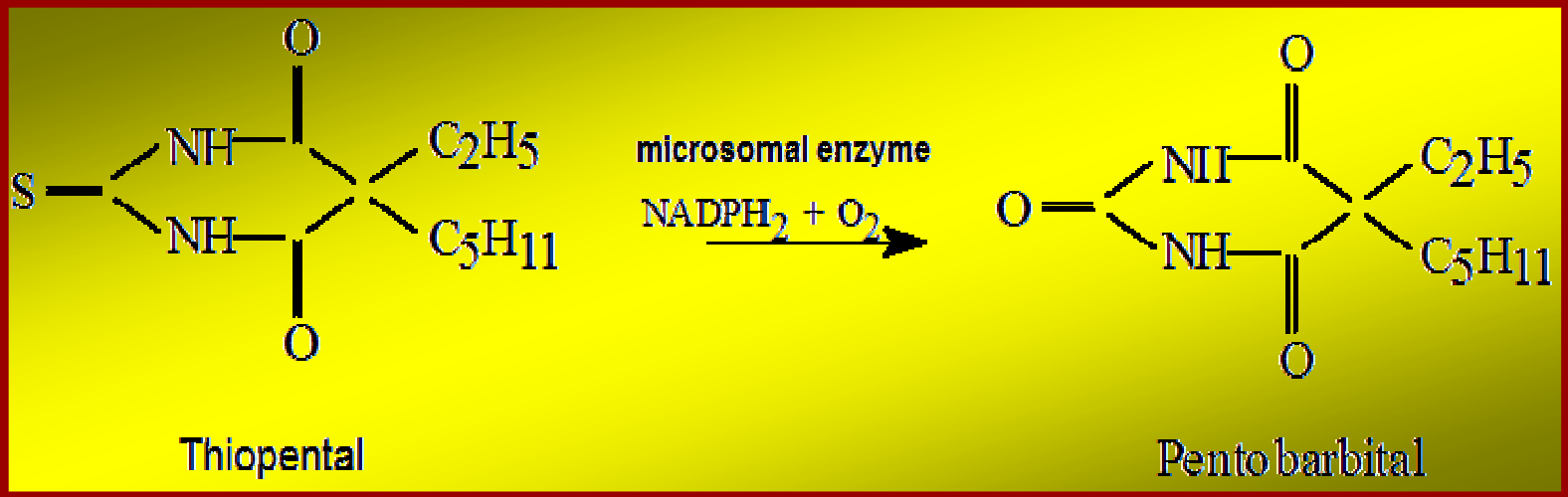
S-atoms of heterocyclic compounds (chlorpromazine, or other phenothiazines tranquilizers) undergo several processes of oxidation resulting in sulfoxides, and then sulphones.
E.g. thioridazine (Mellaril), phenothiazine neuroleptic (piperidyl derivative), undergoes a double S-oxidation.



S - oxidation of thioridazine

8. Desulphurization

Thiobarbiturics are desulphurised oxidatively and converted into the corresponding oxibarbiturates. So, thiopental is metabolized to pentobarbital, an active compound from the hypnotics group.

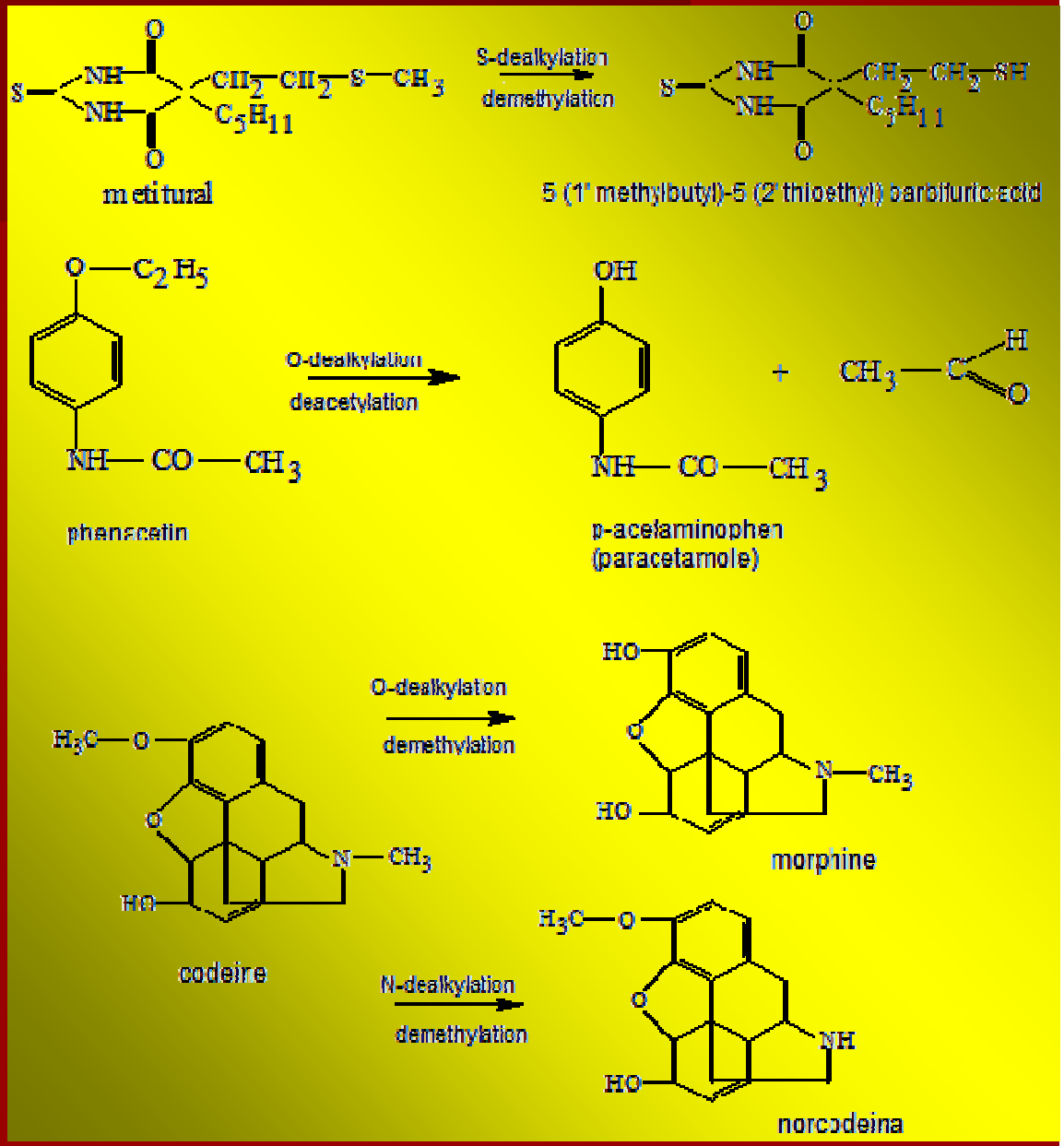


Oxidative desulfurization of thiopental

9. Dealkylation

By oxidative dealkylation the alkyl groups are removed under the action of microsomal enzymes. Depending on the compounds to which the alkyl groups are connected to:

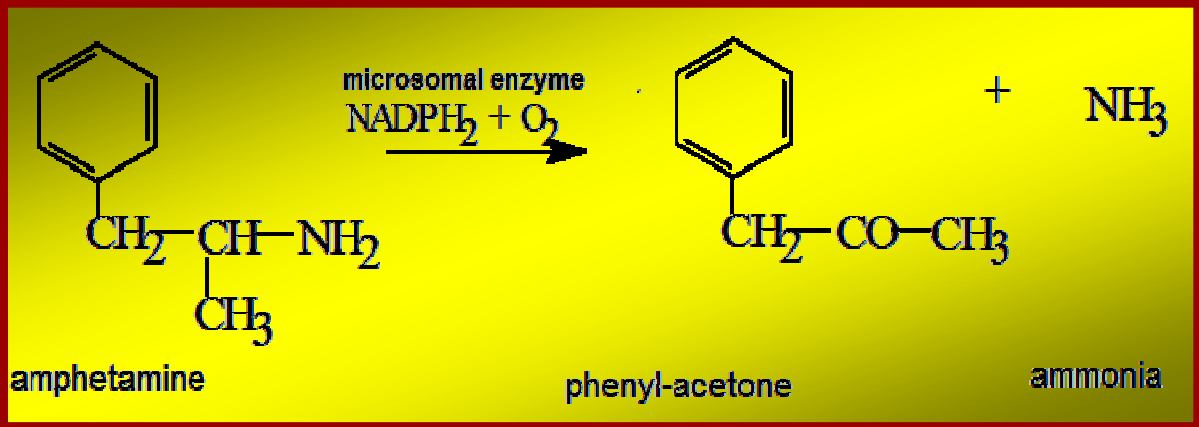
- **O-dealkylation,**
- **S-dealkylation,**
- **N-dealkylation**



Oxidative dealkylation of drugs

10. Oxidative deamination

Aside from mono-amino-oxidase (MAO), a mitochondrial enzyme, in the liver it is also known another microsomal enzyme , which deaminates amphetamine.



Oxidative deamination of amphetamine

B. Microsomal reduction

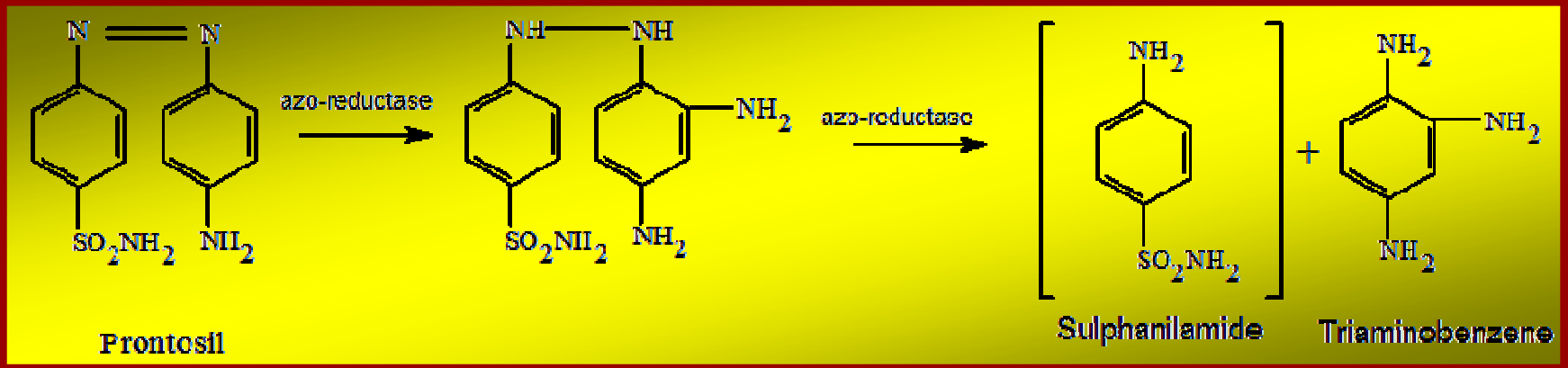
Microsomal reduction reactions occur under the action of reductases in the smooth endoplasmic reticulum of liver cells.

There are three processes, that catalyze the reduction of azo and nitro groups, as well as the dehalogenation processes.

1. Azo-reduction

Under the action of microsomal azo-reductase, azoic bonds are undone, after passing through an intermediate hydrazo compound:

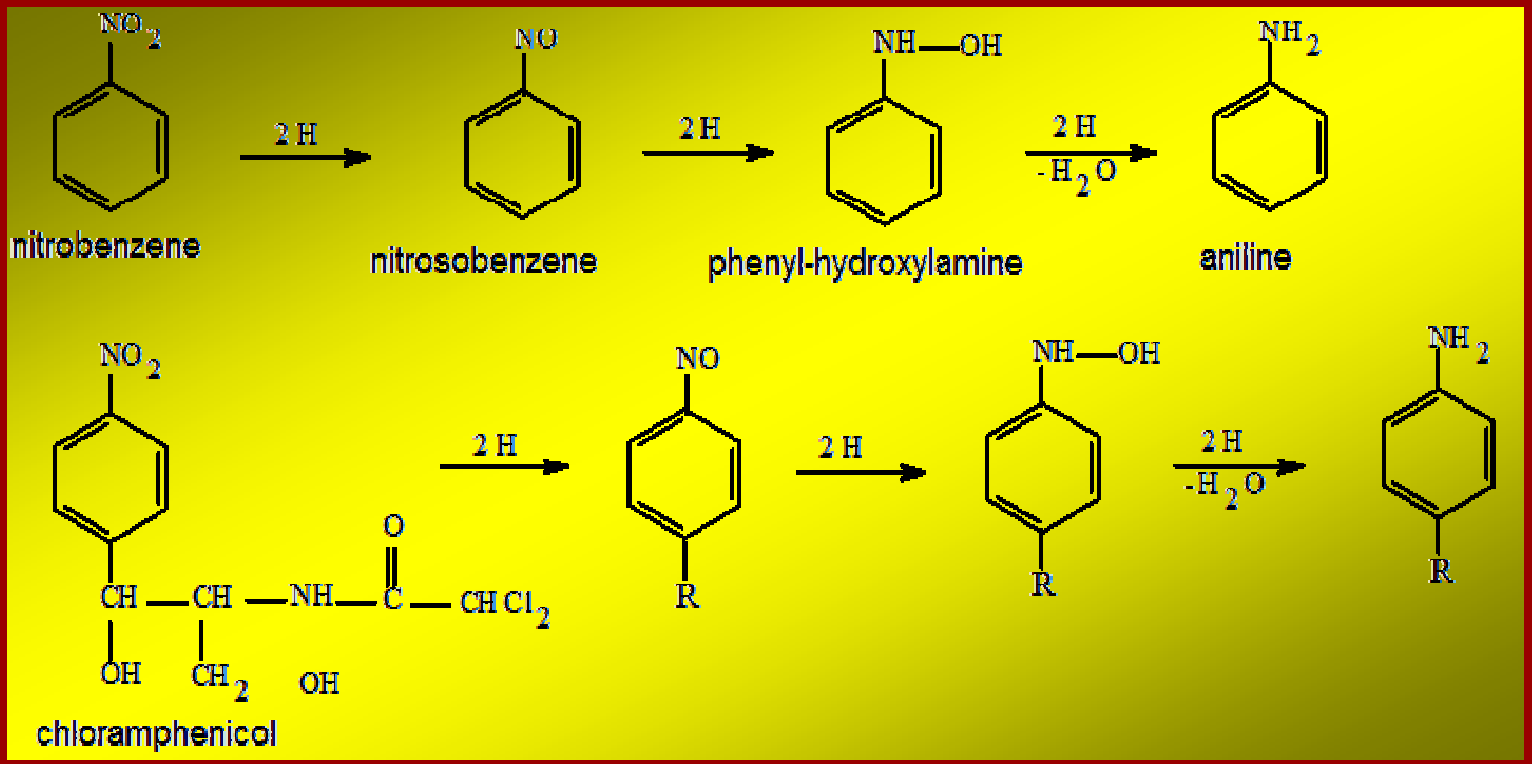
Eg. prontosil is metabolized by azo-reduction in a sulphanilamide (active compound) and a triaminobenzene. After the discovery of this pathway, prontosil was abandoned, and nowadays only sulphanilamide derivatives are used.



Azo-reduction of prontosil

2. Nitro-reduction

aromatic nitro compounds (e.g. nitrobenzene, chloramphenicol) are reduced by the action of microsomal nitroreductaze.



Microsomal nitro-reduction of drugs

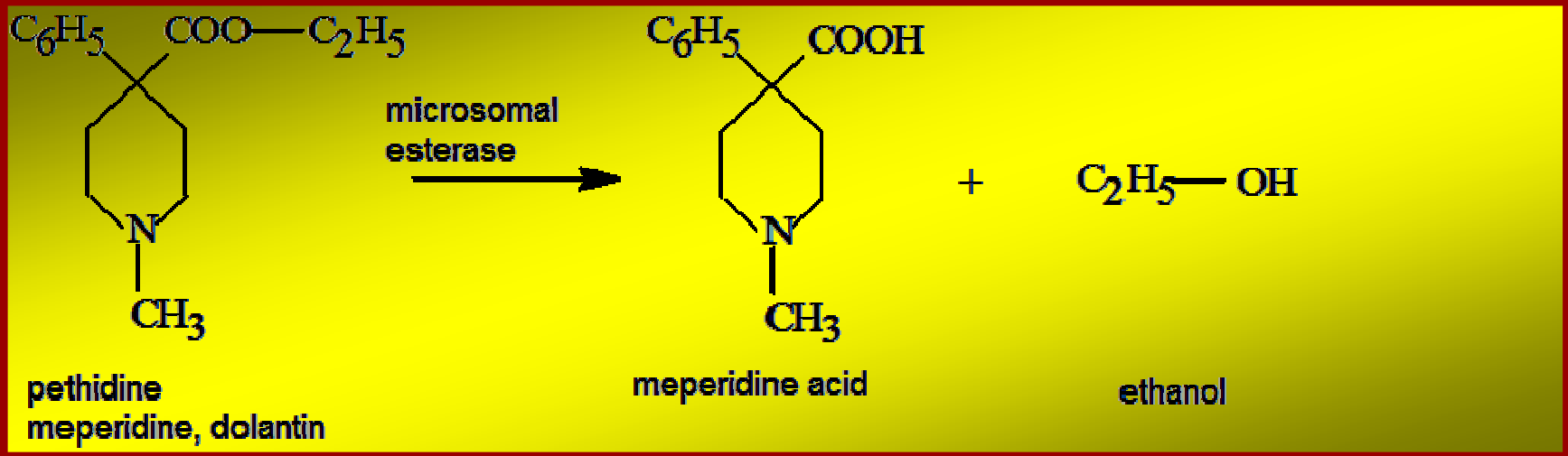
3. Dehalogenation

Halogenated compounds are metabolized by microsomal enzymes.
Ions of: Cl, Br and I are undone, and replaced with H.
Ions of Fluorine remain stable!
Halothane, a volatile narcotic, is metabolized by reductive dehalogenation:

Schematic reductive dehalogenation of halothane

C. Microsomal hydrolysis

- Esters and amides are metabolized by hydrolysis, catalyzed by esterases and amidases in blood and liver.
- Pethidine (Mialgin, Dolantin), widely used analgesic substance, is metabolized by microsomal esterases.



Microsomal hydrolysis of pethidine

II. Non microsomal

biotransformations

A. Non microsomal oxidation

1. Oxidation of alcohols

Primary alcohols are metabolized to aldehydes. The process is catalyzed by alcohol dehydrogenase, found in the cytoplasm of liver cells, kidney and lung and uses NAD or NADP as coenzyme.

This reaction is reversible:

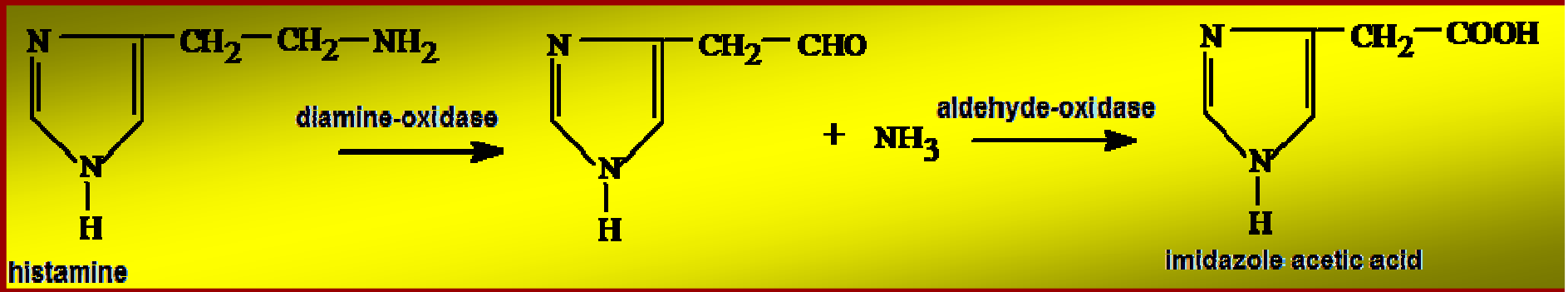
2. Oxidation of aldehydes

The aldehydes are oxidized in the corresponding carboxylic acid, through this enzymes : aldehyde oxidase, xanthine oxidase and NAD-specific aldehyde dehydrogenase.

3. Oxidative deamination

- Non microsomal tissue or plasma enzymes (mono and di-amino oxidases) deaminates primary, secondary and tertiary amines.

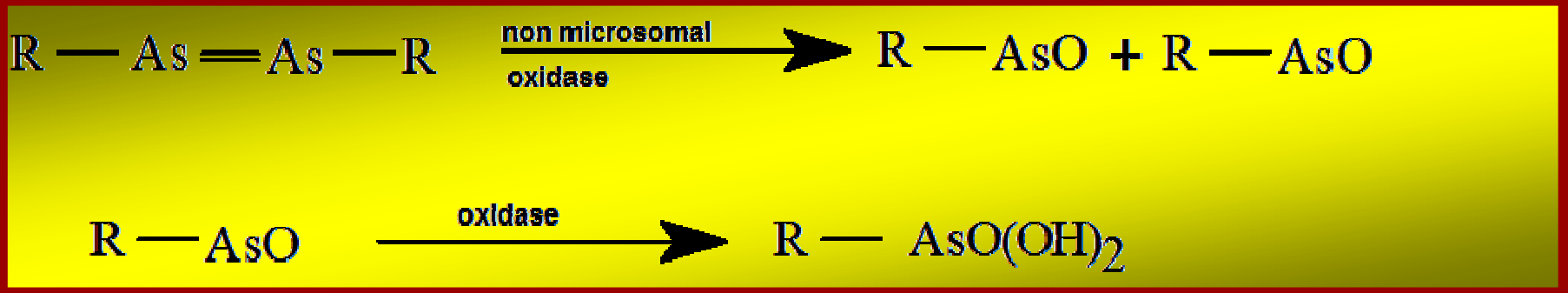
- Histamine is deaminated by a non microsomal diamine oxidase and is converted to aldehyde, and then under the action of aldehyde oxidase in imidazole acetic acid:



Non microsomal oxidative deamination of histamine

4. Oxidative cleavage of arsenobenzenes

- Arsenobenzenes are split in the organism by the action of an oxidase, with an unspecified location, in the corresponding arsenoxides, and then into arsenic acids :



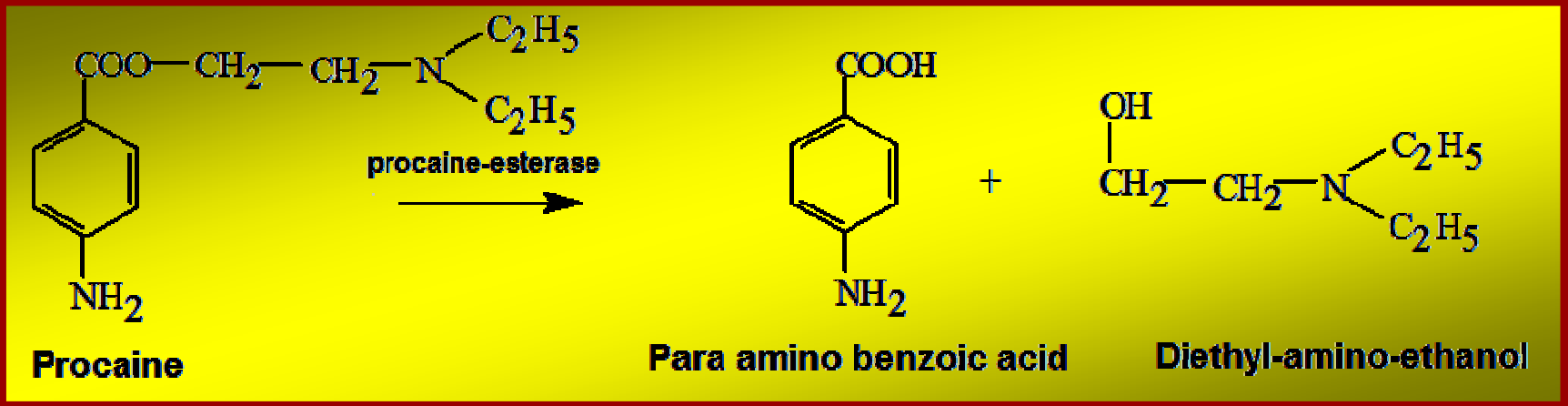
B. Non microsomal reduction

1. Aliphatic dehydroxylation

- Non microsomal reduction process, that can be found in the metabolism of norepinephrine.

C. Non microsomal hydrolysis

- Numerous non microsomal esterases in plasma, red blood cells or in the cytoplasm of cells hydrolyze drugs or other substances.
- Acetyl cholinesterase, one of the esterases, has a physiological role in the splitting of acetylcholine.
- hydrolysis of procaine in p-aminobenzoic acid and diethylaminoethanol is catalyzed by plasma procaine esterase



Non microsomal hydrolysis of procaine

Biotransformation

by the action of digestive microflora

Microflora of the digestive tract is able to mediate the metabolic transformations by hydrolysis and reduction reactions.
e.g. phthalylsulfathiazole is transformed by GI microflora in sulfathiazole.

Antibiotics and chemotherapies may affect the eubiosis of the environment and destroy the GI microflora, affecting the metabolism of other drugs.

We can mention here:

- reduction of azo-compounds,
- hydrolysis of esters and glycosides,
- splitting of cyclic and heterocyclic compounds.

Conjugation of

drugs

Conjugation or synthesis reactions represent phase II of metabolization, because many substances have undergone a process of biotransformation (phase I), so that subsequently undergo conjugation reactions.

During the biotransformation, drugs can be subjected to various reactions:

- oxidation,
- reduction, or
- hydrolysis,

resulting in the introduction or dissolution of functional groups which increase the polarity of the molecule and serve as centers for the second phase of the metabolic response, conjugation.

By synthetic reactions of conjugation, drugs (in their original form) or their metabolites *are combined with endogenous compounds*, as: glucuronic acid, glycine, glutathione, sulfate, methyl, acetyl groups etc.

Conjugation pathways of drug
(synthesis Cristina, R.T. 2006)

Type of reaction	Conjugated group or compound	Functional group to which links
Acetylation	Acetyl radical	Amino, sulfonamide, hydrazino
Methylation	Methyl radical	Hydroxyl, amino, thiol
Sulphono-conjugation	Sulfate radical	Hydroxyl, amino
Glucuronide conjugation	Glucuronic acid	Hydroxyl, carboxyl, amino, thiol
Peptide conjugation	Glycine, glutamine and other amino acids.	carboxyl
Mercaptation	Cysteine or glutathione	Epoxy, halogen, nitro, sulfonamide

1. Acetylation

Metabolic pathway of compounds with -NH2 and –OH groups.

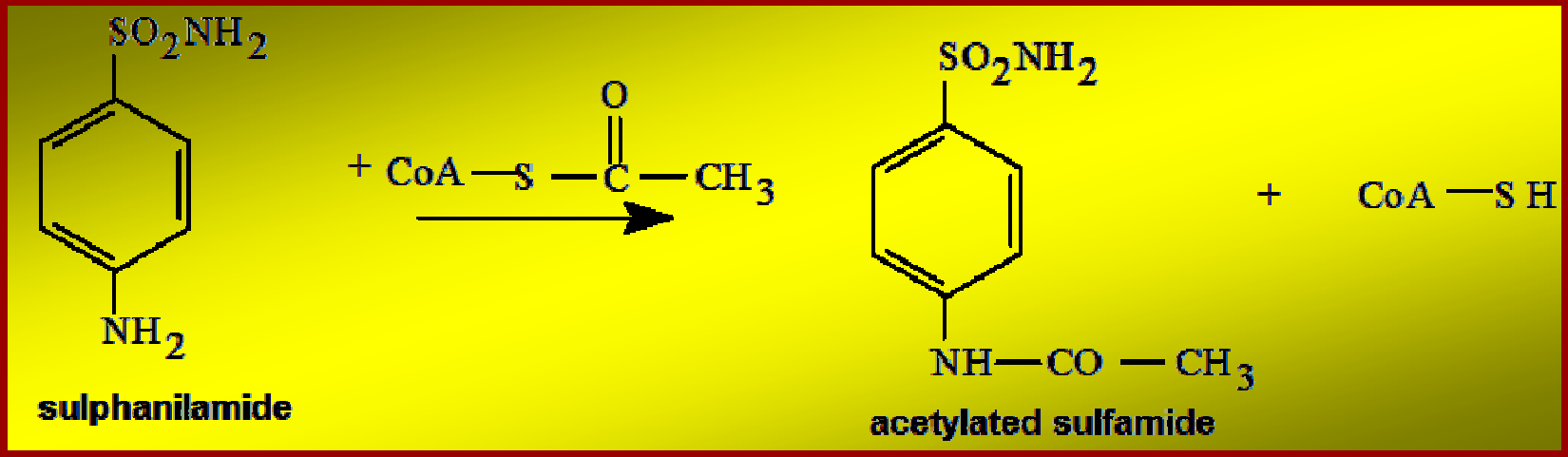
The most important acetylation is found in primary amines, in: aniline derivatives, sulfonamides, aminophenazone ,etc..

Acetylation involves the enzymatic transfer of the acetyl group from acetyl-CoA with the help of acetyl-transferase.

Sulfonamides, especially classic ones, are metabolized by acetylation at N4 (amino nitrogen).

Dogs, for eg., are unable to acetylate aromatic amines, producing instead a reverse reaction of "deacylation" of acetylated aliphatic amino acids.

Chickens manage "deacetylation" of acetylated aryl amines.

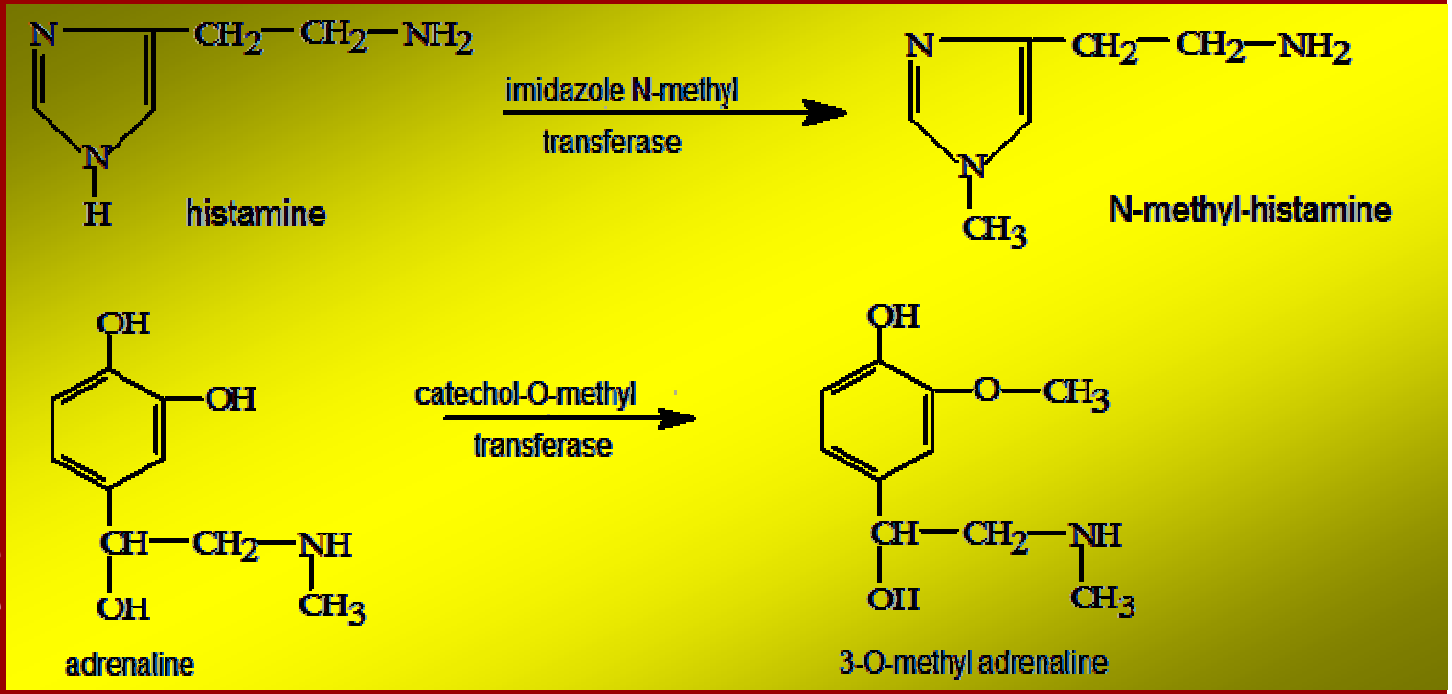


Acetylation of sulfonamides

2. Methylation

Conjugation reaction which transfers methyl groups from coenzyme S-adenosyl-methionine, under the action of methyltransferases.

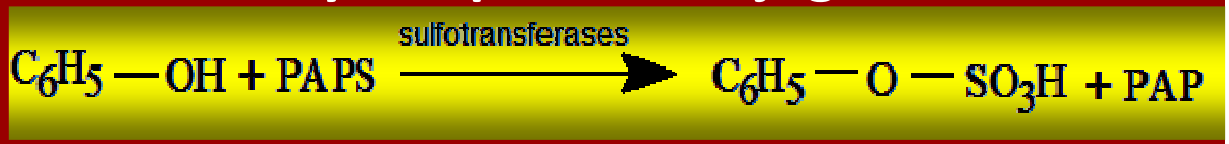
Numerous endogen compounds and some drugs (as phenols, amines, thiols etc) are metabolized this way. Histamine and adrenaline are metabolized through methylation.



Methylation of histamine and adrenaline

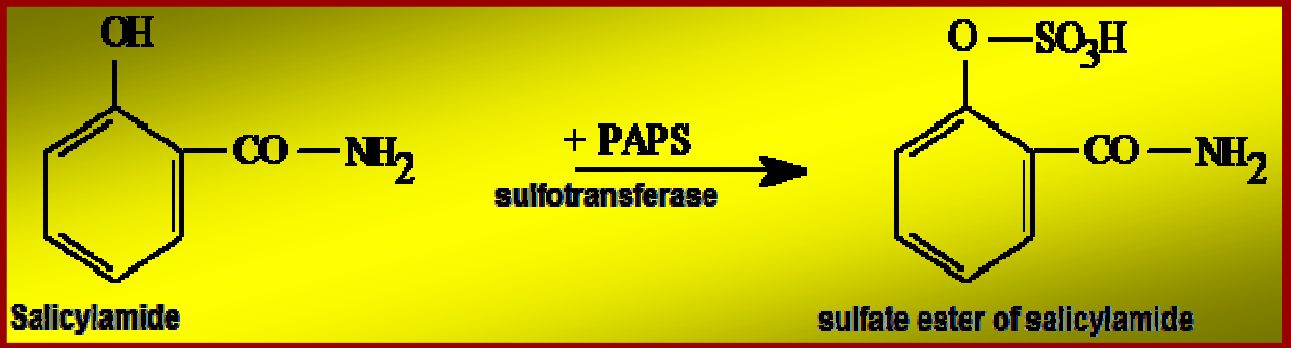
3. Sulphono-conjugation

- Takes place by: binding the sulphate radical to alcohol, phenol, aromatic amine and sterol type compounds, in hepatic cytosol.
- Eg. Phenol is transformed by sulphono-conjugation in phenyl-sulfate:



$$C_6H_5-OH + PAPS \xrightarrow{\text{sulfotransferases}} C_6H_5-O-SO_3H + PAP$$

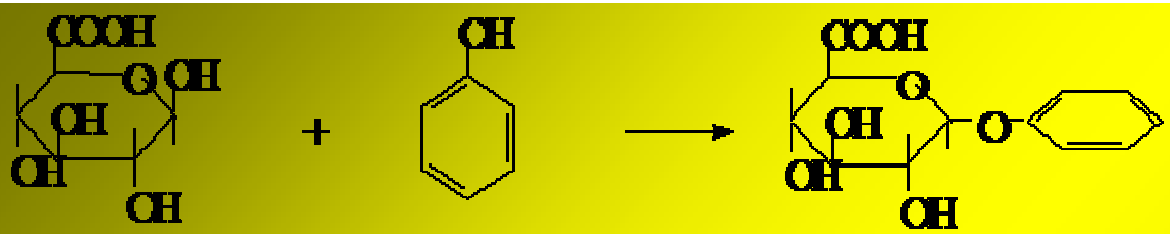
by sulphono-conjugation ethanol gives ethyl sulfate:
C2H5-O-SO3H,
aromatic amines (aniline) give sulphamates: C6H5-NH-SO3H
one of the metabolic pathways of salicylamide:



Sulphono-conjugation of salicylamide

3. Glucuronide - conjugation

- The most important metabolic pathway of conjugation, to all mammals except cats.
- is achieved by binding glucuronic acid to: alcohols, phenols, organic acids, amines, thiols and hydroxylamino compounds, in: liver, kidney and gastrointestinal tract, under the action of microsomal enzymes.
- Involves the transfer of the glucuronyl group from UDPG (uridine-diphosphate-glucuronic acid) coenzyme to drug.
- alcohols and phenols = conjugated in ether glucuronides,
- and carboxylic acid in ester glucuronides.
- amines form N-glucuronides and
- Thiols in S-glucuronides



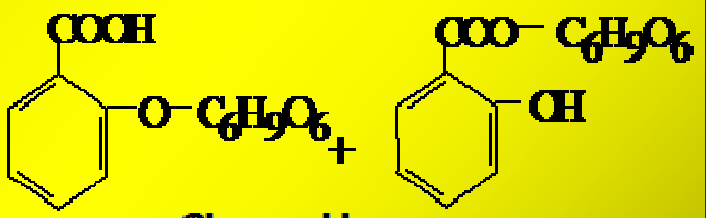
glucuronic acid

phenol

phenyl-glucuronide

O-glucuronides

salicylic acid

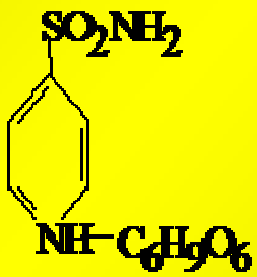


ether type

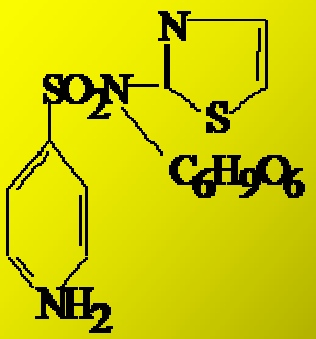
Glucuronides

ester type

N-glucuronides



Sulfanilic amide N_4 glucuronide

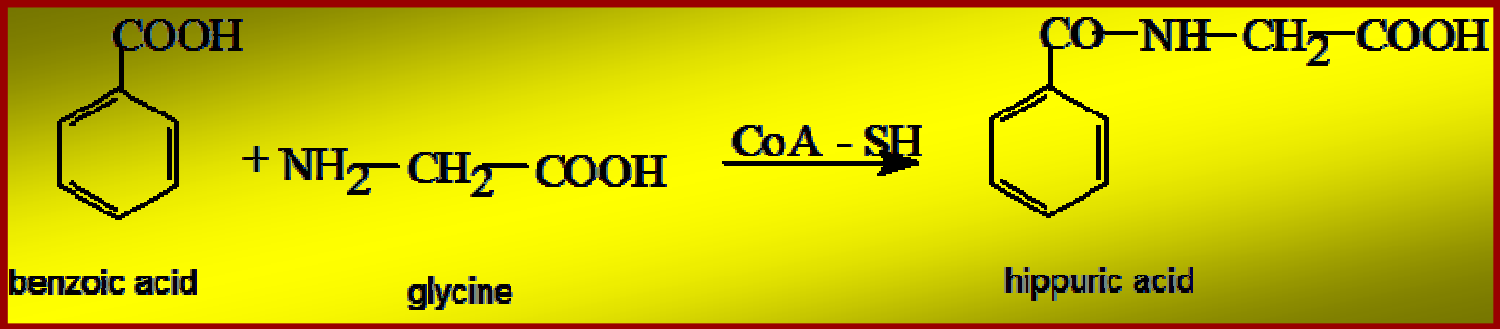


sulfathiazole N_1 glucuronide

Glucuronide -conjugation of drugs

4. Peptide conjugation

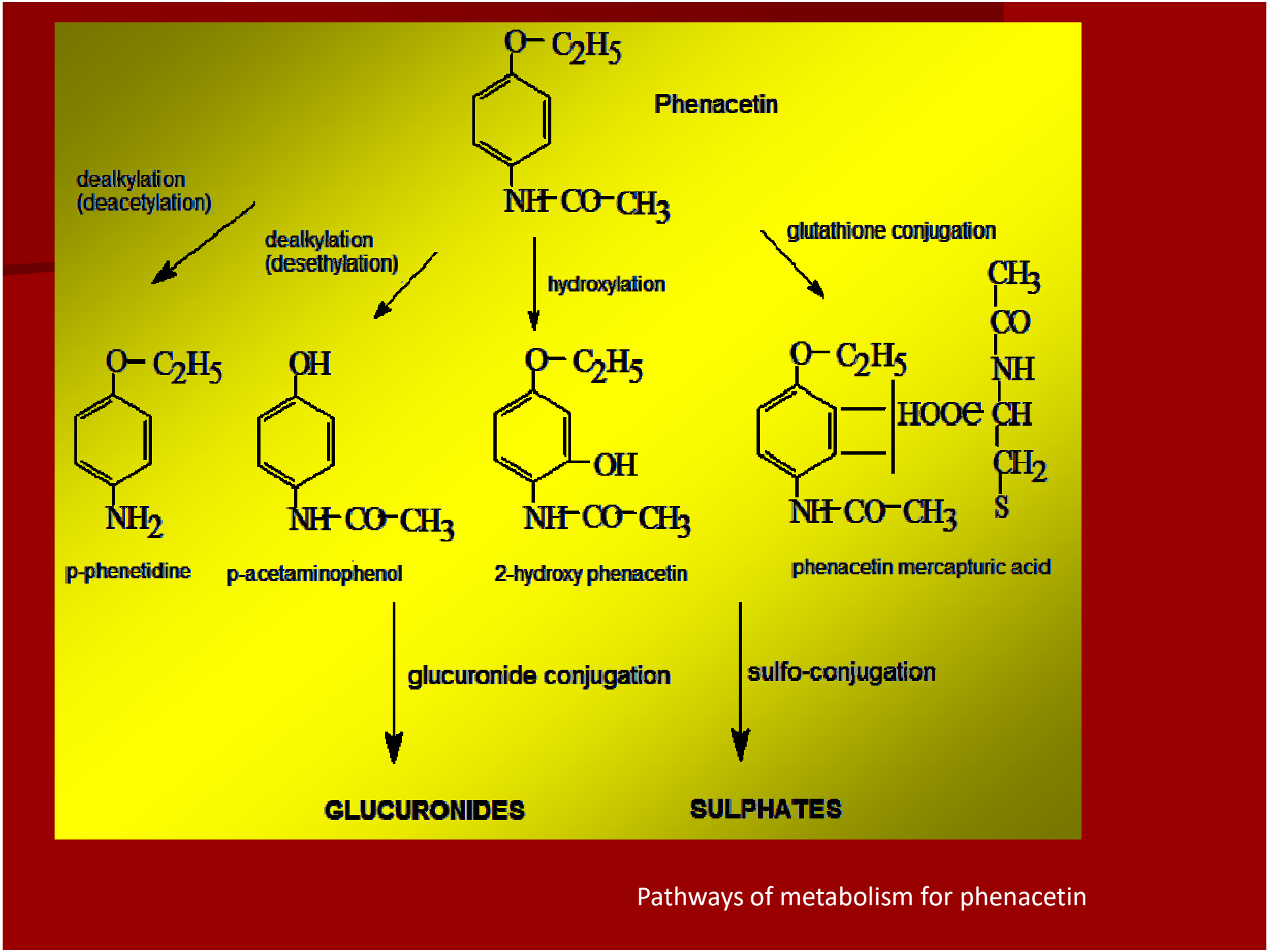
- The linking of some amino acids, especially, glycine (glycochol) or glutamine, with the formation of peptide-conjugates is a common reaction for carboxylic acids.
- The process requires the presence of coenzyme A.
- Benzoic acid is metabolized by conjugation with glycine, resulting hippuric acid.



Glicino - conjugation of benzoic acid

5. Mercaptation

- Conjugating drugs with cysteine or glutathione gives mercapturic acids.
- The reaction takes place within the kidney.
- Among the best-known drugs that are metabolized by mercaptation are: arecoline, nitrofurans, some sulfonamides.
- Phenacetin (substance which is metabolized in several ways) suffers among other things, a process of mercaptation through glutathione conjugation



Pathways of metabolism for phenacetin

Thank you for your attention!