فريق طوفان الأقصى

METABOLISM

Modified N.11

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Metabolism of lipids VIII:

Cholesterol

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Resources

- This lecture
- Lippincott's Biochemistry, Ch. 17

Chole ster ol

Chole: Bile

Stereos: solid

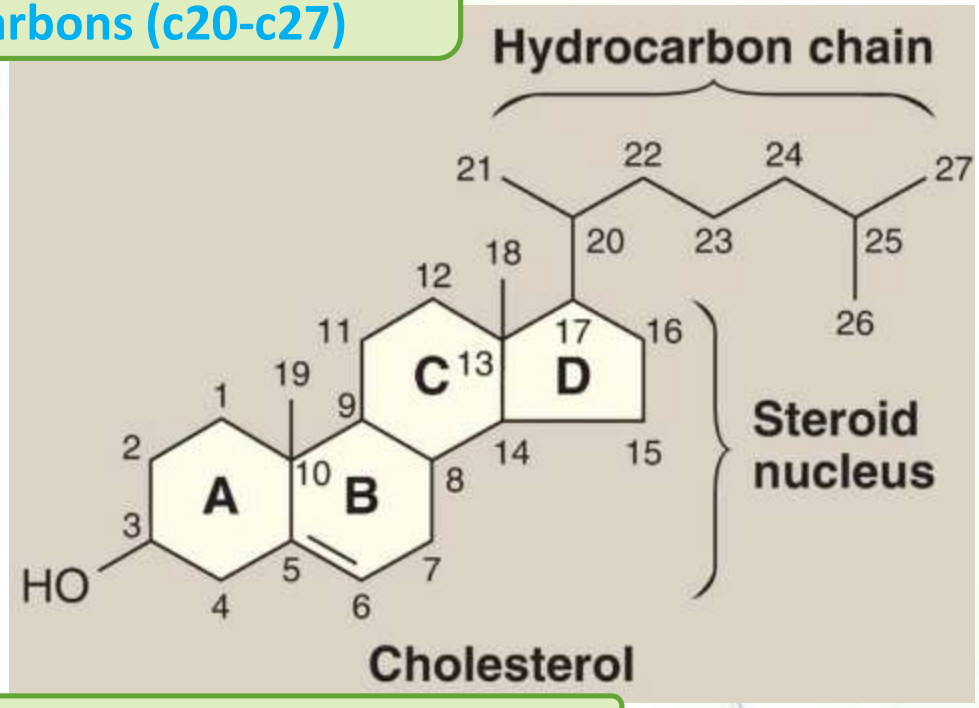
ol: alcohol

NOTE: has a hydroxyl group

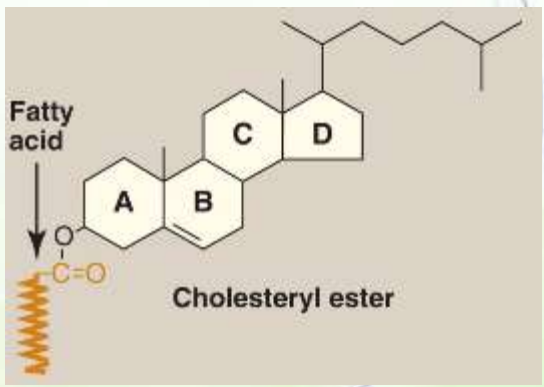
Structure of cholesterol

- Cholesterol is a very hydrophobic compound.
- It is a 27-carbon molecule that consists of:
  - Four fused hydrocarbon rings (A–D) of 17 carbons called the steroid nucleus
  - Two methyl groups (C18 and 19)
  - Eight-carbon, branched hydrocarbon chain attached to carbon 17 of the D ring.
    - Ring A has a hydroxyl group at carbon 3.
    - Ring B has a double bond between C5 and c6.
- Most plasma cholesterol is esterified with a fatty acid attached at carbon 3.

NOTE: it is made of 8 carbons (c20-c27)



NOTE: a double bond

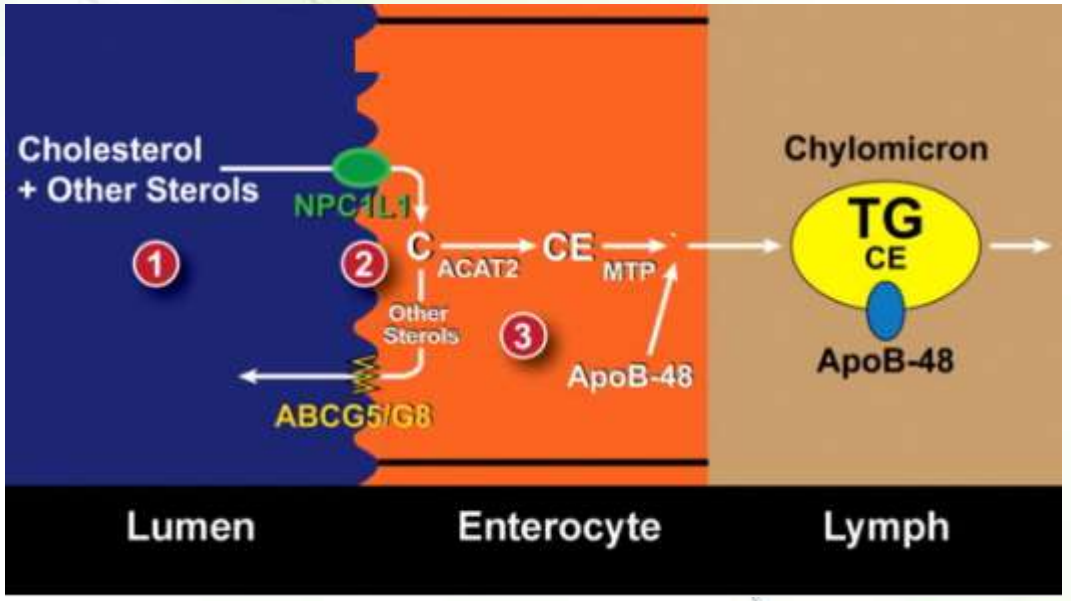


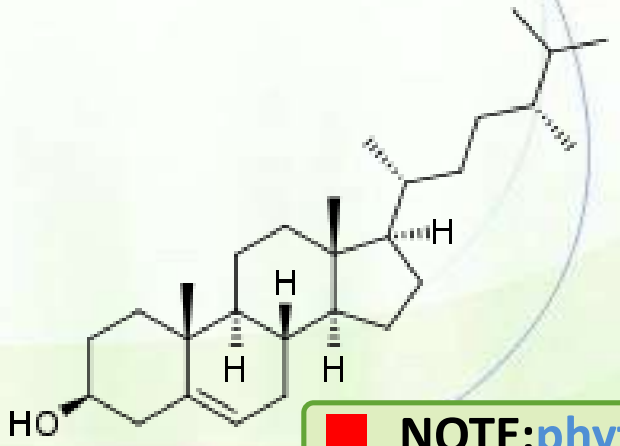
NOTE: it is a hydrophobic molecule except the hydroxyl group which makes it an amphipathic molecule, so you can draw a line to divide it into two parts..a hydrophobic part (which is the larger part) and a hydrophilic part (the smaller one).

- **The complement in this slide:** cholesterol is esterfied by a fatty acid , changing the amphipathic structure of cholesterol , and making it more hydrophobic (the attachment occurs on carbon #3 of the hydroxyl group).

Intestinal absorption of cholesterol

- Intestinal uptake of cholesterol is mediated by the Niemann-Pick C1-like 1 protein, the target of ezetimibe, and pumped out by ABCG5/8.
  - Defects in the efflux transporter (ABCG5/8) result in the rare condition of sitosterolemia increasing the risk of MI.
- Plant sterols (phytosterols) are poorly absorbed by humans (5% vs. 40% for cholesterol) and are actively transported back into the intestinal lumen.
  - Plant sterols reduce the absorption of dietary cholesterol.
    - A dietary strategies to reduce plasma cholesterol levels.





NOTE:phytosterols

The complement in this slide:

the way the cholesterol is absorbed :

- Via a carrier protein (NPC1L1) which is targeted by ezetimibe drug(inhibitor) .
- Efflux or pumped out(in the opposite direction): via another protein carrier (ABCG5/8) (if it is defective it will cause an increase of cholesterol inside the body which results in sitosterolemia disease and increases the risk of myocardial infarction , because of the accumulation of LDL and cholesterol in blood vessels).

Cholesterol is a mammalian molecule which exists in plasma membrane so it isn't found in bacteria or plants but plants have a similar molecule called phytosterol , so it can compete with cholesterol in binding to the previous carrier protein, so it reduces the absorption of cholesterol. However ,it is poorly absorbed so it not that efficient .

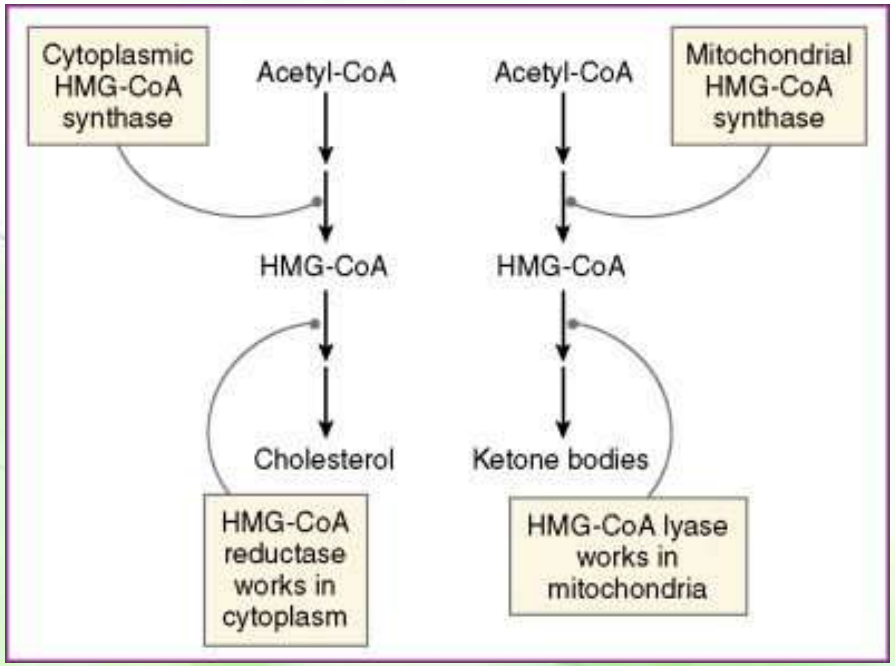
Notes regarding synthesis of cholesterol

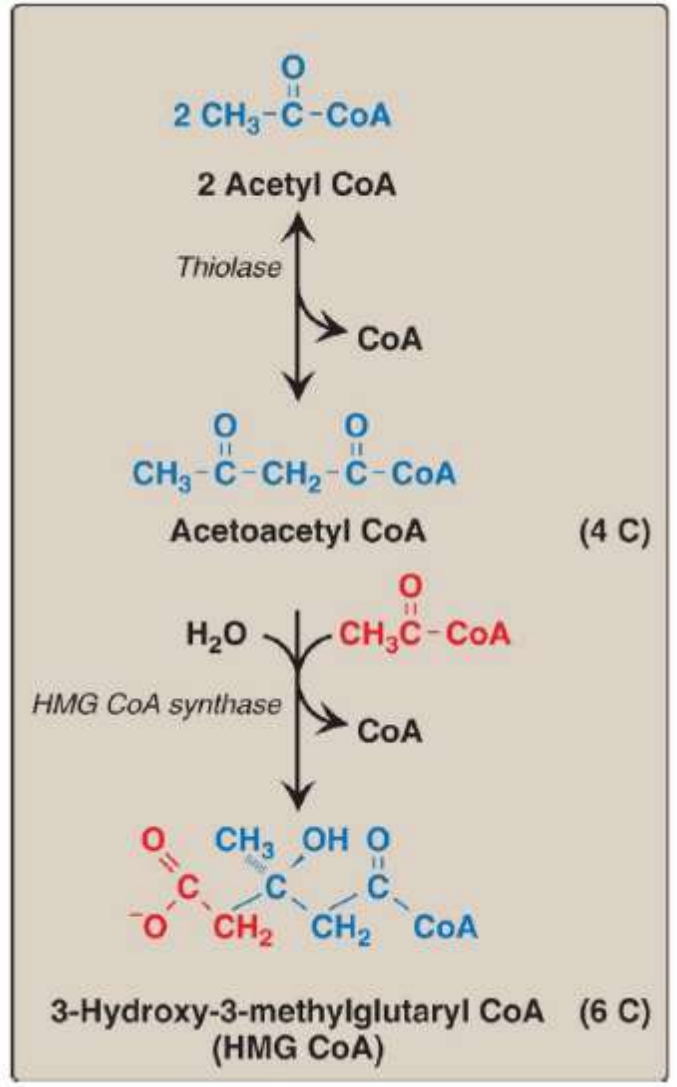
NOTE: synthesis means anabolic metabolism so we need a reducing agent which is NADPH in this pathway.

- All the carbon atoms in cholesterol are provided by acetyl coenzyme A (CoA).
- NADPH is the reducing agent.
- The pathway is endergonic, and energy is provided by the hydrolysis of
  - The thioester bond of acetyl CoA
  - ATP
- Synthesis requires enzymes in the cytosol, the membrane of the smooth endoplasmic reticulum (SER), and the peroxisomes.
- The pathway is regulated to balance the rate of cholesterol synthesis/excretion.

The first reactions...

- Similar to the synthesis of ketone bodies.
- Liver parenchymal cells contain two isoenzymes of the HMG CoA synthase.
  - A cytosolic enzyme for cholesterol synthesis.
  - A mitochondrial enzyme for ketone body synthesis.



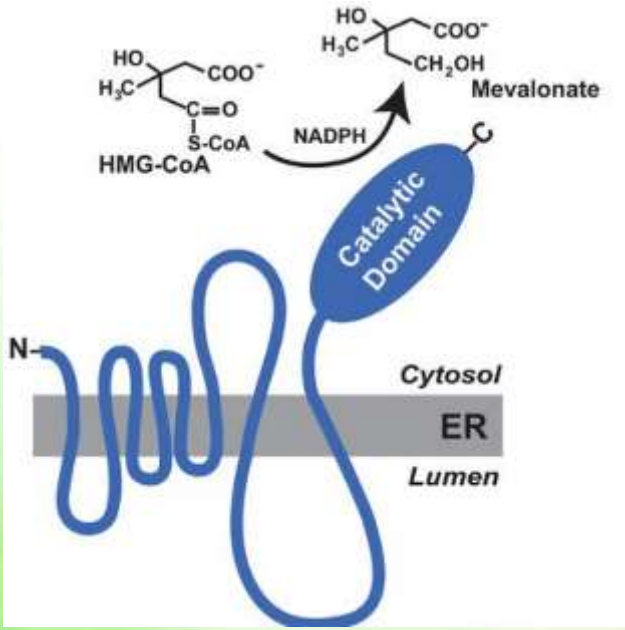


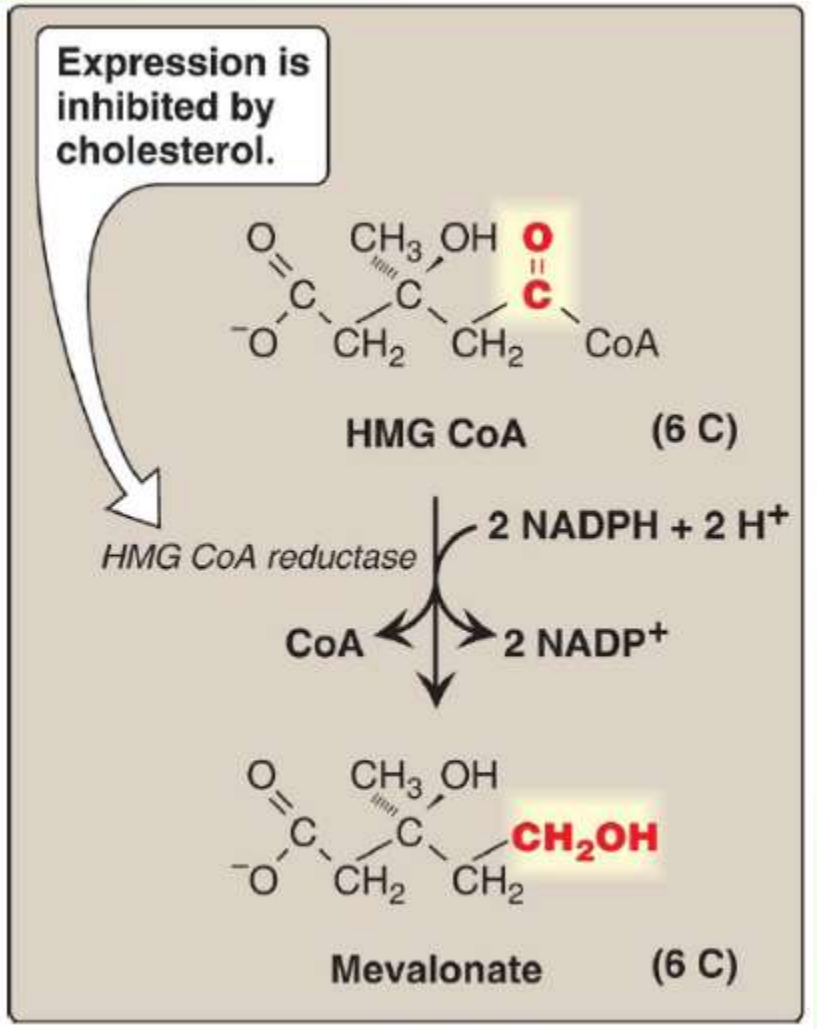
The complement in this slide:

1) Condensation of two Acetyl CoA molecules to give Acetoacetyl CoA by thiolase enzyme which catalyzes the reversible reaction.
2) Condensation of Acetyl CoA and Acetoacetyl CoA to give HMG CoA by (HMG CoA synthease)this enzyme is found in the mitochondria , however , its isoenzyme is found in the cytosol so the synthesis of cholesterol occurs in the cytosol while the synthesis of ketone bodies occurs in the mitochondria.

Synthesis of mevalonate

- HMG CoA is reduced to mevalonate by HMG CoA reductase.
  - A rate-limiting reaction and a committed step.
  - Two molecules of NADPH are oxidized.
  - CoA is released making the reaction irreversible.
- HMG CoA reductase is an integral membrane protein of the SER, with its catalytic domain projecting into the cytosol.





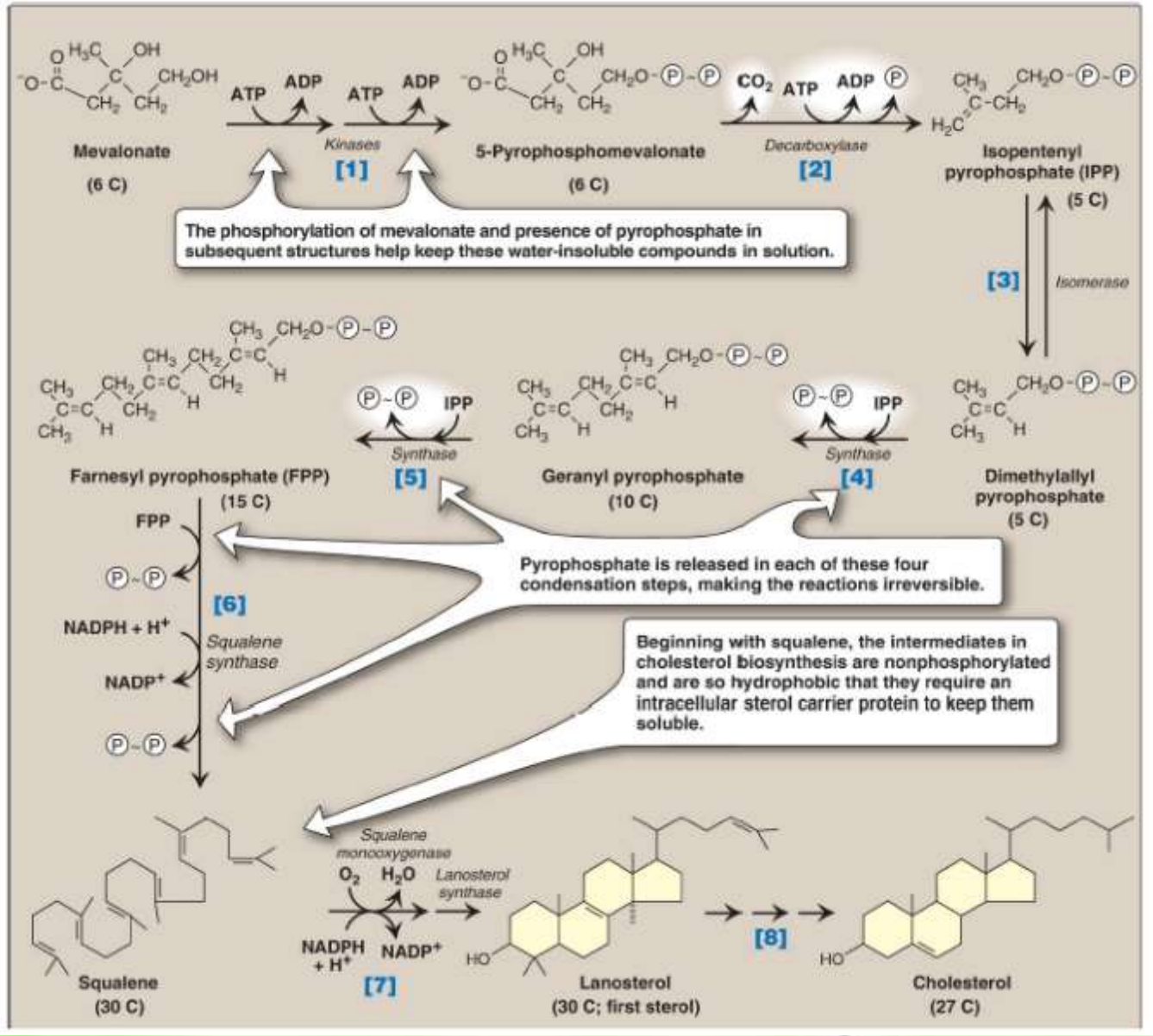
The complement in this slide:

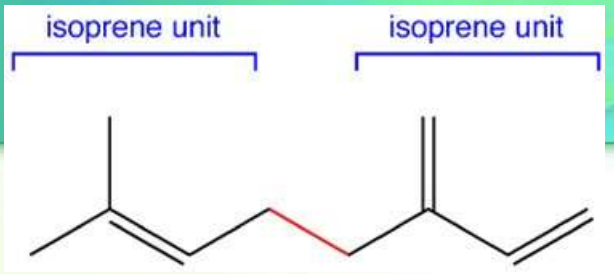
Here we convert the HMG CoA into mevalonate by(HMG CoA reductase) and this reaction requires energy which can be obtained from the release of CoA . this reaction also needs electron carrier which is NADPH .

This reaction happens in the cytosol although the enzyme is found in SER membrane, and this can be explained by which the catalytic domain of the enzyme(that is bound to the SER) is exposed to the cytosol which makes sense.

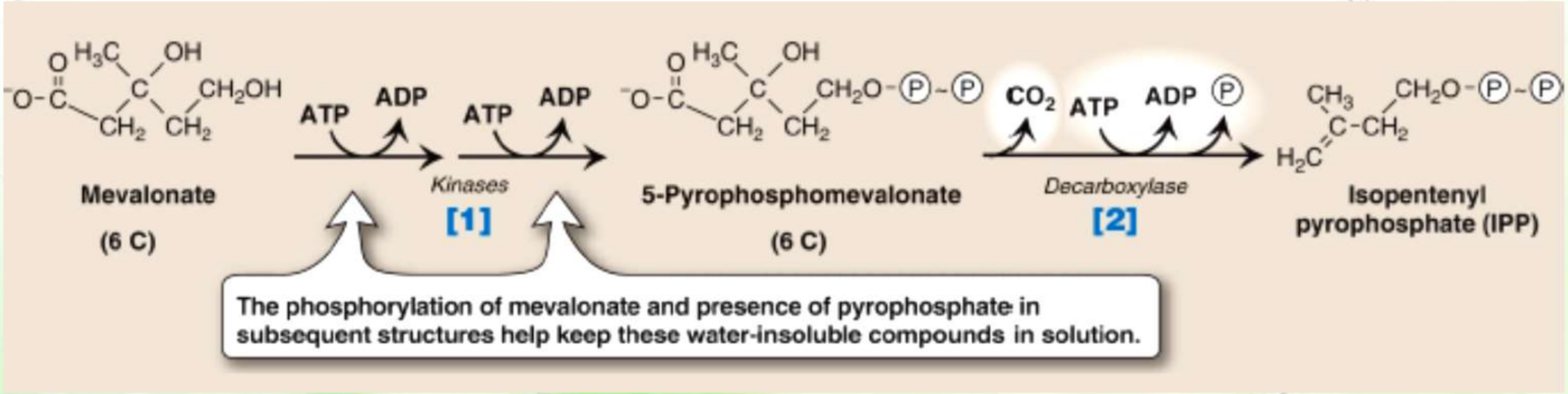
Synthesis of cholesterol

- **NOTE:** Remember that mevalonate is a six carbon molecule because it is resulted from the condensation of three acetyl CoA molecules





- [1] Mevalonate is activated by transferring 2 phosphate groups from ATP.
- [2] A five-carbon isoprene unit, isopentenyl pyrophosphate (IPP), is formed by the decarboxylation of 5-pyrophosphomevalonate.
  - The reactions require ATP.
  - IPP is the precursor of the isoprenoid family with diverse functions,.
    - Nonsterol isoprenoids include ubiquinone (or coenzyme Q).



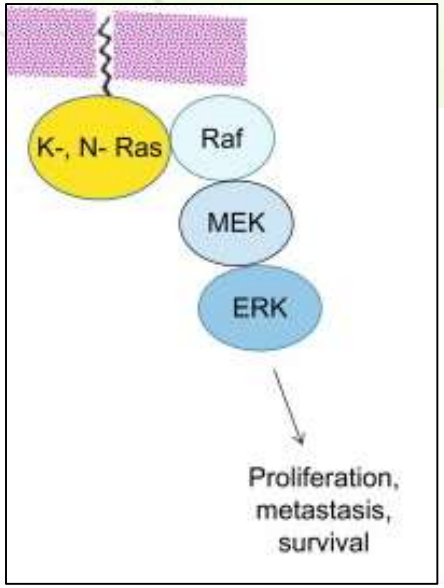
The complement in this slide:

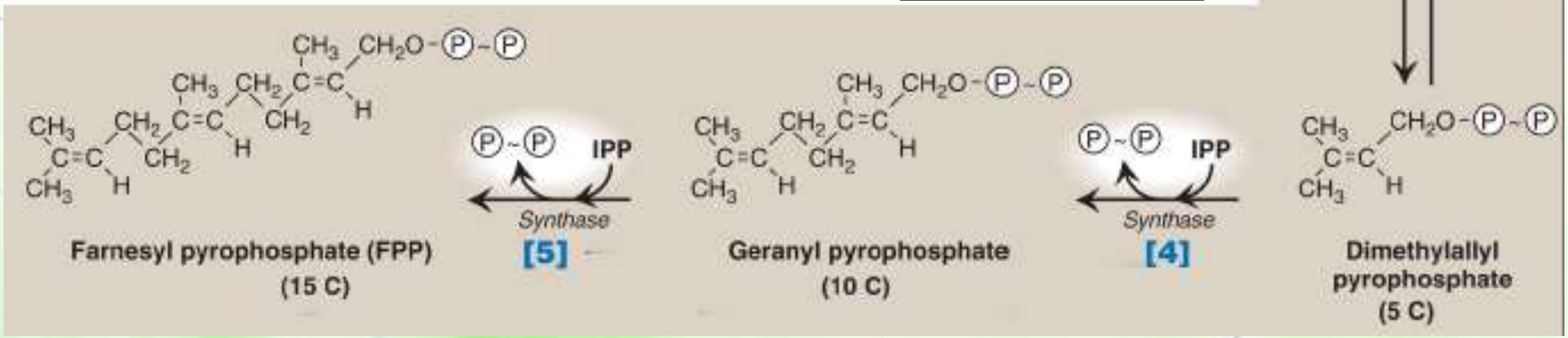
1) Mevalonate is activated and converted to 5-pyrophosphomevalonate (the reaction needs two ATP molecules).
2) Decarboxylation of 5-pyrophosphomevalonate to IPP (the reaction needs one ATP).

IPP is basically an isoprene(a 5 carbon molecule) (the structure is shown in previous slide) isoprene can make many molecules with different functions like coenzyme Q.

From 5 to 15

- [3] IPP is isomerized to 3,3-dimethylallyl pyrophosphate (DPP).
- [4] IPP and DPP condense to form 10-carbon geranyl pyrophosphate (GPP).
- [5] A second molecule of IPP then condenses with GPP to form 15-carbon farnesyl pyrophosphate (FPP).
  - Covalent attachment of farnesyl to proteins, a process known as prenylation, is one mechanism for anchoring proteins (for example, Ras) to the inner face of plasma membranes.





The complement in this slide:

4) Condensation of two IPP molecules to form GPP(a10 carbon molecule)(the energy of this reaction comes from the release of pyrophosphate which will be immediately cleaved , making the rxn more exergonic , because we have reduced the amount of the product >> pulling the rxn forward .

5) Condensation of another IPP with the GPP to form FPP (15C).

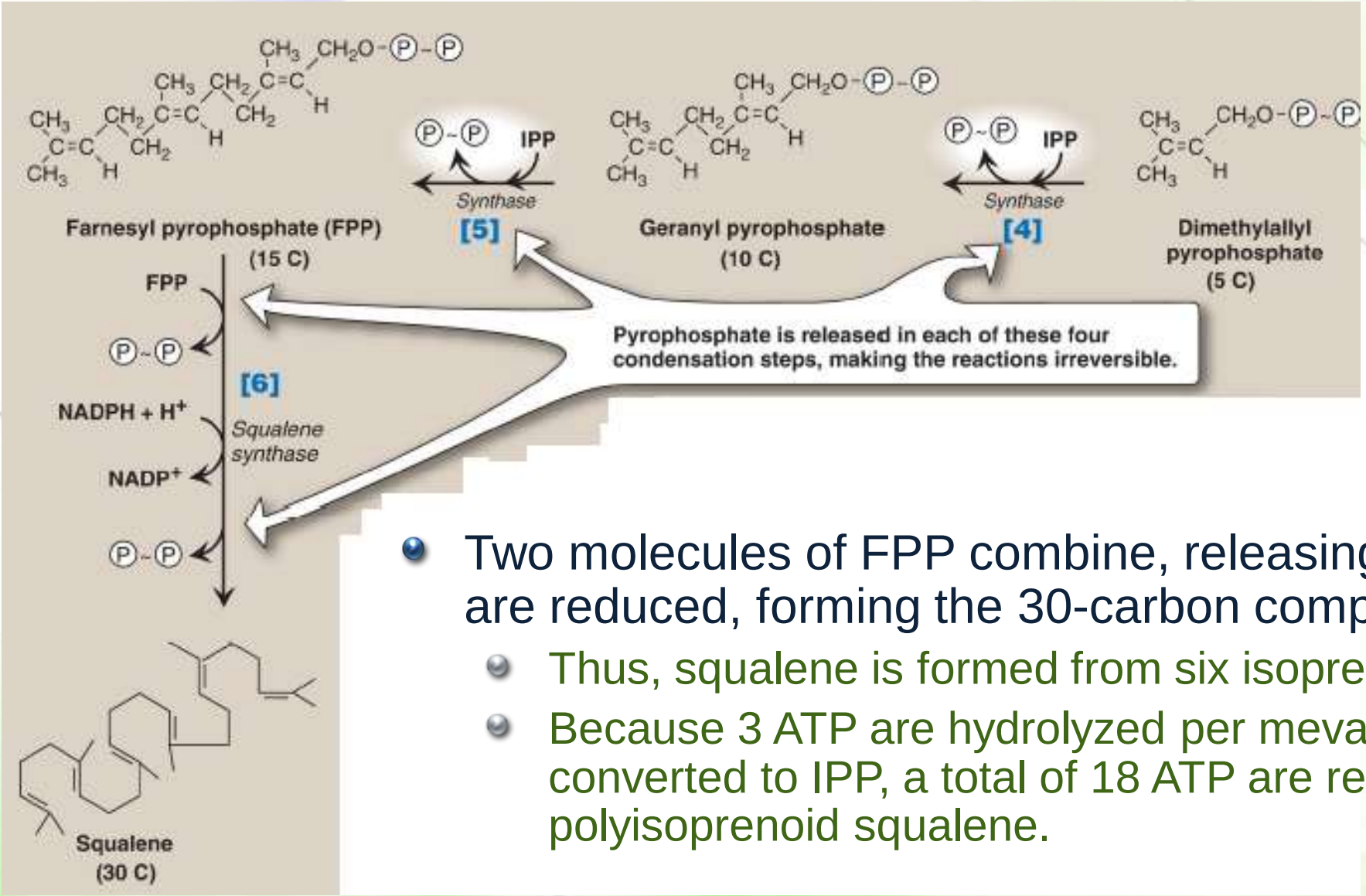
Both FPP and GPP are hydrophobic so they can be added to some proteins in order to attach these proteins to the plasma membrane .

- **The complement in this slide:** Extra information: Ras is proto-oncogene which means that if it is mutated it will cause a cancer. Ras is a protein that is responsible for growth factor signal cascade, when it binds to its receptor, it will become active and able to activate another protein like RAF.

Inhibition of farnesyl transferase (which is the enzyme that is responsible to prenylation of Ras) was a hot topic of research to kill cancer cells because Ras (whether it is normal or oncogene) must be anchored to plasma membrane to be functional.(Ras is important in cell proliferation).

Drug companies tried this mechanism to create anti-cancer drugs and it worked on animals but it didn't work on humans **(the doctor said that this is an extra info , if you want to understand it more, kindly go back to the recorded lecture)**

The synthesis of squalene



- Two molecules of FPP combine, releasing pyrophosphate, and are reduced, forming the 30-carbon compound squalene.
  - Thus, squalene is formed from six isoprenoid units.
  - Because 3 ATP are hydrolyzed per mevalonate residue converted to IPP, a total of 18 ATP are required to make the polyisoprenoid squalene.

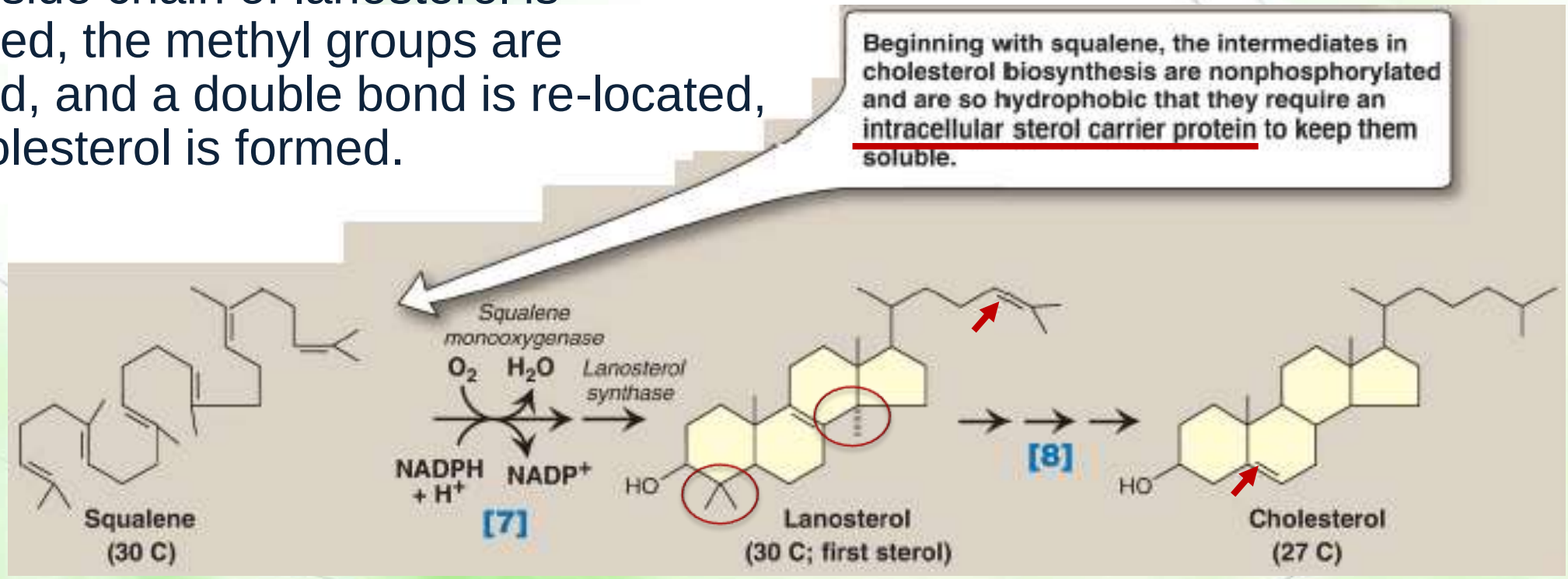
- **The complement in this slide:**

6) Condensation of two FPP to form squalene (the energy of this reaction comes from the release of both pyrophosphates).

Squalene is a hydrocarbon chain not cyclic and it is very hydrophobic (because it does not have the pyrophosphate which makes it hydrophilic).

And finally...

- [7] Squalene is converted to the sterol lanosterol by SER-associated enzymes that use molecular oxygen (O2) and NADPH.
  - The hydroxylation of linear squalene triggers the cyclization of the structure to lanosterol.
- [8] The side chain of lanosterol is shortened, the methyl groups are removed, and a double bond is re-located, and cholesterol is formed.

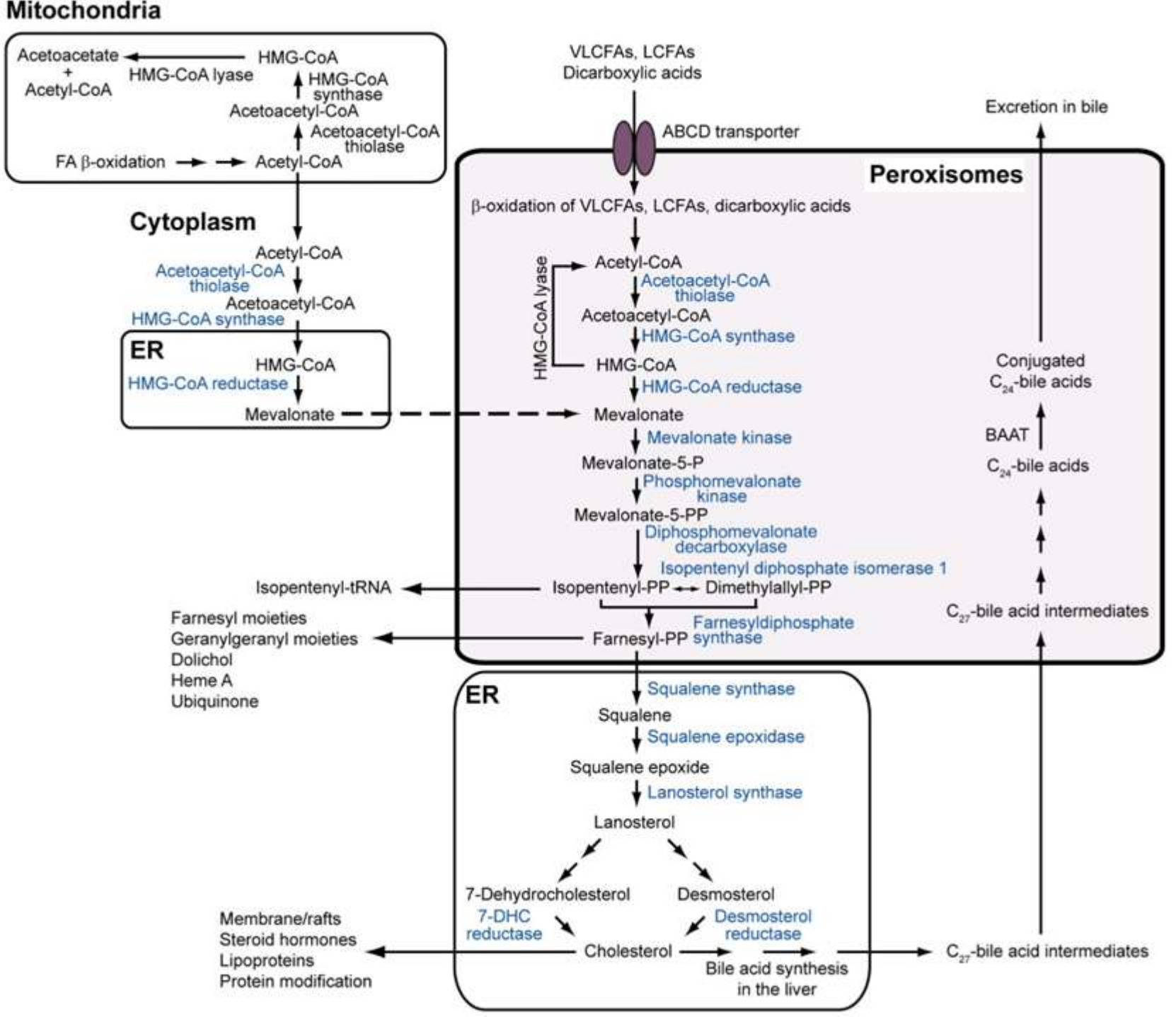


- **The complement in this slide:**

Because the squalene isn’t soluble we need a protein carrier for it which called intracellular sterol carrier protein.

7) By cyclisation of squalene it is converted to lanosterol which also binds to intracellular sterol carrier protein , because it is also hydrophobic .

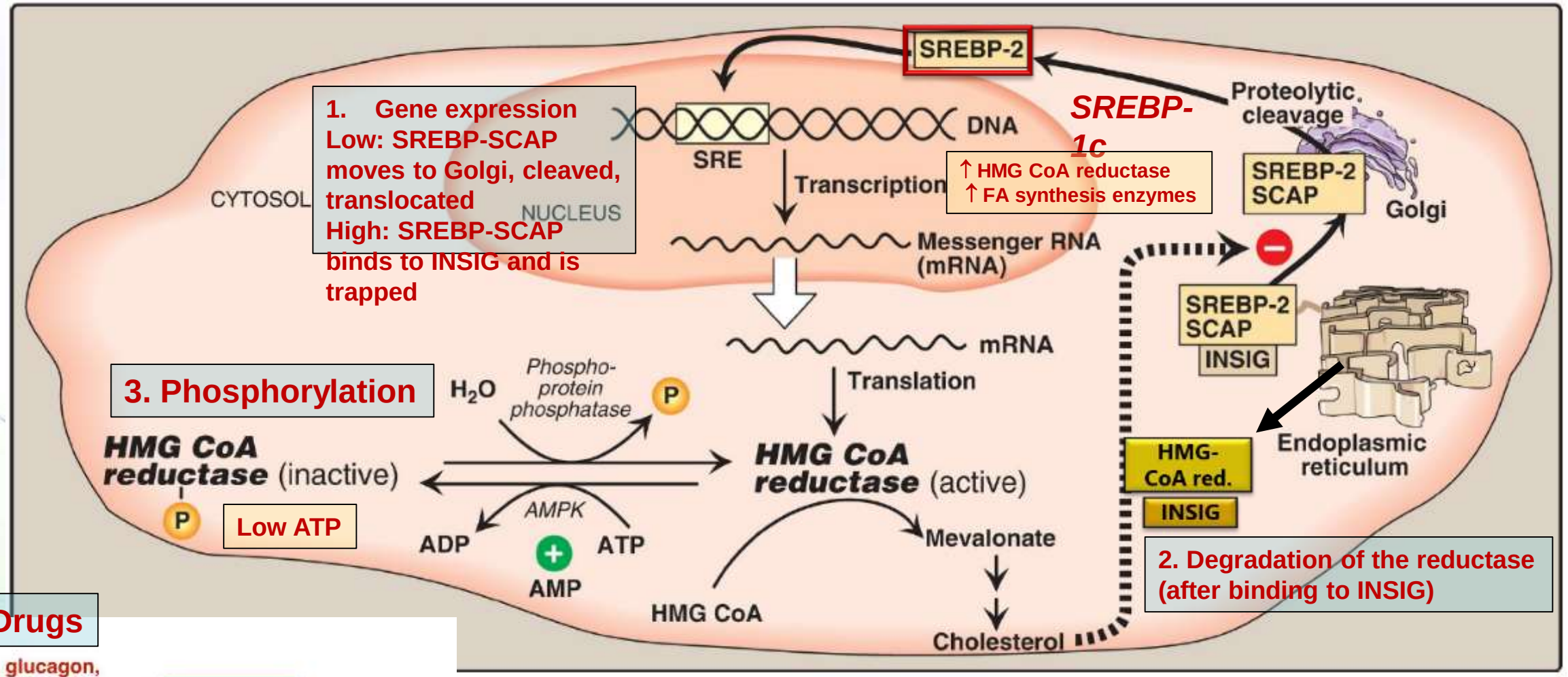
8) Removing methyl groups and alkene group and change the position of the double bond of lanosterol (30c) to convert it into cholesterol (27c). (look at the figure in previous slide to understand)



Do not memorize

- **NOTE:** **just to understand how cholesterol is produced in different organelles.**

Regulation of cholesterol synthesis



5. Drugs

statins, glucagon, cholesterol ⊣ HMG-CoA reductase ← insulin

4. Hormones
(through phosphatase and PKA)

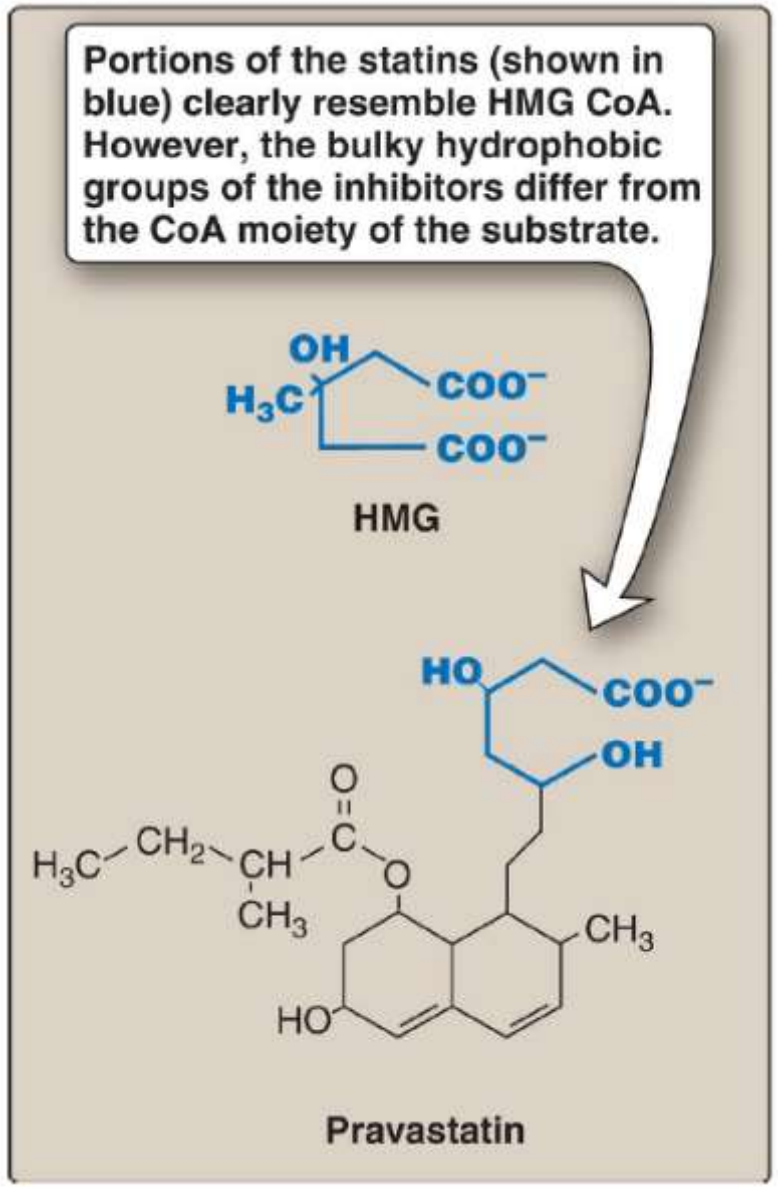
SRE: Sterol regulatory element
SREBP: SRE binding protein
SCAP: SREBP cleavage–activating protein
INSIG : Insulin-induced gene

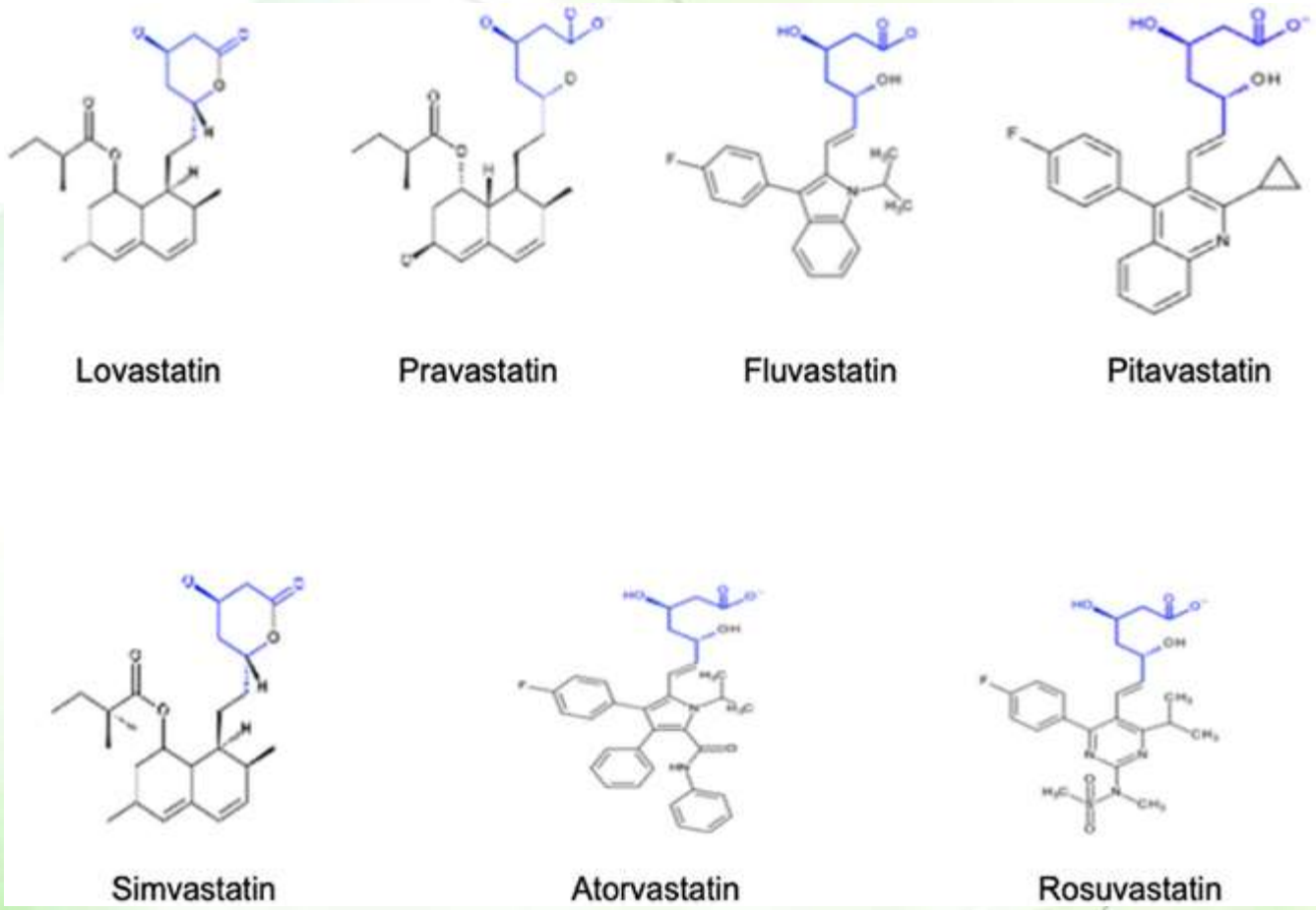
- Because a lot of ATP molecules have been utilized , production of cholesterol should be highly regulated at different levels
- Let’s talk about the first level (transcriptional level) : remember the transcriptional SREP (sterol response element binding protein) it’s not only responsible for the induction of the production of the enzyme responsible for fatty acids synthesis, but also for steroid as well.
- This SREBP enters the nucleus and binds to the sterol regulatory element (this element is found in a number of promoter regions of many enzyme genes , one of them is the HMG CoA reductase enzyme)
- So the SREBP binds to the promoter element and stimulates the production of mRNA and it’s transcription to HMG CoA reductase

- How the SREBP is activated ?
- It’s fond in the ER bund to protein called SCAP and to another inhibitor protein called INSIG
- When INSIG binds to (SREBP / SCAP) complex , INSIG will trap the complex inside the ER
- And this happens when the cholesterol level is high
- Now , when cholesterol level is decreased the INSIG will be removed from the complex , allowing it to leave the ER towards Golgi
- Inside Golgi , release of SREBP and SCAP and cleavage of SREBP
- The cleaved SREBP will leave Golgi towards the nucleus and start the mechanism explained in the previous slide
- Level 2 : INSIG can bind to the HMG CoA reductase and trapping it in the ER , and also it degrades the enzyme
- Level 3 (post-translational level) : high AMP >> activates AMP kinase > phosphorylation of reductase enzyme (inactivating it) .. And vice versa , activation of phosphates > dephosphorylation of the reductase > activating it
- Level 4 (hormonal level) : glucagon in starvation, more cAMP > activating PKA > activation AMP kinase > phosphorylation of reductase
- Insulin : activates the phosphates, dephosphorylating the reductase

Statins

Each type of the statins has it's own efficacy and kinetics and mechanism





- **The complement in this slide:**The fifth mechanism is pharmacological by drugs which are called statins (they contain group that looks like 3-hydroxy-3-methylglutaryl (HMG) which is the substrate of reductase enzyme,so they will bind to the active site of the enzyme and blocking it (inhibiting cholesterol synthesis)).
- Statins are important (back to slide 24) if cholesterol level is decreased, this will activates the transcription of reductase, so even if a person control his own diet and he didn't take enough cholesterol in the diet , cells can overcome the dietary response by producing cholesterol using reductase enzyme, so statins(which inhibit reductase) block this additional pathway , and reduce the amount of cholesterol.

  There are many statins each one its advantages, beside managing cholesterol level they have a protective role from cancer cells

  NOTE: inhibition of cholesterol synthesis is better way than managing cholesterol by diet

Elimination of cholesterol

- The intact steroid nucleus is eliminated from the body by:
  - conversion to bile acids(protonated)and bile salts(unprotonated), a small percentage of which is excreted in the feces.
  - secretion of cholesterol into the bile, which transports it to the intestine for elimination.

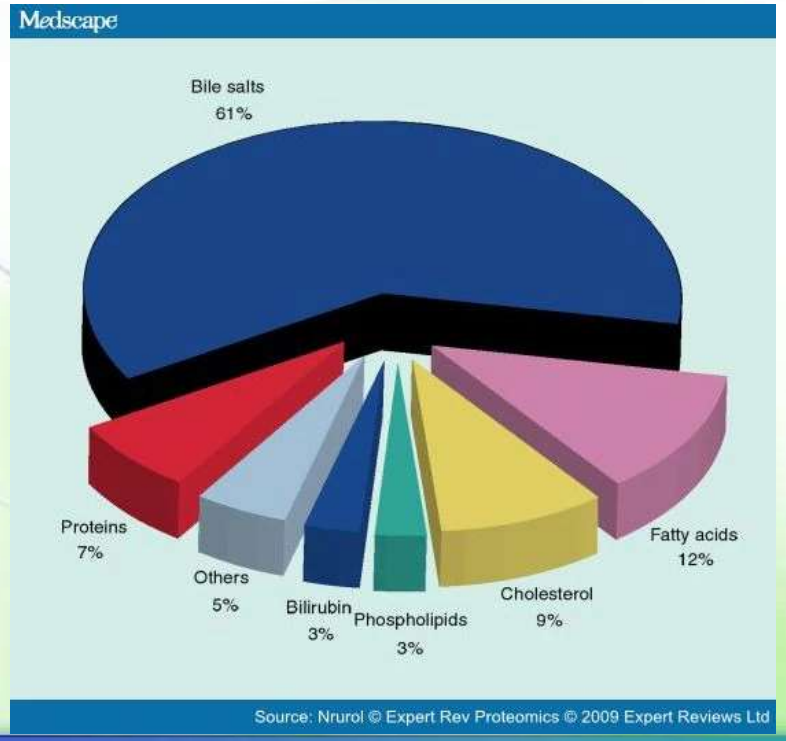
- *Note: The terms bile acid and bile salt are frequently used interchangeably.*

- **How cholesterol is eliminated from our bodies ?**
- **Cholesterol isn't broken down(degraded) in our bodies , it is eliminated by one of these pathways**

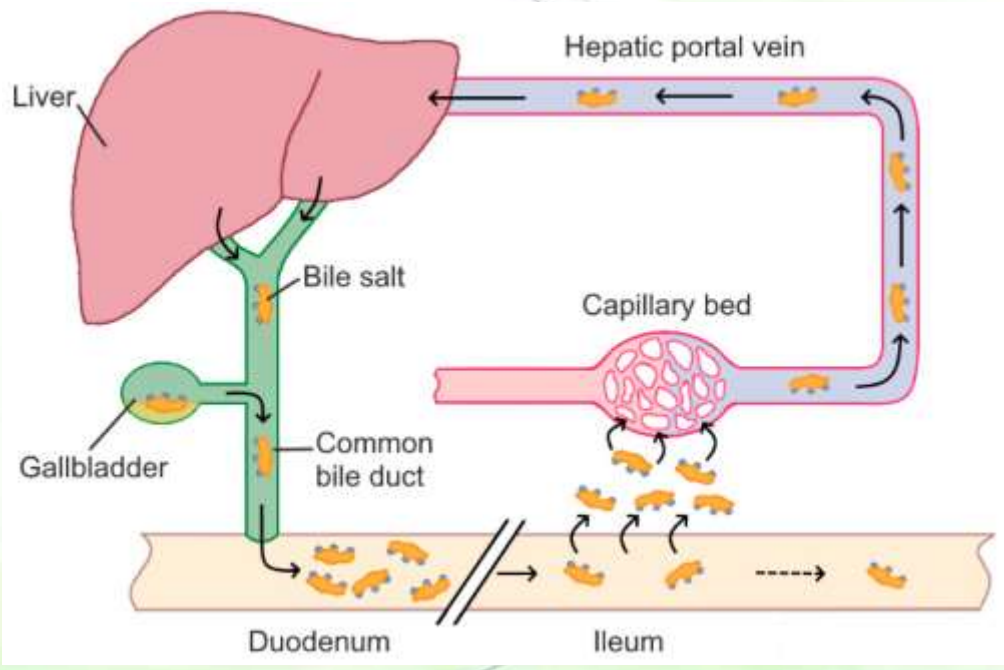
- **Bile acid : protonated**
- **Bile salt: unprotonated**

What is bile?

- Bile consists of a watery mixture of organic and inorganic compounds.
  - Phosphatidylcholine (PC) and conjugated bile salts are the most important organic components of bile.
- Bile can either pass directly from the liver, *where it is synthesized*, into the duodenum through the common bile duct, or be stored in the gallbladder.



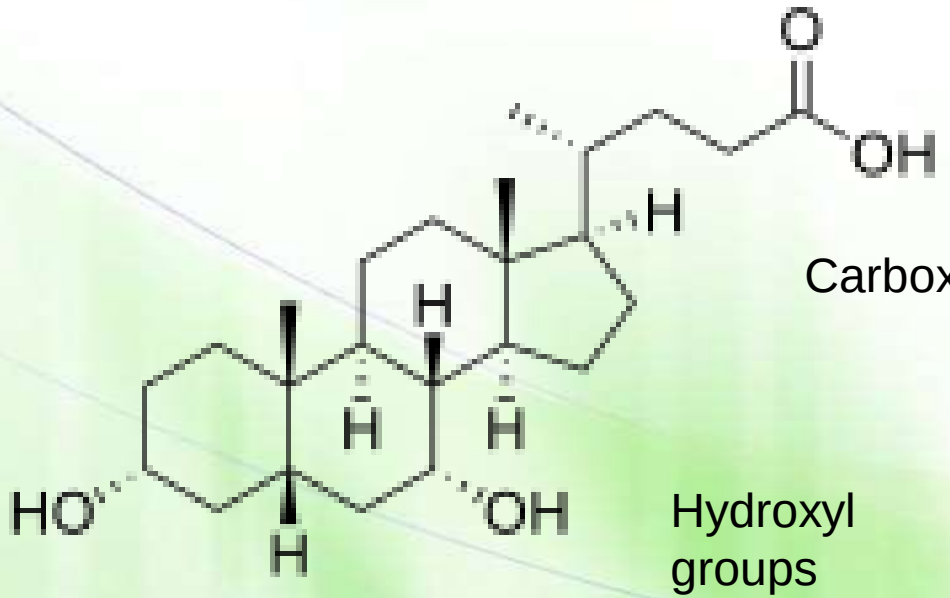
Recycled back to the liver



- **The complement in this slide:**
- Bile consists of a watery mixture of organic and inorganic compounds.
- It has large amounts of bile salts , it also contains Fatty acids , cholesterol phospholipids and bilirubin and so on .
  Bile acids and bile salts are synthesized in the liver(remember : cholesterol is also synthesized in the liver) and it is pumped out through common bile duct , some of them are stored in the gallbladder and the rest will be released into the duodenum then to the intestine .
- They can be recycled by the portal vain back to the liver . See the picture in the previous slide

Structure and protonation states

- The bile acids contain 24 carbons, with two or three hydroxyl groups and a side chain that terminates in a carboxyl group.
- The carboxyl group has a pKa of ~6.
  - In the duodenum (pH ~6), 50% exist as bile acids (protonated) and 50% exists as bile salts (deprotonated).



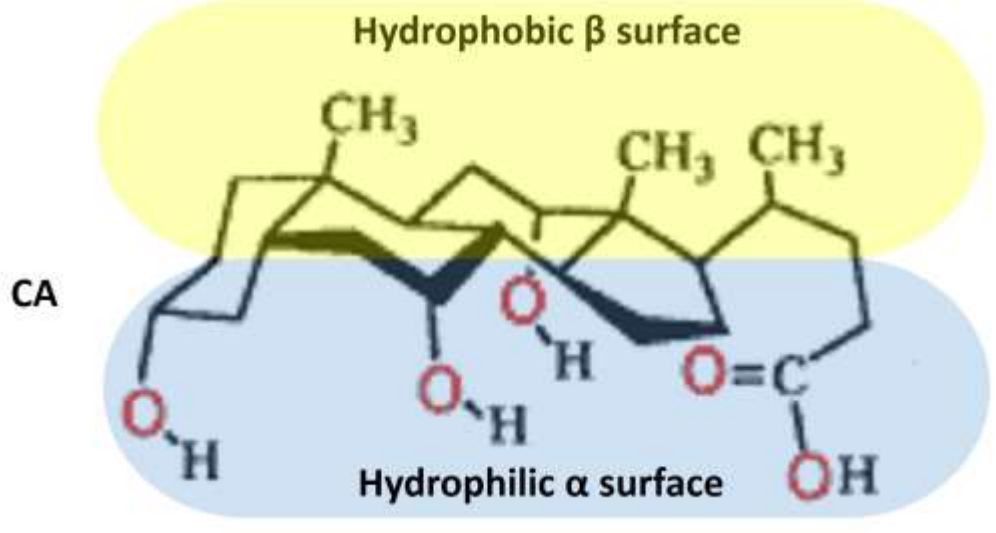
Carboxyl group

Hydroxyl groups

- **But in the intestine it will be mainly deprotonated because ph in intestine is nearly 8**

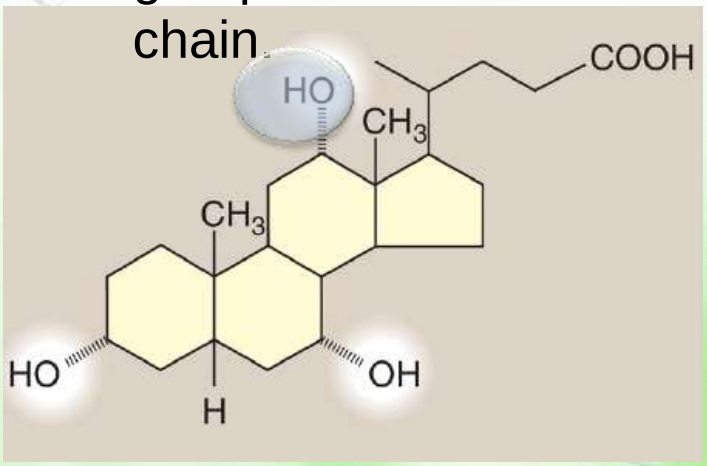
- **The hydrocarbon chain that was in the cholesterol , now it ends with a carboxyl group**

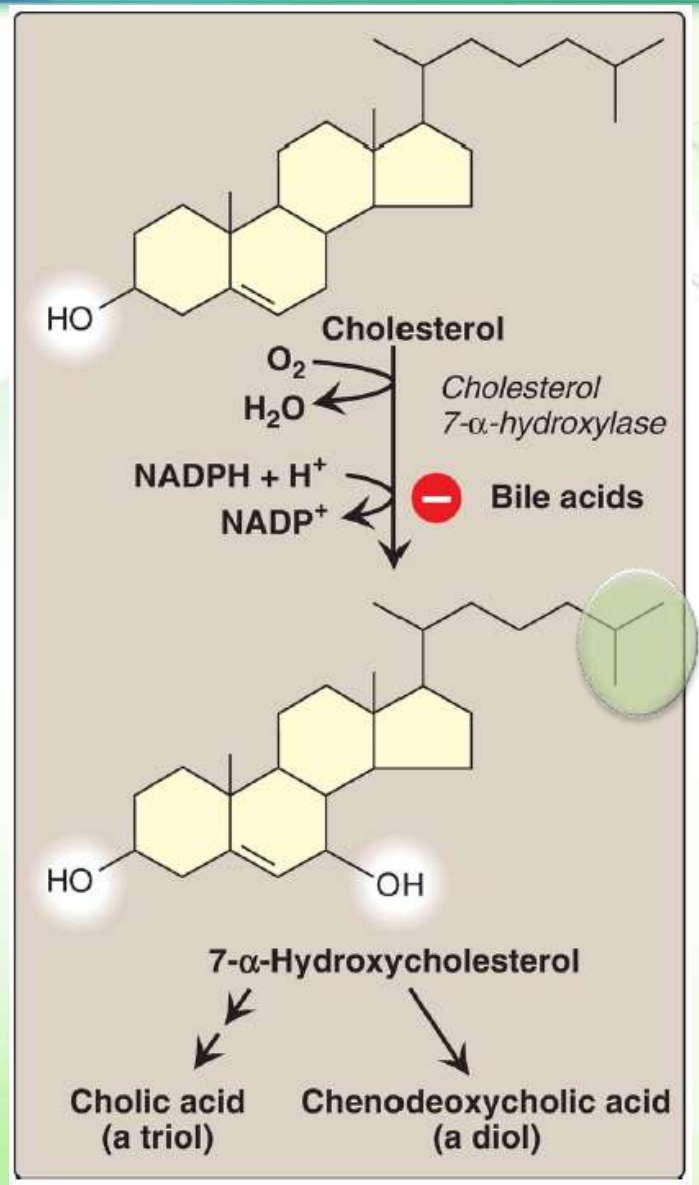
- **NOTE: bile acids are amphipathic,which means all the hydrophilic groups exists on one side & hydrophobic exits on the other side.**



Synthesis of primary bile acids

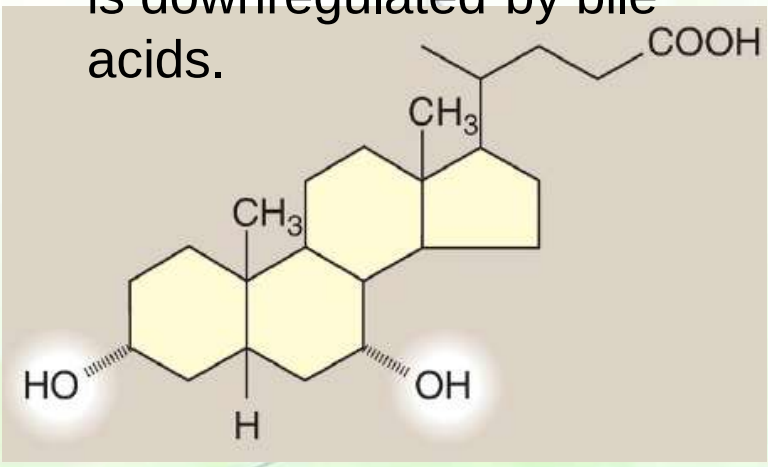
1. Cholesterol is hydroxylated by *7-α-hydroxylase*.
2. The double bond in ring is removed.
3. The hydrocarbon chain is shortened by three carbons.
4. Introducing a carboxyl group at the end of the chain.





Primary bile acids

- The rate-limiting step is catalyzed by *7-α-hydroxylase*, a SER-associated *cytochrome P450 monooxygenase* found only in liver.
- Expression of the enzyme is downregulated by bile acids.

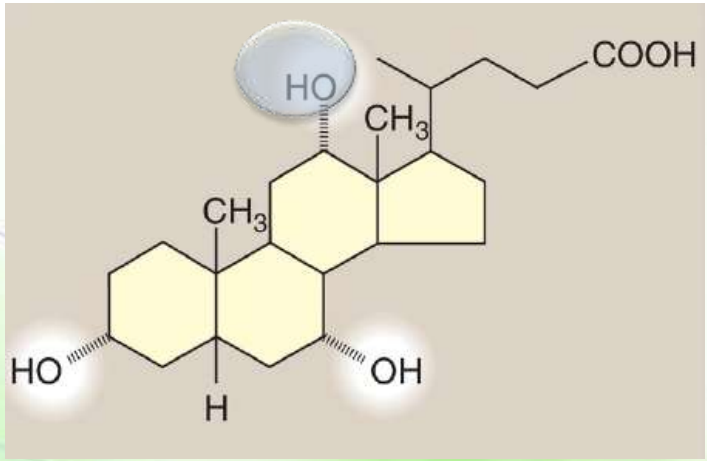


- **The complement in this slide:**
- One of the main differences between cholesterol and bile acids is the presence of hydroxyl group on carbon 7 , so there is an enzyme (cholesterol 7-alpha-hydroxylase) that inserts hydroxyl groups into cholesterol.
  This reaction is the committed step (rate limiting step) in the production of bile acids
- It is a target of bile acid (when bile acid increases the enzyme will be inhibited)

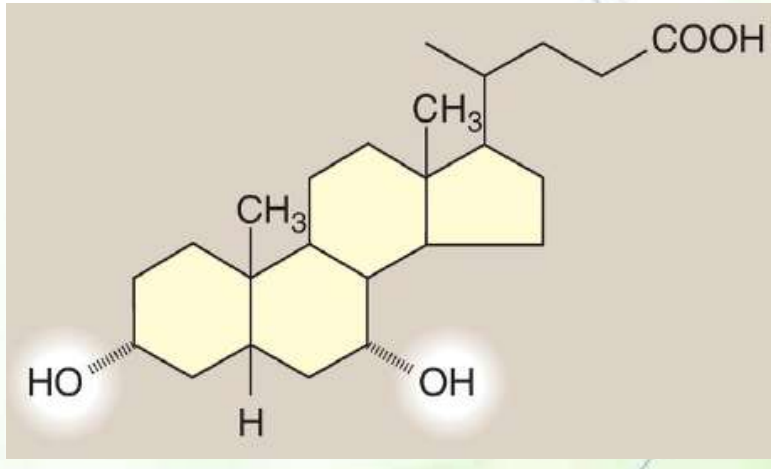
Then it will be modified by many things such as

- 1.The double bond of the cholesterol B ring is reduced
- 2.The hydrocarbon chain is shortened by three carbons
- 3. Introducing a carboxyl group at the end of the chain

- We have 2 primary bile acids , the only difference between them is the hydroxyl group (the blue circle) in choline acid



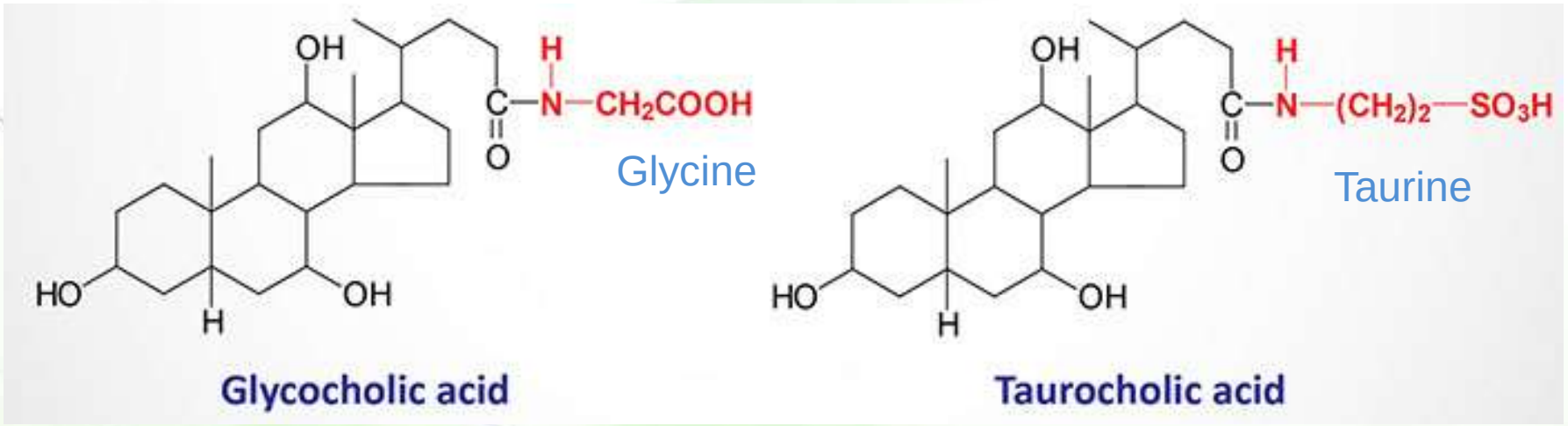
Cholic acid

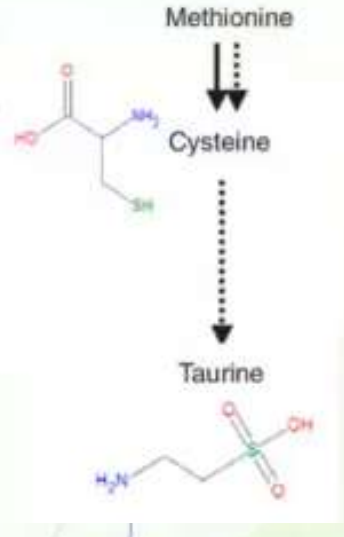


Chenodeoxycholic acid

Conjugation

- In the liver, bile acids are conjugated to either glycine or taurine (an end product of cysteine metabolism) forming more amphipathic and ionized compounds and better emulsifiers.
- The ratio of the glycine to taurine forms in the bile is ~3/1.





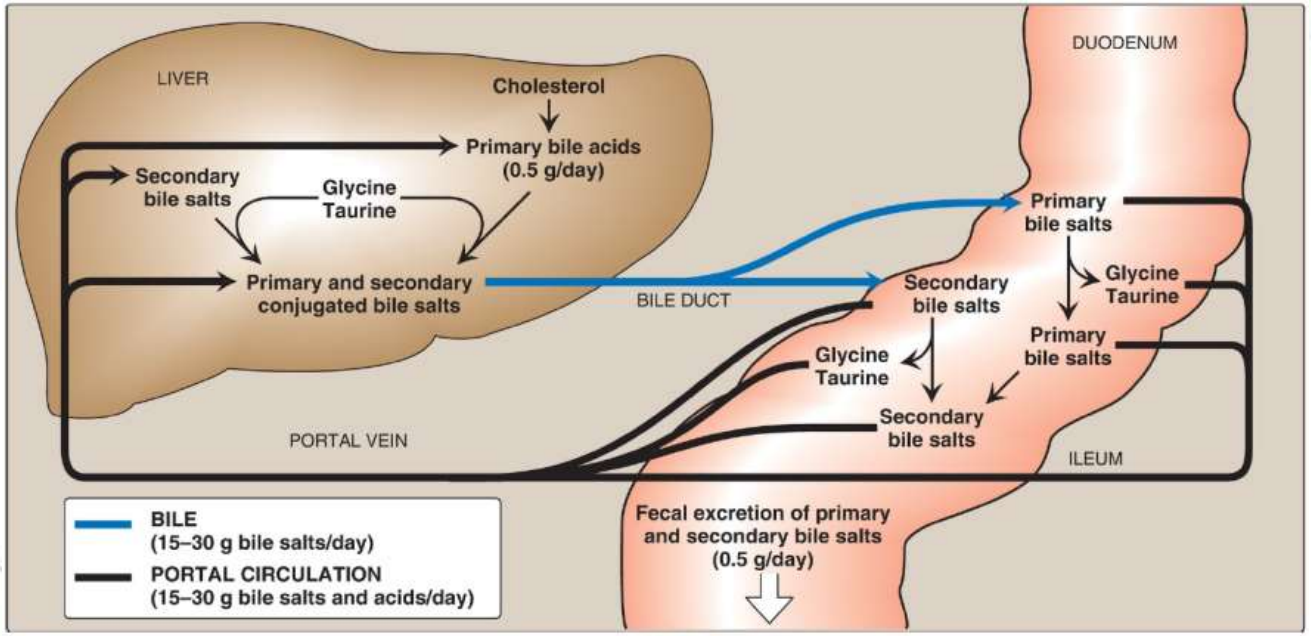
OR Glycochenodeoxycholic acid

OR Taurochenodeoxycholic acid

Also, primary bile acids

- The complement in this slide:
- The glycine and taurine are conjugated to the carboxyl group
- Glycine and taurine make the bile acid more amphipathic , more capable of dissolving lipids (emulsifing lipids) in the intestine .

Bacterial actions



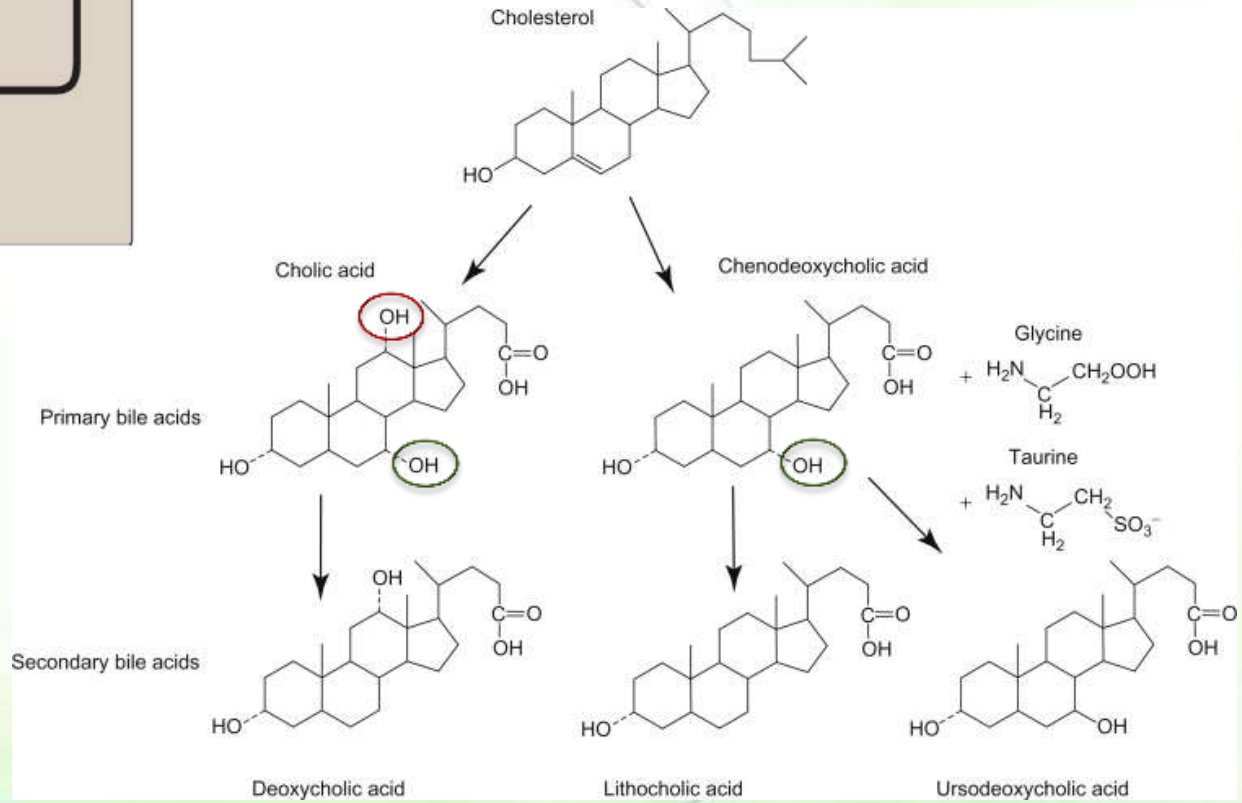
Primary bile acids: cholic acid and chenodeoxycholic acid

Secondary bile acids (by bacteria): deoxycholic acid and lithocholic acid

Bacteria can also deconjugate bile acids.

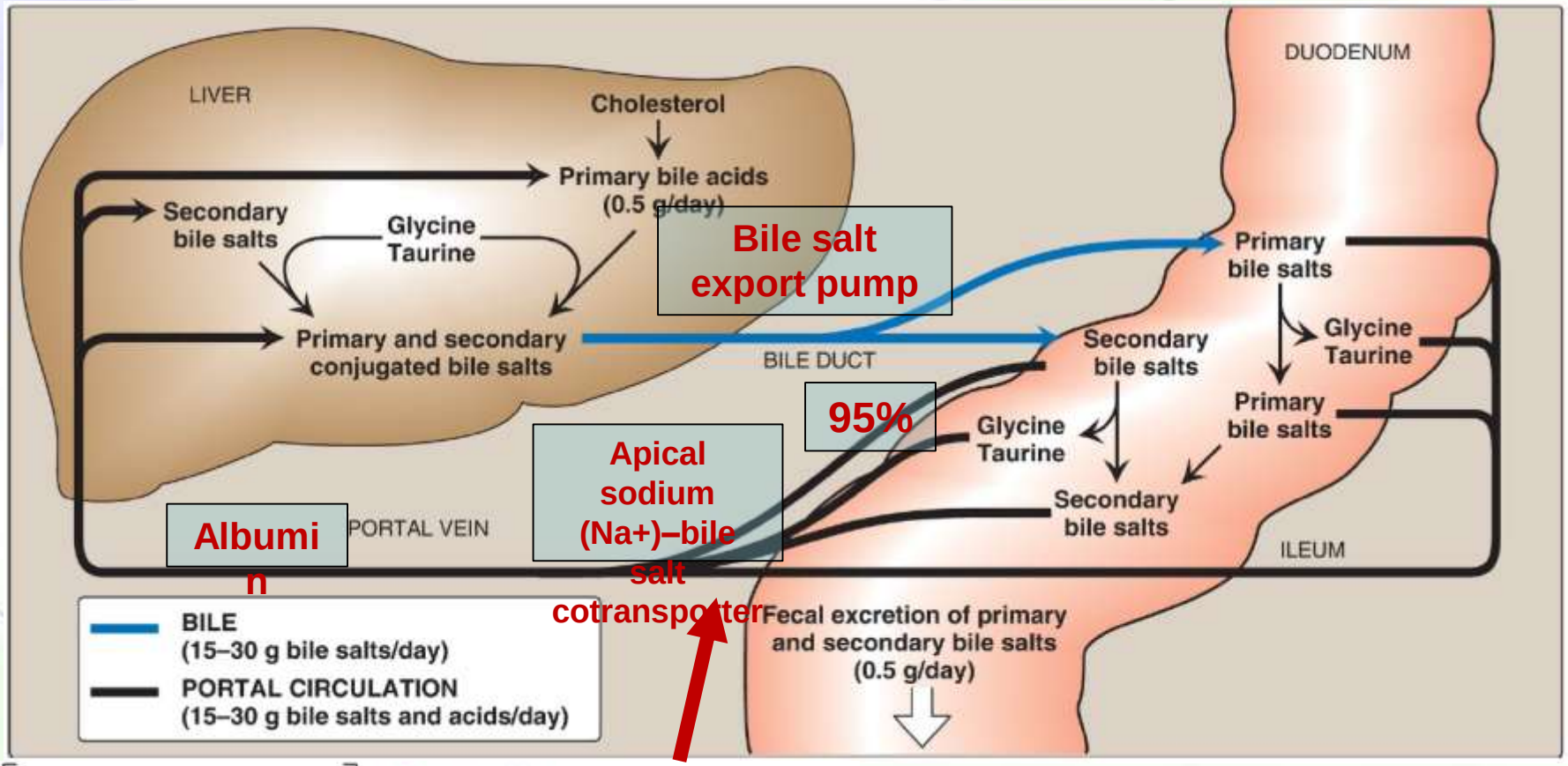
- **Bacteria can remove the glycine and taurine as well**

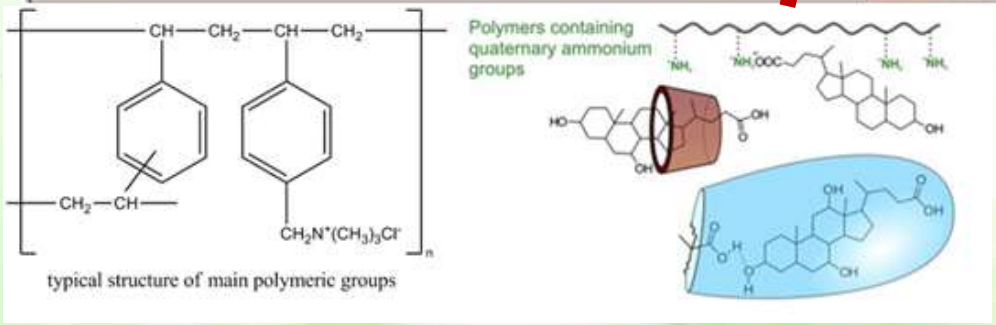
The doctor said don't worry about the structures, just try to understand them



- The complement in this slide:
- Production of bile acids in the liver , then released through the bile duct to the duodenum (deconjugation of glycine or taurine may occurs here)
- In the intestine the primary bile acids may be converted into secondary bile acids (the bacteria is responsible for this conversion)
- Then recycling of bile acids (primary or secondary) into the liver , in the liver conjugation may occur another time returning back to the duodenum

Enterohepatic circulation





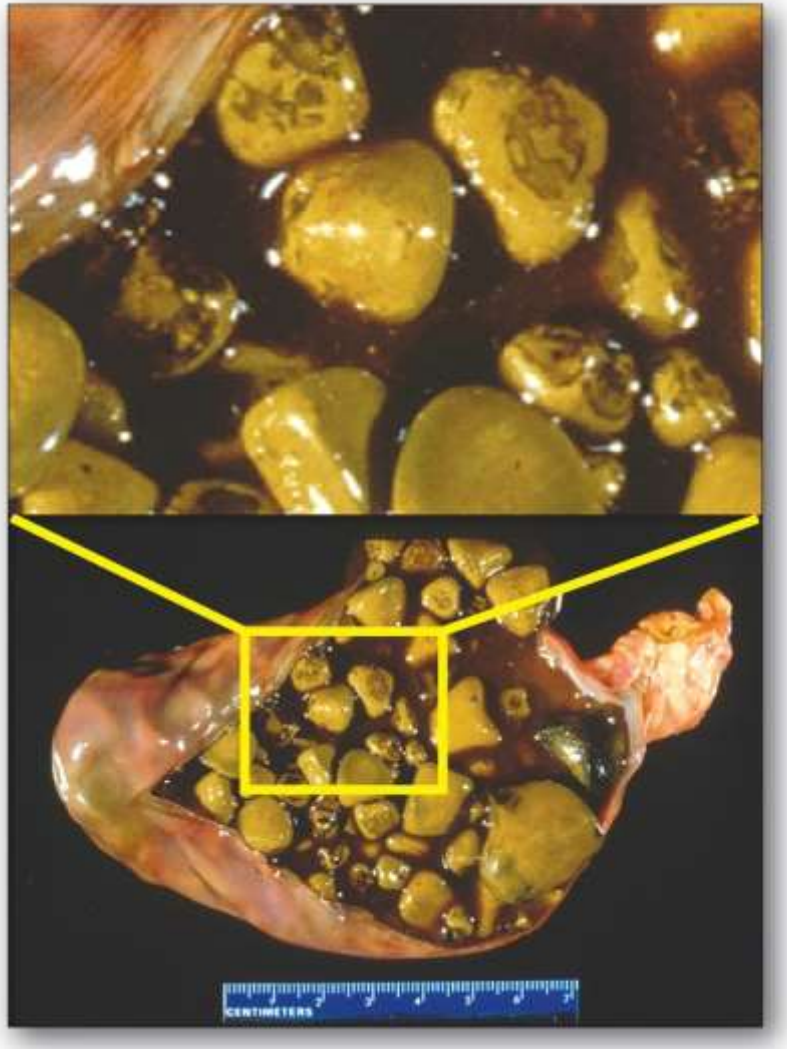
Bile acid sequestrants, such as cholestyramine, bind bile salts in the gut and prevent their reabsorption, thereby promoting their excretion.

- **The complement in this slide:**
- 95% of the bile acids will be recycled by binding to Albumin
- Albumin is the main carrier of bile acids and it takes them back to the liver
- 5% of bile acid will be eliminated (excreted)

- **The complement in this slide:**Scientists were thinking of reducing the amount of bile acid so the enzyme continues to convert the cholesterol into bile acid, this will reduce the amount of cholesterol in the plasma and this will reduce the recycling of bile acids .. Increasing the amount that is eliminated (to be more than 5%)
- So they established " bile acid sequestrants" , the drugs bind to the bile acid and inhibit the recycling >> increase elimination >> increase the conversion of cholesterol to bile acid >> reducing the amount of cholesterol in the plasma

Bile salt deficiency: Cholelithiasis

- ↑Cholesterol or ↓bile acids → insolubility → gallbladder stones (cholelithiasis)
  - *"Lithiasis" refers to the presence of stones that can originate in any part of the urinary tract.*
- Treatment: cholecystectomy
  - Alternatively: oral administration of chenodeoxycholic acid results in a gradual (months to years) dissolution of the gallstones.



- **The complement in this slide:**
- If cholesterol increases in the liver or bile acid decreases in the gallbladder, this causes insolubility of cholesterol or other components of bile >> formation of gallbladder stones (choletithiasis)
- Drinking small amounts of water >> that makes things worst
- Cholecystectomy: removal of the gallbladder.. Patients will suffer initially from problems in absorption of lipids through bile acids which causes diarrhea (they should change their lifestyle at first until their bodies adapt)

سُورَةُ آلِ عِمْرَانَ

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

أَمْ حَسِبْتُمْ أَن تَدْخُلُوا۟ ٱلْجَنَّةَ وَلَمَّا يَعْلَمِ ٱللَّهُ ٱلَّذِينَ
جَٰهَدُوا۟ مِنكُمْ وَيَعْلَمَ ٱلصَّٰبِرِينَ ﴿١٤٢﴾

V2: Slide 14

Three instead of two