# **Drug metabolism and Cytochromes** P450 & **Bile**

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# Drug metabolism

- **Cytochromes: are** heme-containing proteins
- Heme is made up of a porphyrin ring containing an atom of iron.

- In the electron transport chain, they are involved as carriers of electrons
- The major respiratory cytochromes are classified as a, b, or c, depending on the wavelengths of the spectral absorption peaks.

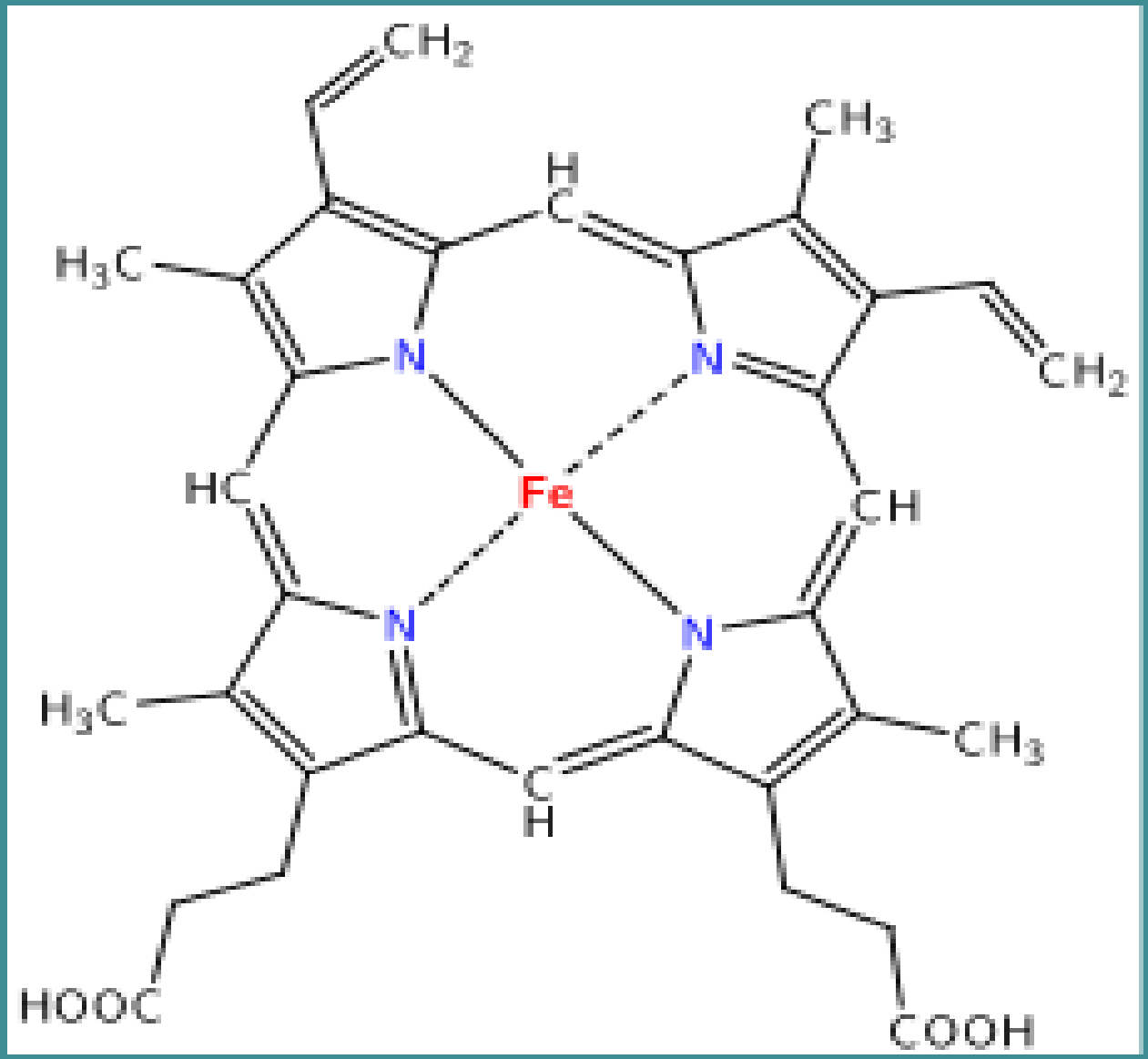
- Cytochromes are also found in the endoplasmic reticulum eg P450, *b*5

- **Cytochrome P450** family are found associated with the membrane of the smooth endoplasmic reticulum particularly in liver. The cytochrome P450 got its name because when reduced and complexed with carbon monoxide it exhibited a spectral absorbance maximum at 450 nm.

- Cytochrome P450 uses iron to oxidise molecules to makes them water-soluble and thus easy to dispose out of body. (Oxidation means the addition of oxygen to a molecule or the removal of hydrogen from a molecule)

- The iron acts as an electron carrier, undergoing alternate reduction to the ferrous +2 states and oxidation to the ferric +3 state.

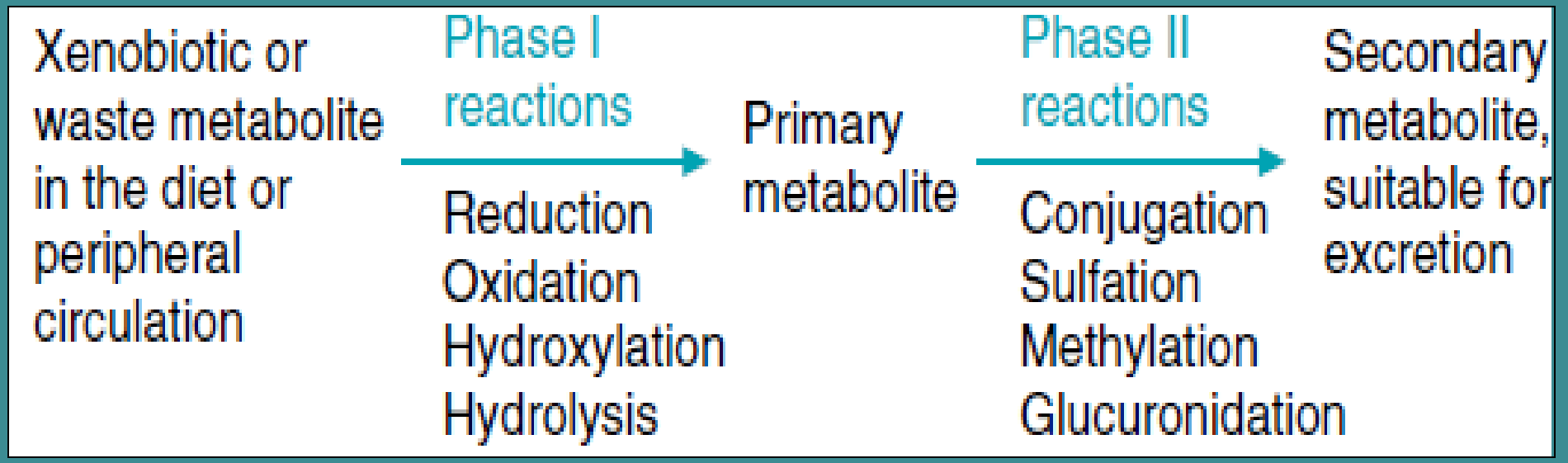
# Heme



**Drug metabolism reactions can be divided into phase I & phase II**

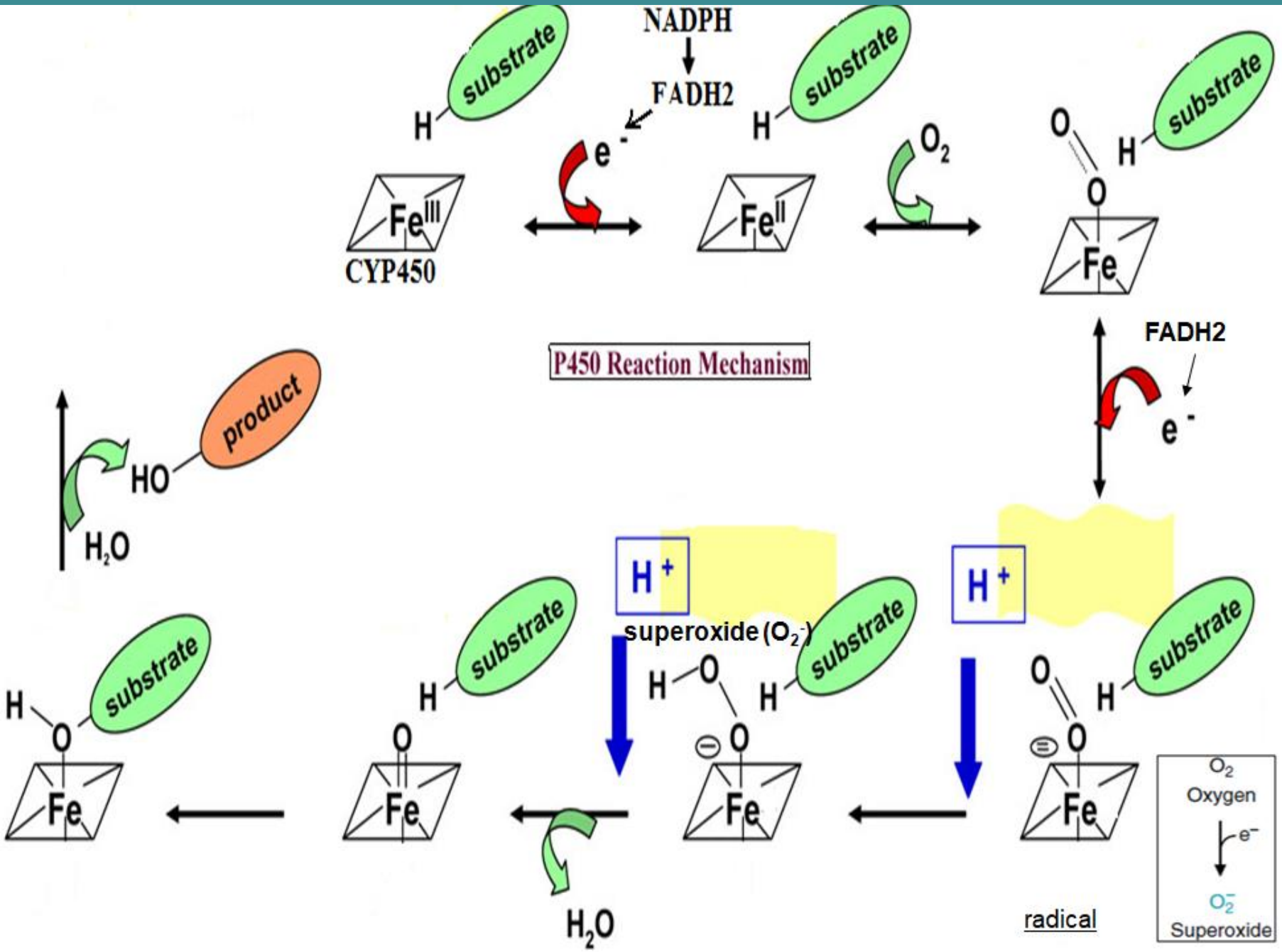
1- Phase I reactions involve oxidation, reduction, hydroxylation, hydrolysis, cyclization or decyclization reactions. Oxidation is the most common phase I reactions and it involves the addition of oxygen or removal of hydrogen by mixed function oxidases in the liver.

2- Metabolites that are not sufficiently polar may undergo phase II metabolism which involves Sulfation ($SO_4^{-2}$), Methylation (example methylation process helps convert the amino acid (homocysteine) into a amino acid (methionine), Glucuronidation (D-Glucuronic Acid is a sugar acid formed by the oxidation of the C-6 carbon of glucose), and conjugation of the metabolite or drug with large molecular groups that further reduced the biological activity of the metabolite (if any) and increase its solubility even further. Conjugation occurs with glucuronic acid, sulfonates, glutathione or amino acids. Functional groups that are often attached to these large molecules include carboxyl, hydroxyl, amino and sulhydryl groups.

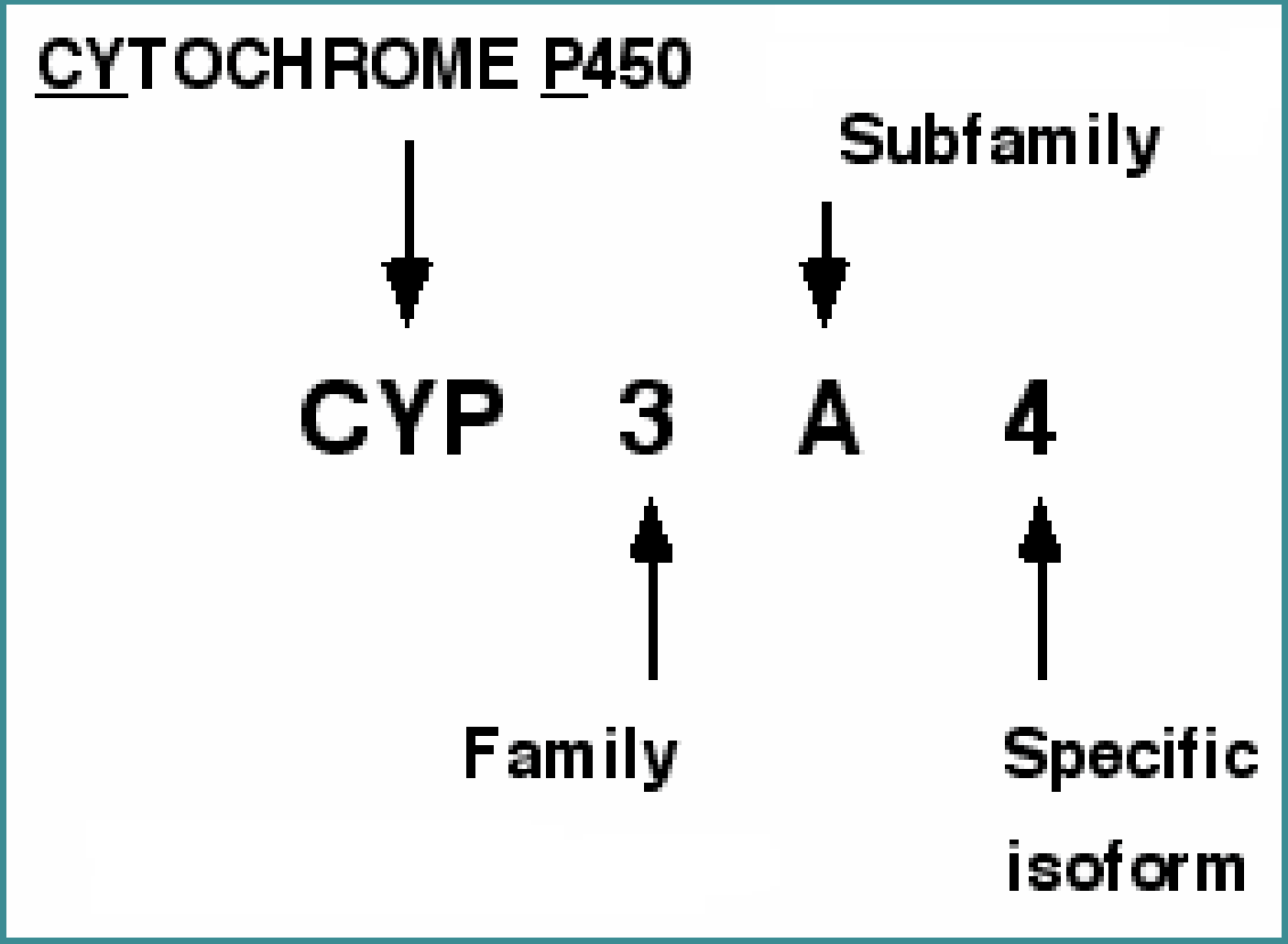



Xenobiotic are chemical substances that are foreign to animals life examples: drugs, flavoring, food additives, pesticides, industrial chemicals

# P450 Oxidation mechanism


NADPH
FADH2
substrate
H
e -
$Fe^{III}$
CYP450
$Fe^{II}$
$O_2$
O
H
Fe
P450 Reaction Mechanism
FADH2
e -
product
HO
$H_2O$
$H^+$
superoxide ($O_2^-$)
radical
$O_2$
Oxygen
e⁻
$O_2^-$
Superoxide
$H_2O$

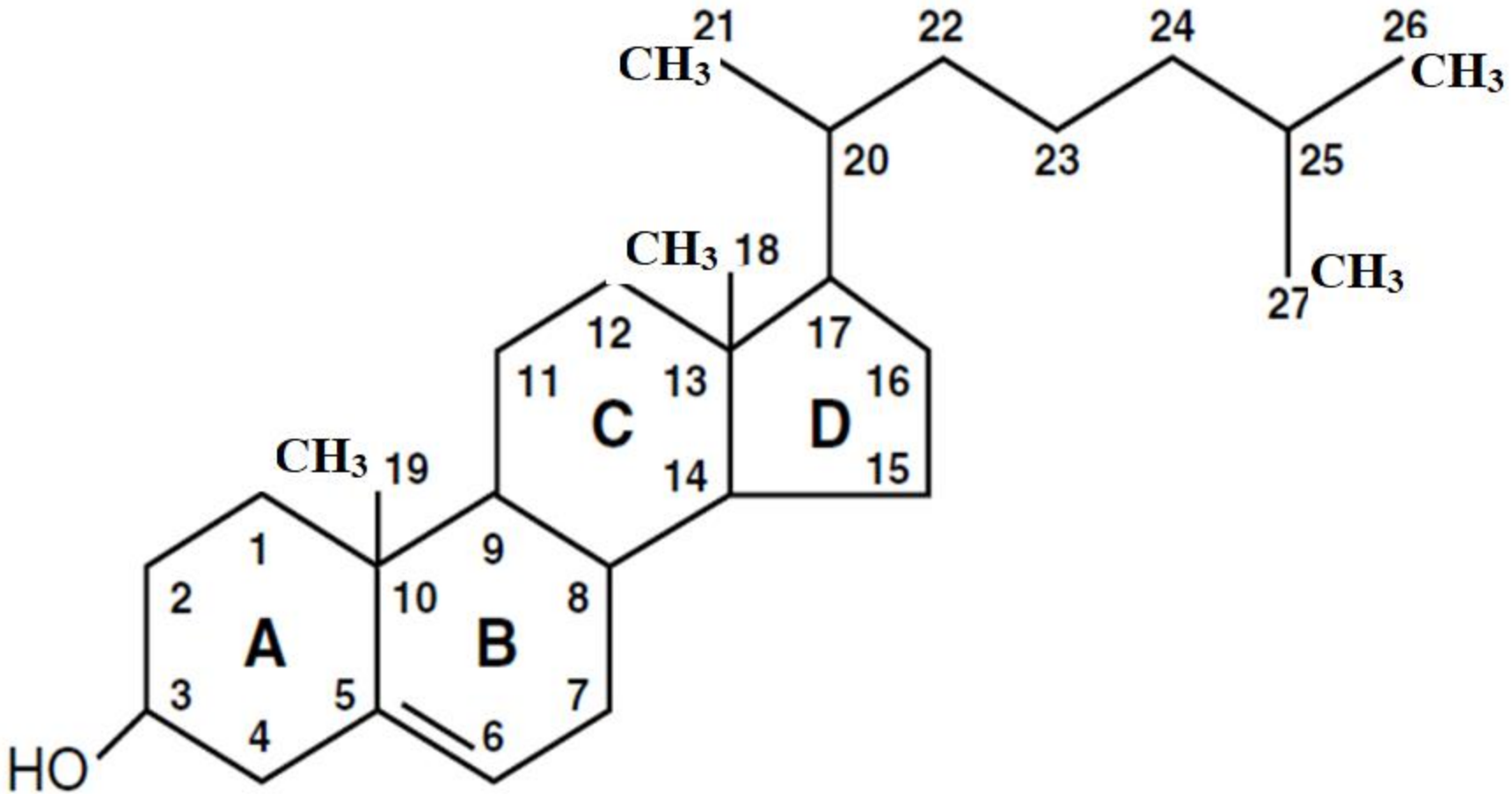
# Nomenclature of CYP450




CYP3A4 is a P450, the CYP denotes the cytochrome P450, the 3 denotes the family, the A denotes subfamily, and the 4 denotes the specific isozyme (enzymes with different primary structure but catalyze the same reaction).

- **Role of cytochromes P- 450 in the metabolism of Steroid hormones**
- Cholesterol is the precursor of all steroid hormones.

- Steroid hormones contain 21 or fewer carbon atoms, whereas cholesterol contains 27.
- The first stage in the synthesis of steroid hormones is the removal of a six-carbon unit from the side chain of cholesterol to form pregnenolone. The removal is accomplished by Cytochrome $P450_{SCC}$ (desmolase) that cleaves the bond ($P450_{SCC}$ is Cholesterol Side-Chain Cleavage Enzyme).
- Desmolase that include $P450_{SCC}$ is found in the mitochondria of tissues that synthesize steroids (mainly the adrenal glands and gonads)
- Other steroid hormones are produced from progesterone by reactions that involve members of the P450 family.




**Cholesterol ($C_{27}H_{46}O$)**

# Few points on CYP & Drugs

- Different people have different activity of CYP due to genetic variation that result in higher or lower expression of CYP than normal.
- This can lead to differences in drug metabolism: poor drug metabolizer, normal drug metabolizer or ultra drug metabolizer.
- For ultra drug metabolizer the patient may not get the benefit of drug as it get detoxified very fast. Also some of drugs intermediates are toxic specially if accumulated at high concentrations so if a patient is a high drug metabolizer this may lead to patient toxicity.
- Some drugs are given for special purposes that inhibit P450 enzymes to prolong the activity of some other drugs.
- Poorer P450 substrates drugs would last longer in the body before elimination, which is desirable for some drugs
- Some drugs that have a narrow range of effective dose before they become toxic might be overdosed in a poor metabolizer.
- CYP may lead to making some drugs ineffective while activating others.

# Bile

- Is an important product released by the hepatocytes.
- It promotes the digestion of fats from food by emulsifying them in the small intestine.
- The emulsifying components of bile mainly consist of bile acids and bile salts plus free cholesterol.

- **A. Bile acids and bile salts**
- Bile acids are steroids (cholesterol ) consisting of 24 C atoms carrying one carboxyl group and several hydroxyl groups.
- Cholic acid and chenodeoxycholic acid are the most important primary bile acids.
- Cytochrome P450 in the sER is involved in many of the steps.

**Formation of bile acid from cholesterol**:

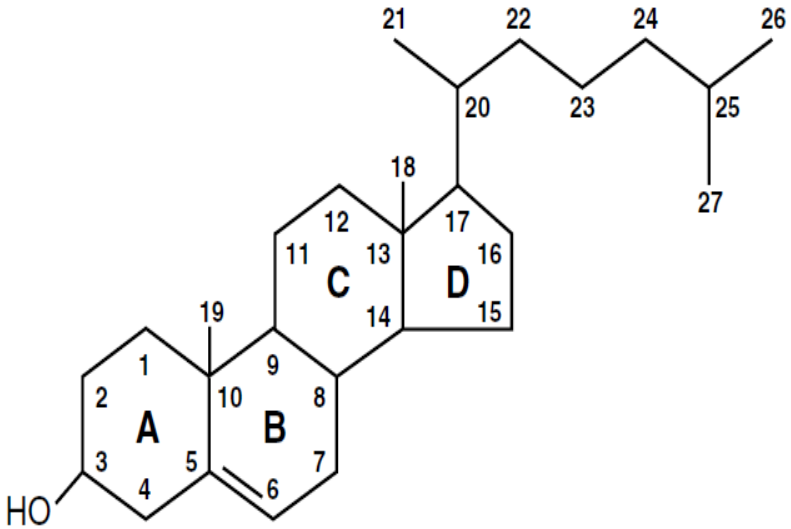
1- The side chain is shortened by three C atoms, and the terminal C atom is oxidized to a carboxylate group (COO-).

2- Cholesterol double bond is removed.

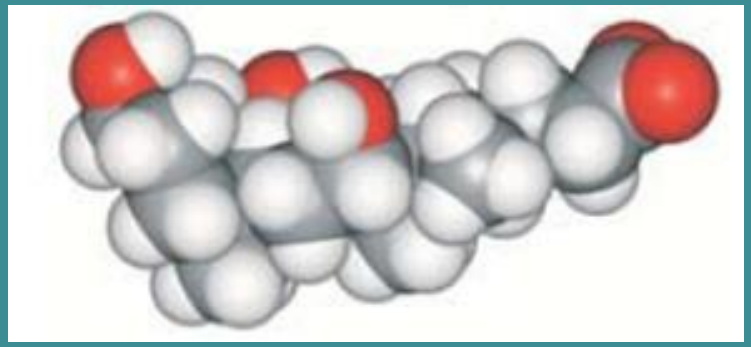
3- Monooxygenases then introduce one or two additional OH groups into steroid ring (to atoms 7, 12 in Cholic acid and atom 7 to chenodeoxycholic acid)

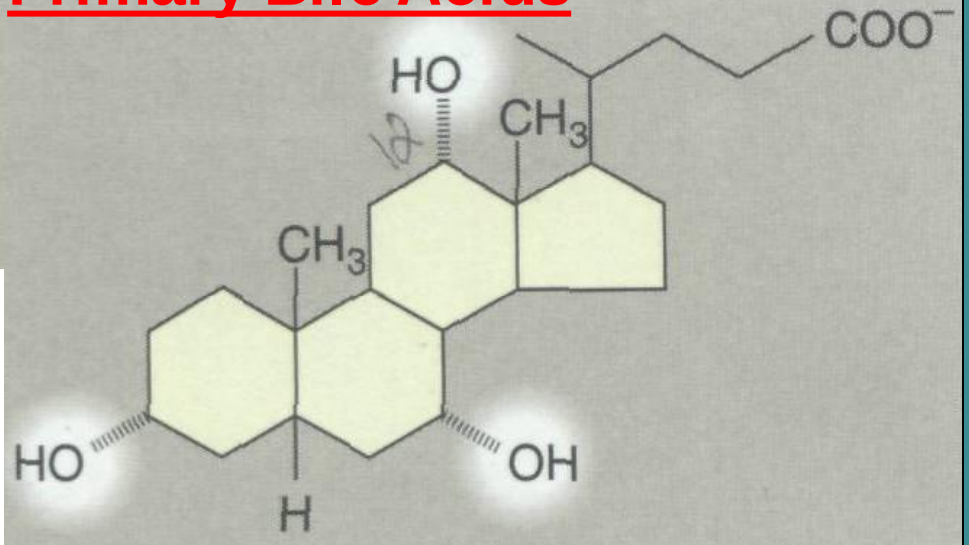
4- During bile acid synthesis it is important that A and B rings is altered from *trans* to *cis* so the hydrophilic groups in the bile acids lie on one side of the molecule.

# Primary Bile Acids

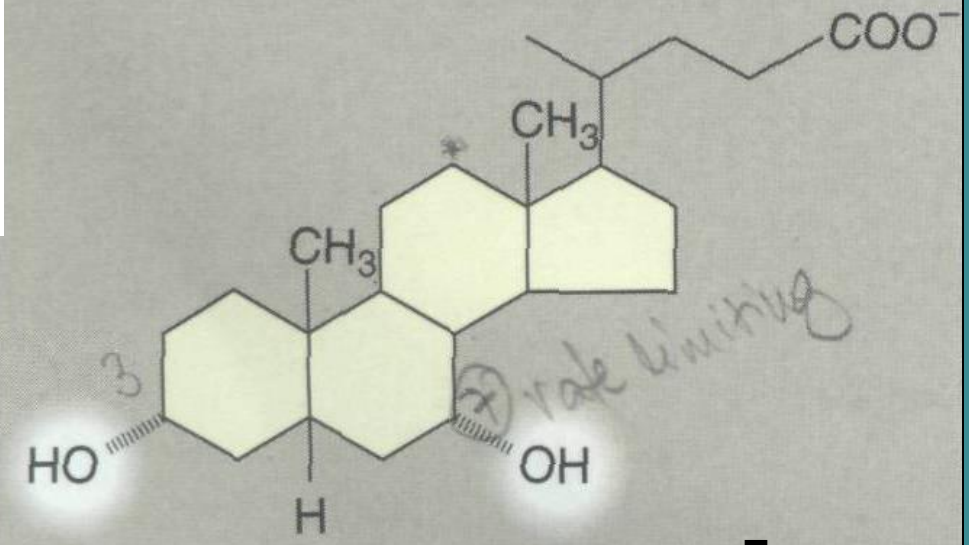



Cholesterol ($C_{27}$)






Cholic acid (24C)

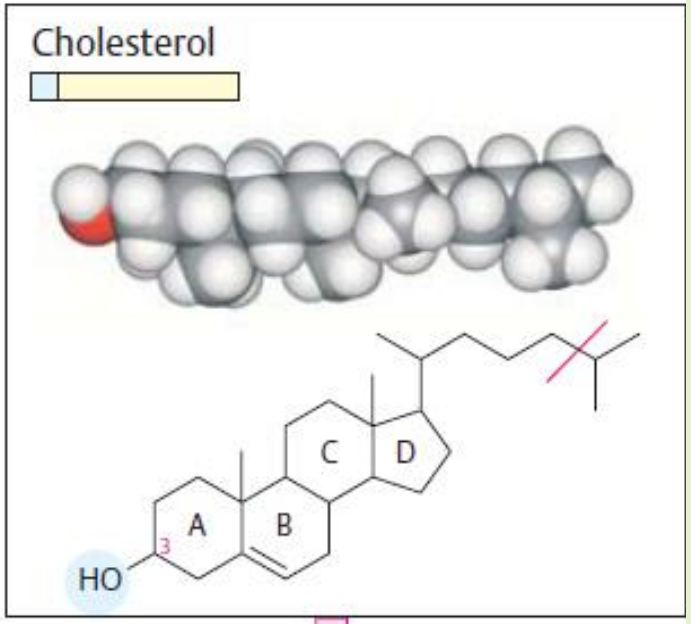



Chenodeoxycholic acid

- **Conversion of bile acid to bile salt**
- Cholic acid and chenodeoxycholic acid, known as **primary bile acids.**
- They are activated with coenzyme A
- Then conjugated with glycine or taurine (an end-product of cysteine metabolism).
- The cholic acid conjugates with glycine and taurine are called the **conjugated bile acids or bile salts**.

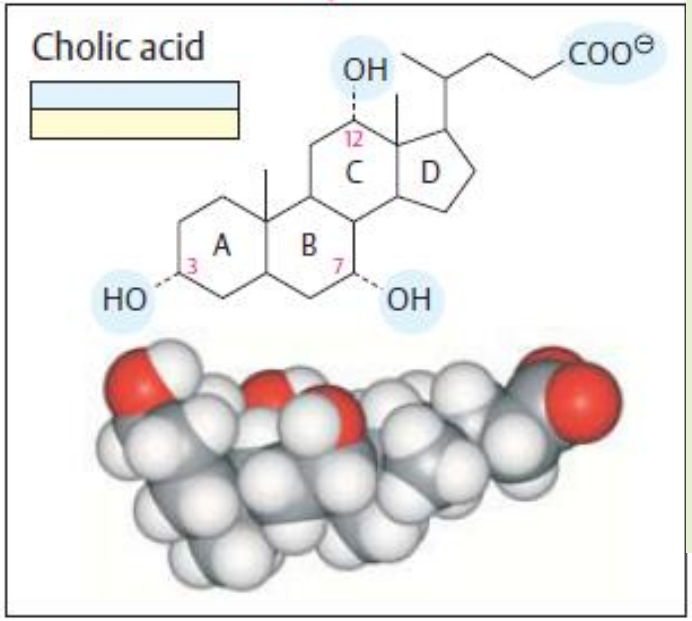
- Bile salts include glycocholic and glycochenodeoxycholic acids, and taurocholic and taurochenodeoxycholic acids
- Bile salts are more amphipathic than the primary bile acids .
- Bile salts are more effective detergents than bile acids because of their **enhanced amphipathic nature.**
- Therefore, only bile salt are found in the bile.

## A. Bile acids and bile salts

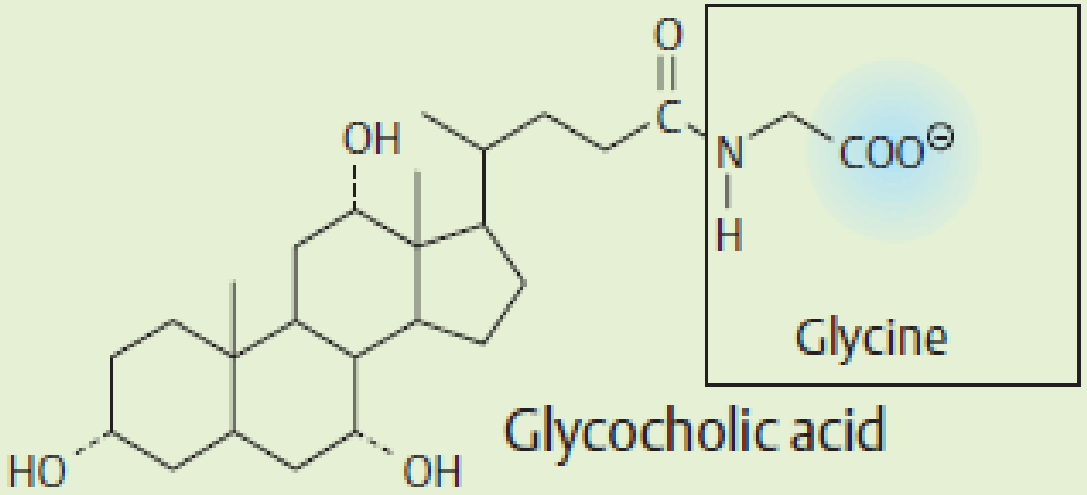



14 steps

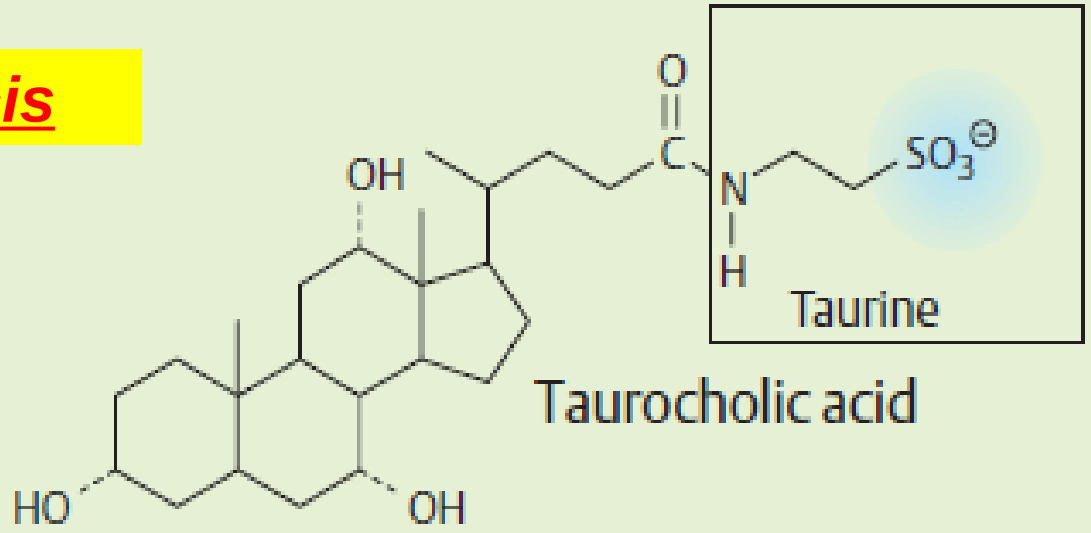
**trans to *cis***




## Bile salts




Glycocholic acid




Taurocholic acid

Bile salts = conjugated bile acids

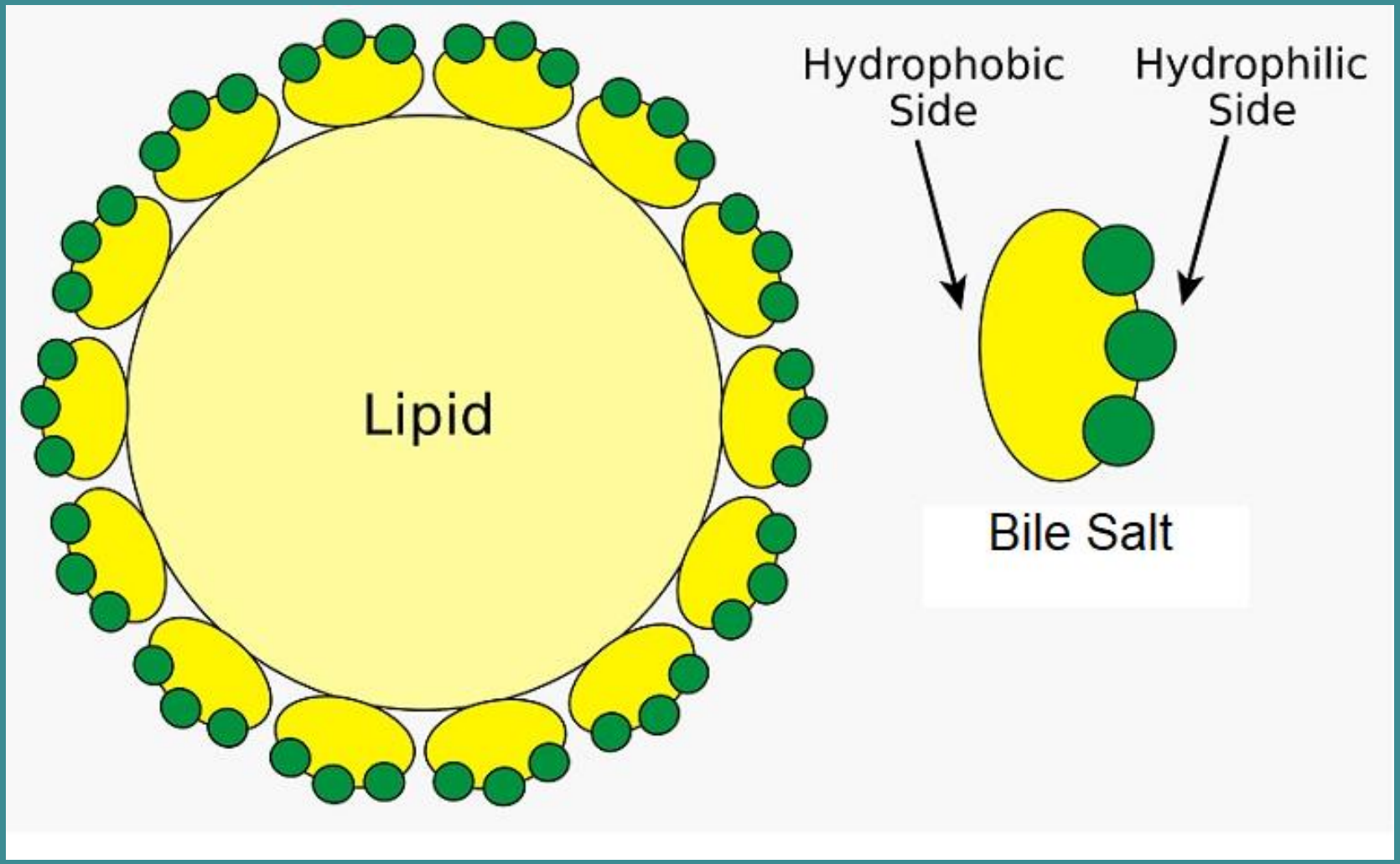
# Action of Bile Salts

**Emulsification** mixes two different substances that normally do not mix together like fat and water through formation of micelles. It greatly increases the surface area of fat, making it available for digestion by lipases.

Bile salt adsorb on the surface of fat droplet broken up by action of the intestinal muscle forming micelles thus greatly increases the surface area of fat, making it available for digestion by lipases.

The hydrophobic side of the bile salts mix with fat droplet and the charged hydrophilic side will be projecting from the surface of micelles thus making the micelles soluble in water and ensure that large fat drops cannot reform because like charges repel each other. Pancareatic lipase digest the fat in micelles then the micelles travel through a layer of water to the microvilli on the surface of the intestinal epithelial cells, where the fatty acids, 2-monoacylglycerols, and other dietary lipids are absorbed, but the bile salts are left behind in the lumen of the gut.

# Micelles

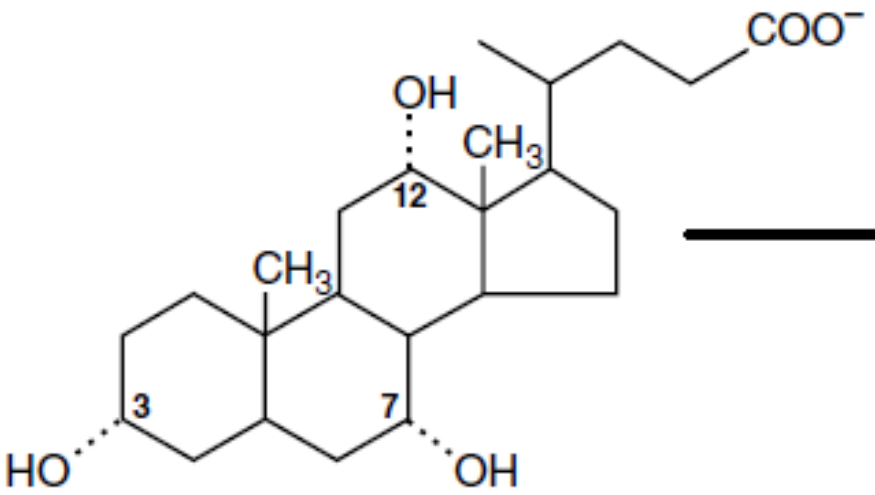


- **Fate of bile in intestine**
- Intestinal bacteria deconjugate and dehydroxylate the bile salts, removing the glycine and taurine residues and the hydroxyl group at position 7 and thus regenerating what is known as secondary bile acid.
- The bile acids that lack a hydroxyl group at position 7 are called secondary bile acid.
- The deconjugated and dehydroxylated bile acids are less soluble and, therefore, less readily absorbed from the intestinal lumen than the bile acids that have not been subjected to bacterial action.
- Lithocholic acid, a secondary bile acid that has a hydroxyl group only at position 3, is the least soluble bile acid. Its major fate is excretion.

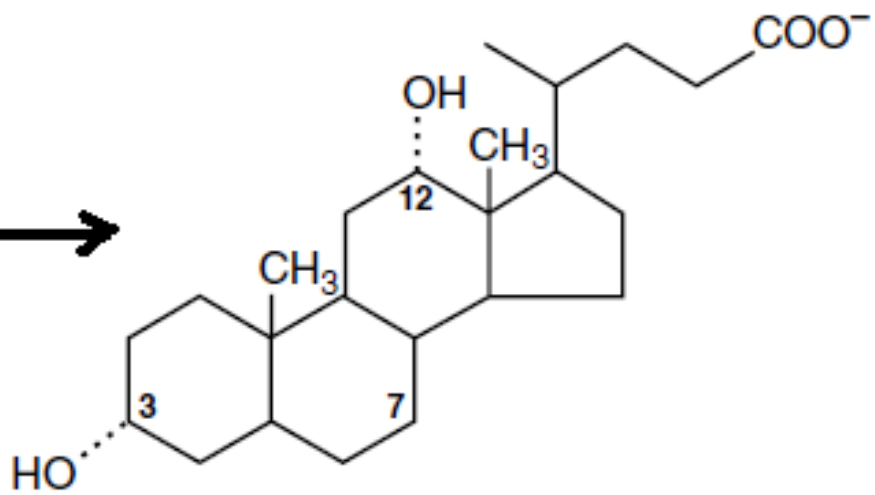
- Greater than 95% of the bile acids are reabsorbed in the ileum and return to the liver via the enterohepatic circulation (via the portal vein). The bile acids are recycled by the liver, which secretes them into the bile. This enterohepatic recirculation of bile salts is extremely efficient. Less than 5% of the bile acid entering the gut are excreted in the feces each day.
- Because the steroid nucleus cannot be degraded in the body, the excretion of bile acid serves as a major route for removal of the steroid nucleus and, thus, of cholesterol from the body.

**Primary bile** acid

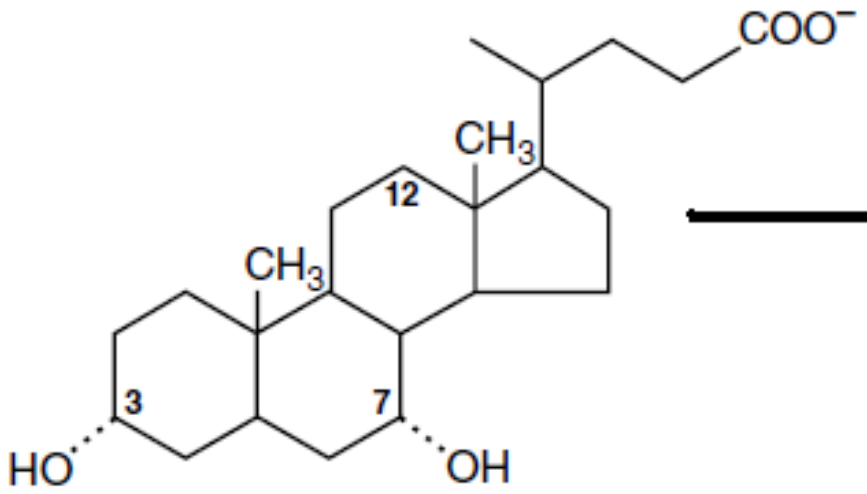
**Secondary bile** acid



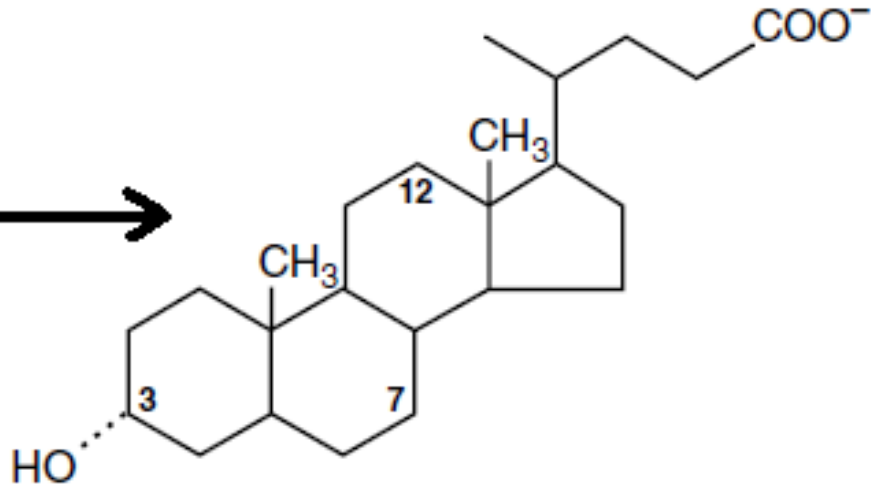

Cholic acid



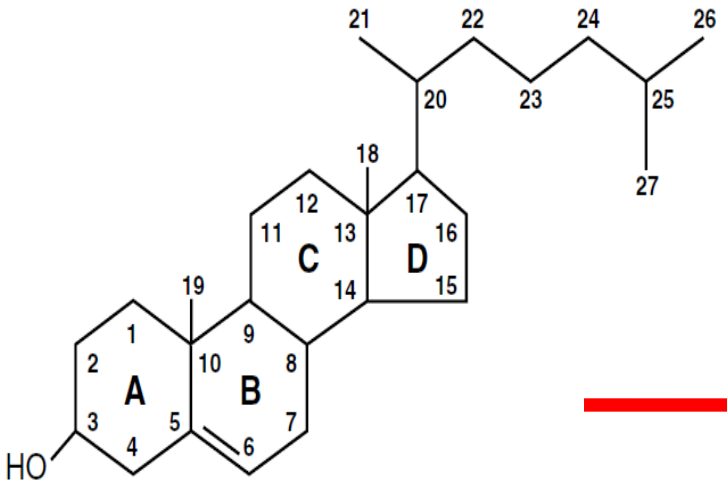

Deoxycholic acid




chenodeoxycholic acid

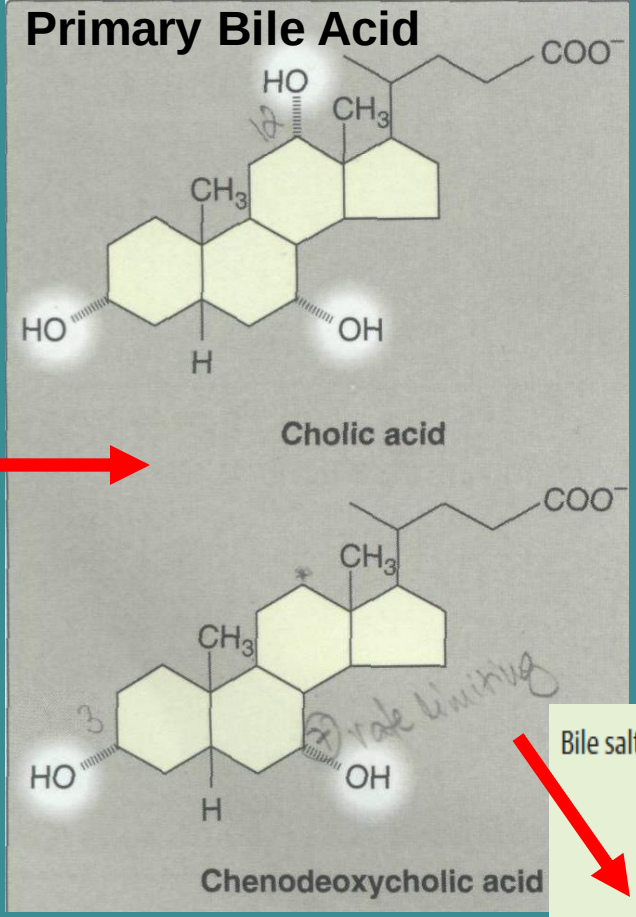



Lithocholic acid

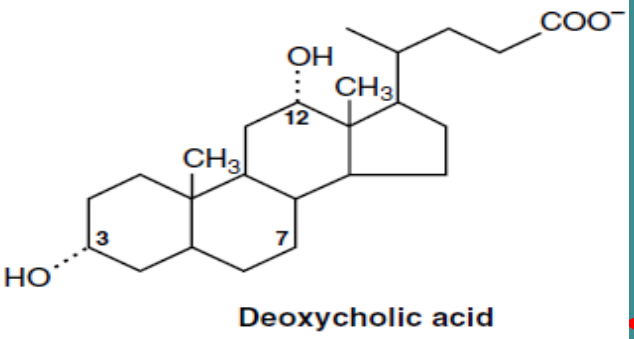


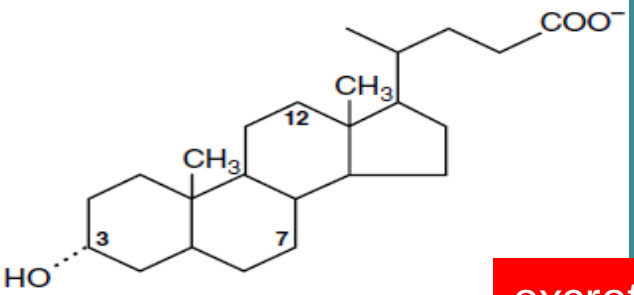

Cholesterol ($C_{27}$)

**Primary Bile Acid**




**Secondary bile acid**




excreted