

## **Lipid metabolism II**

# **Phospholipids and glycolipids**

# **Eicosanoids.**

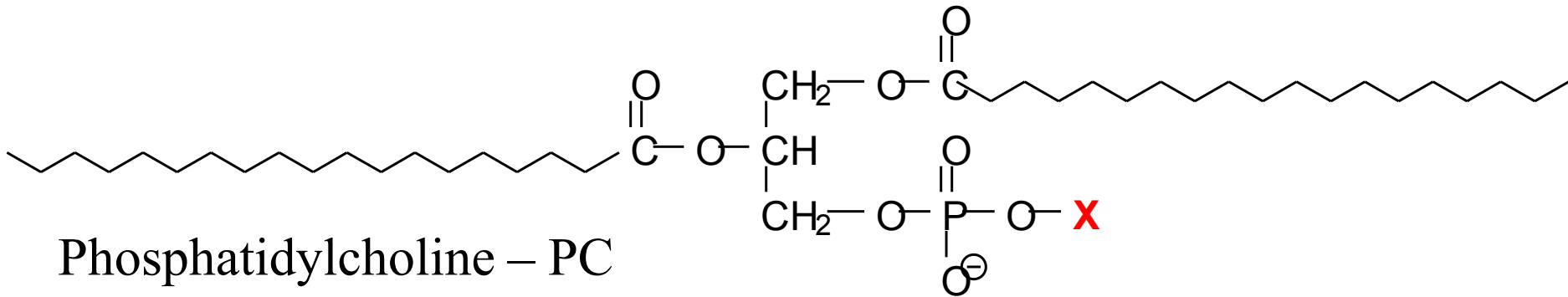
# **Synthesis and metabolism of cholesterol and bile acids**

Biochemistry I

Lecture 9

2012 (E.T.)

# Glycerophospholipids



Phosphatidylcholine – PC

Phosphatidylethanolamine – PE

Phosphatidylserine – PS

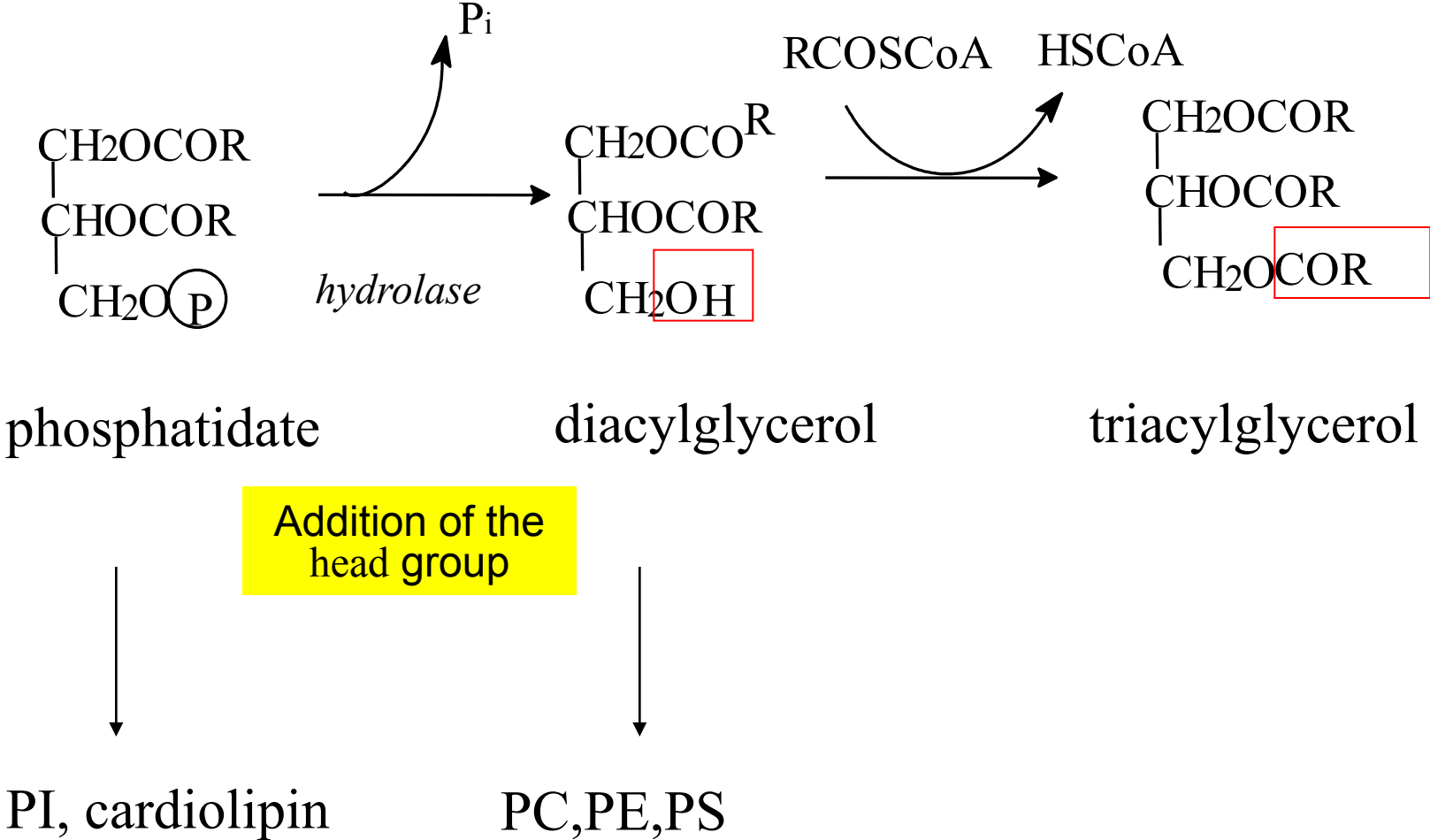
Phosphatidylinositol – PI

Cardiolipin - CL

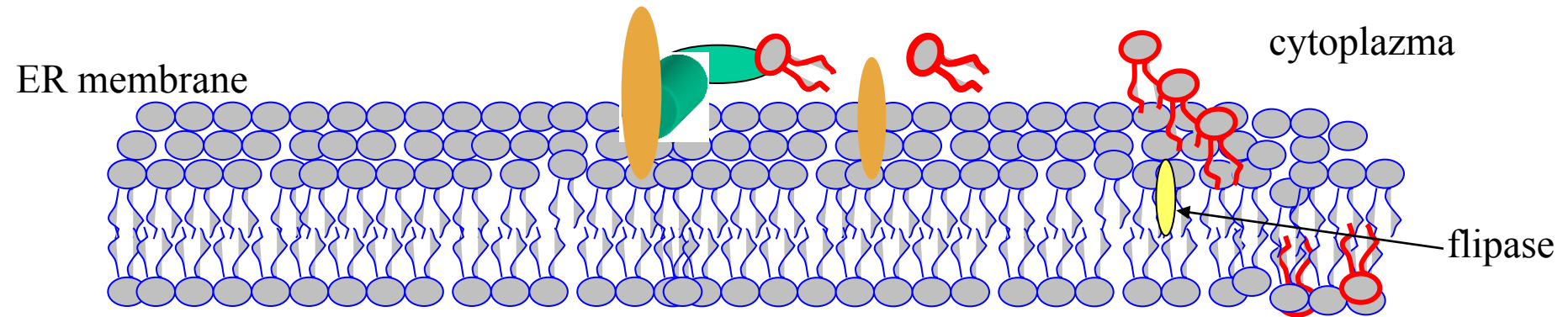
# Biosynthesis of glycerophospholipids

- located in all cells with exception of erythrocytes
- the initial steps of synthesis are similar to those of triacylglycerol synthesis

# Synthesis of triacylglycerols and glycerophospholipids – common aspects



# Location of phospholipids synthesis in the cell

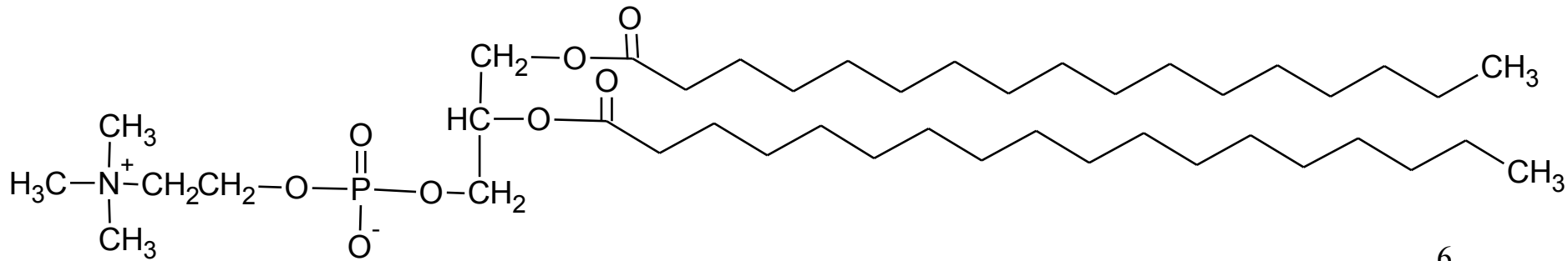


- Synthesis of phospholipids is located on membranes of ER
- Enzymes are integral membrane proteins of the outer leaflet with active centers oriented on cytoplasm
- Newly synthesised phospholipids are built in the inner layer of the membrane
- By the action of flippases are transferred into the outer layer
- *De novo* synthesized membranes are transported via a vesicle mechanism to the Golgi complex and from there to different organelles and the plasma membrane.

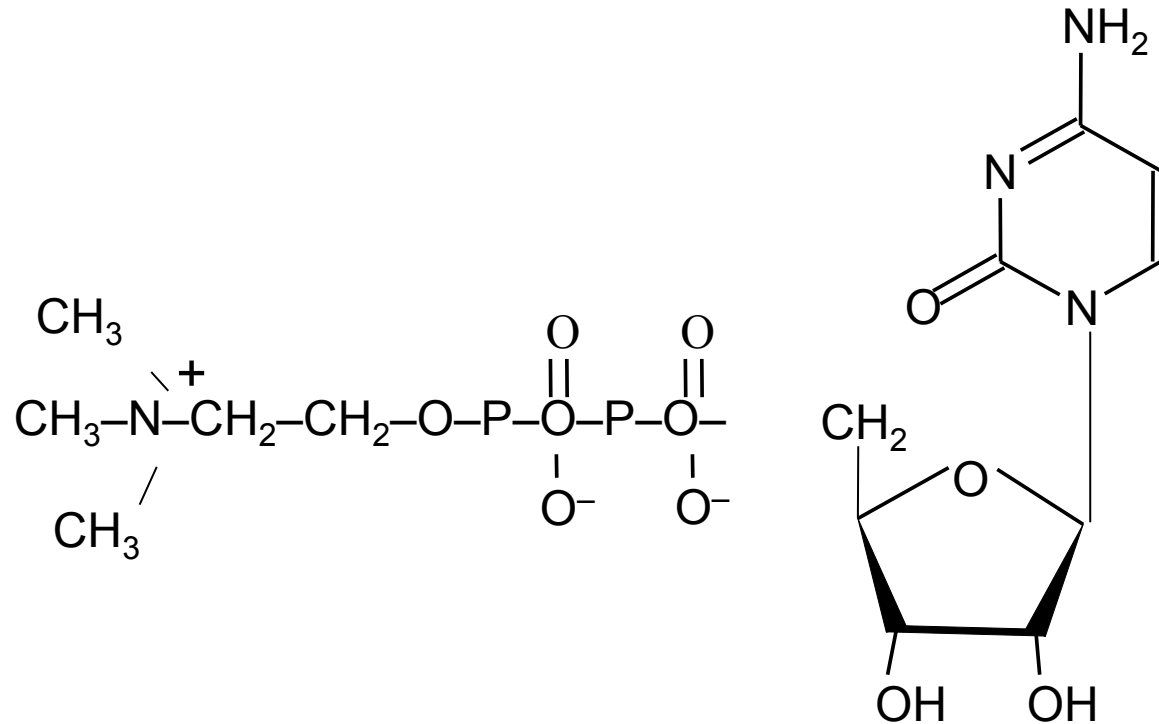
# Synthesis of phosphatidyl choline

Choline must be activated before the synthesis

- 1) Choline + ATP → Choline-P + ADP
- 2) choline-P + CTP → CDP-choline + PP<sub>i</sub>
- 3) CDP-choline + 1,2-DG → fosfatidylcholine + CMP



# CDP-choline

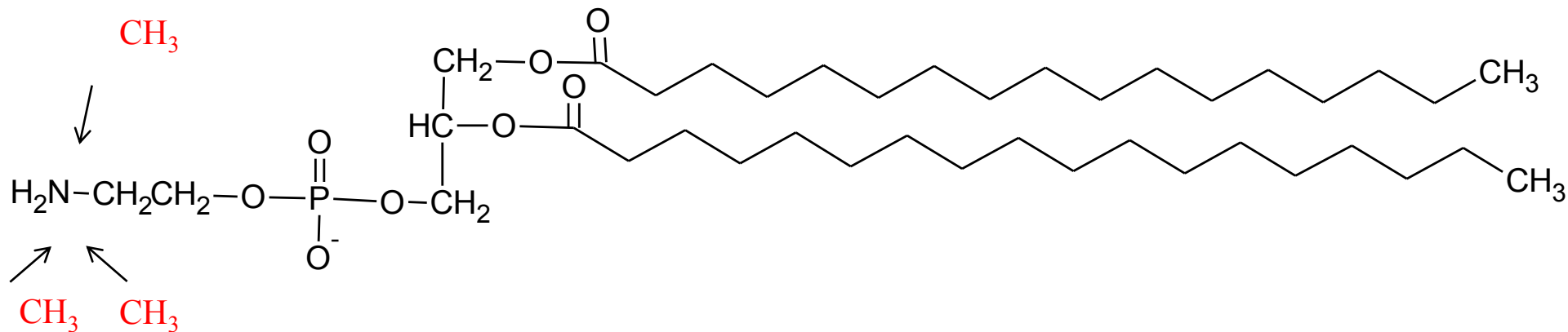


CDP-choline plays a part formally similar to that of UDP-glucose in the synthesis of glycogen.

The biosynthesis of **phosphatidyl ethanolamine** (PE) is similar.

## Conversion of phosphatidyl ethanolamine to phosphatidyl choline

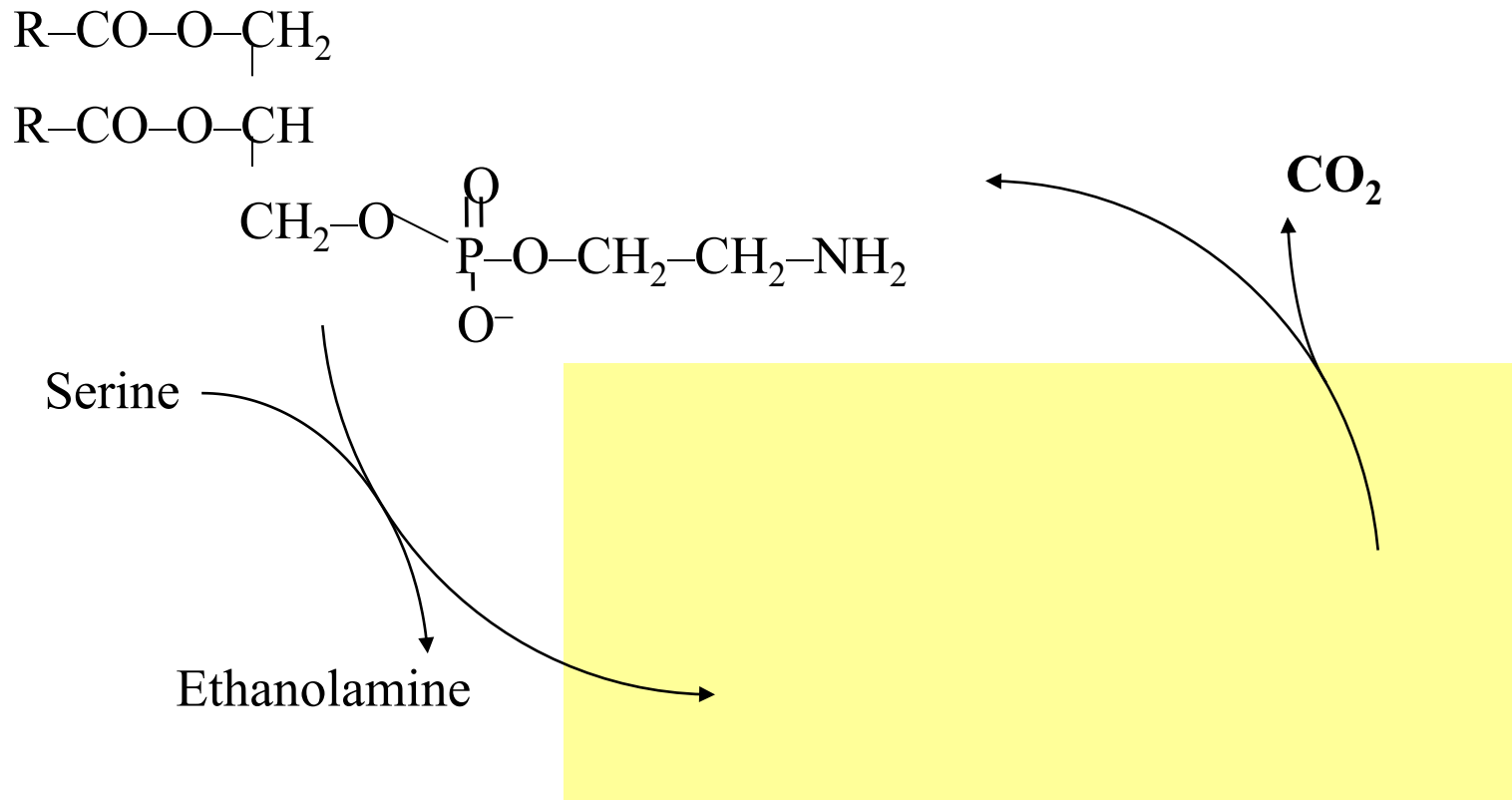
N-methylation of phosphatidyl ethanolamine by SAM





# Biosynthesis of phosphatidyl serine (PS)

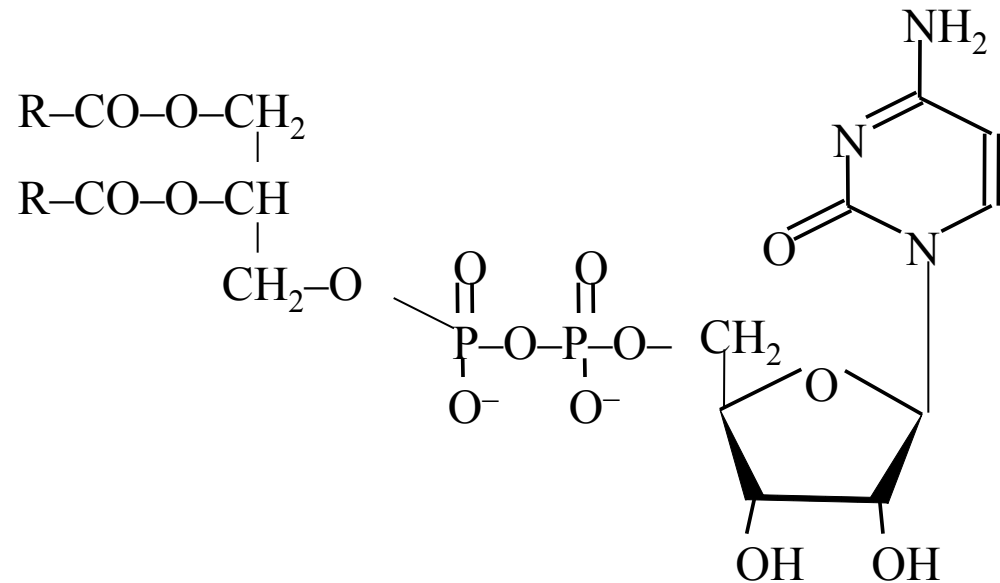
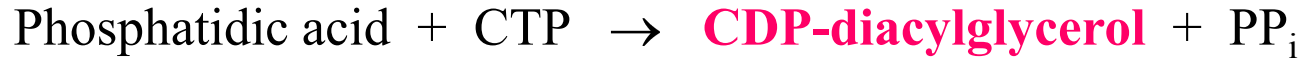
Phosphatidyl ethanolamine + **serine** → **phosphatidyl serine** + ethanolamine



Phosphatidyl serine can be also **decarboxylated** to form **PE**.

## 2 Synthesis of phosphatidyl inositol

### 1. Activation of phosphatidic acid

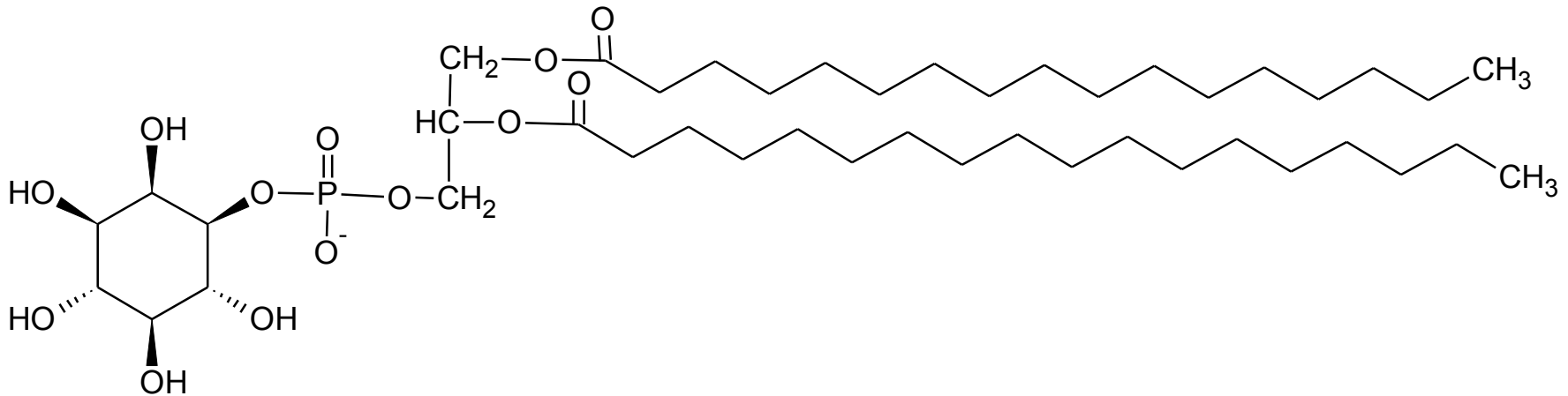


CDP-diacylglycerol =  
activated phosphatidate

## 2. CDP-Diacylglycerol then reacts

with free inositol to give phosphatidyl inositol (PI)

CDP-diacylglycerol + inositol  $\rightarrow$  CMP + phosphatidyl inositol (PI)

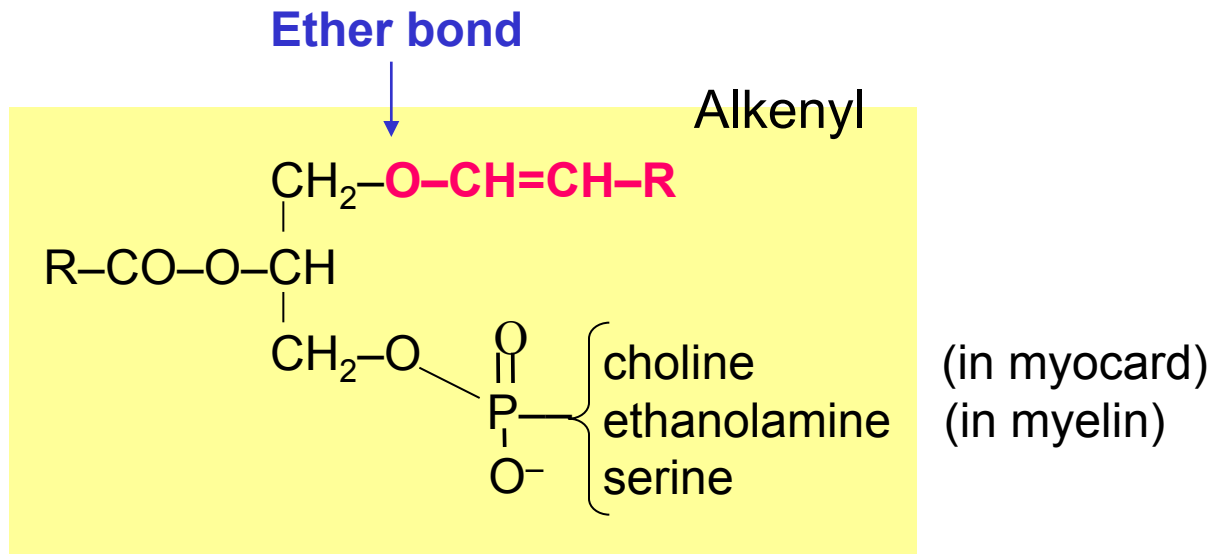


# Products of lipid remodeling

## Plasmalogens

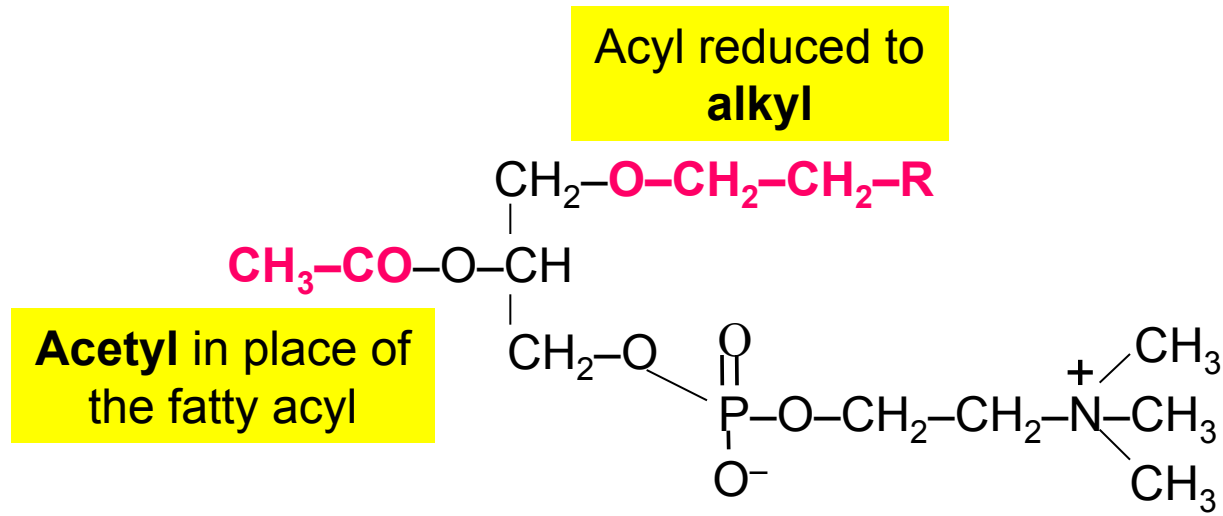
modified glycerophospholipids (alkoxylipids or ether glycerophospholipids).

Plasmalogens represent about 20 % of glycerophospholipids.



# PAF (platelet activating factor)

PAF induces aggregation of blood platelets and vasodilation and exhibits further biological effects, e.g. it is a major mediator in inflammation, allergic reaction and anaphylactic shock.



# Transacylation reactions

## Exchange of acyls on the C-2 in phospholipids:

diacylglycerols:

phospholipids:

oleic acid on the C-2

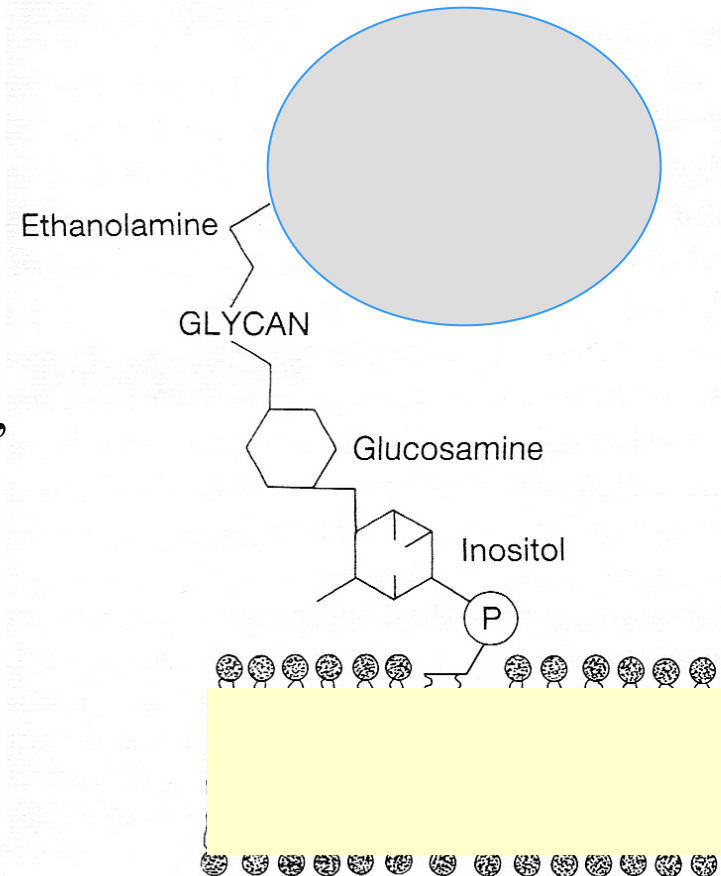
polyunsaturated FA (often  
arachidonic acid) on the C-2

# Significance of glycerophospholipids

- essential structural components of all biological **membranes**
- essential components of **lipoproteins** in blood
- supply polyunsaturated fatty acids for the synthesis of eicosanoids
- act in anchoring of some (glyco)proteins to membranes,
- serve as a component of lung surfactant
- phosphatidyl inositols are precursors of second messengers (PIP<sub>2</sub>, DG)

# Anchoring of proteins to membrane

The linkage between the COOH-terminus of a protein and phosphatidylinositol fixed in the membrane lipidic bilayer exist in several ectoenzymes (alkaline phosphatase, acetylcholinesterase, some antigens).

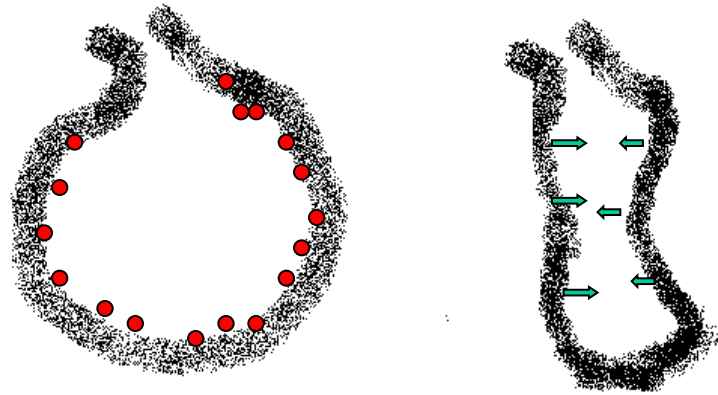




# Lung surfactant

The major component is **dipalmitoylphosphatidylcholine**.

It contributes to a reduction in the surface tension within the alveoli (air spaces) of the lung, preventing their collapse in expiration. Less pressure is needed to re-inflate lung alveoli when surfactant is present.

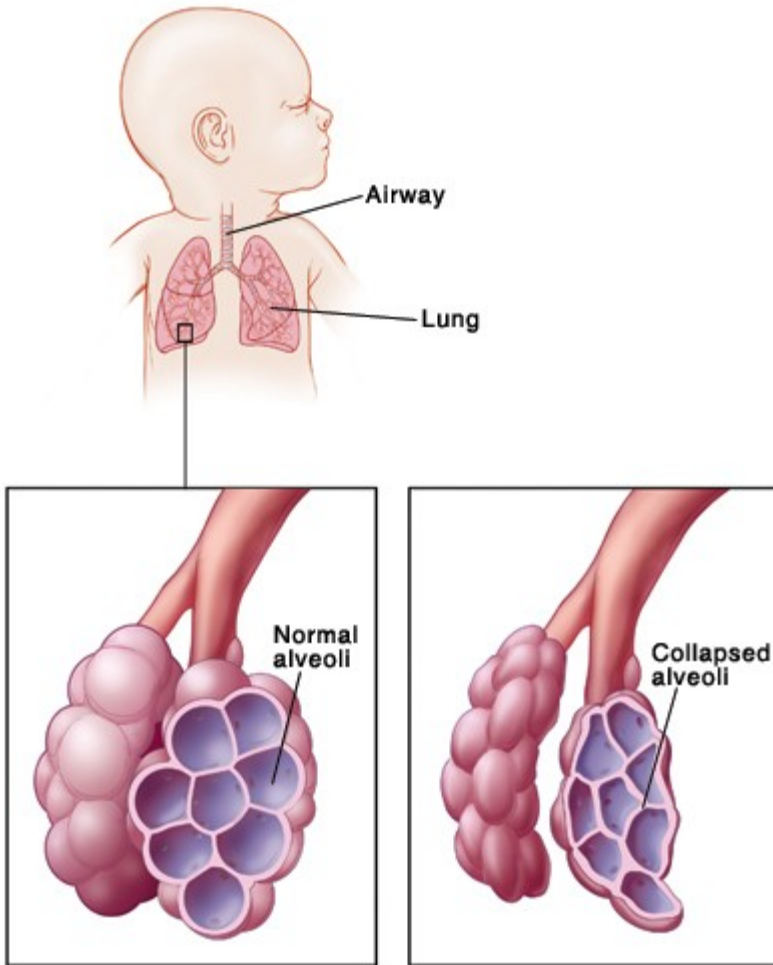


The respiratory distress syndrome (RDS) of premature infants is caused, at least in part, by a deficiency in the synthesis of lung surfactant.

# Respiratory distress syndrome

Treatment:

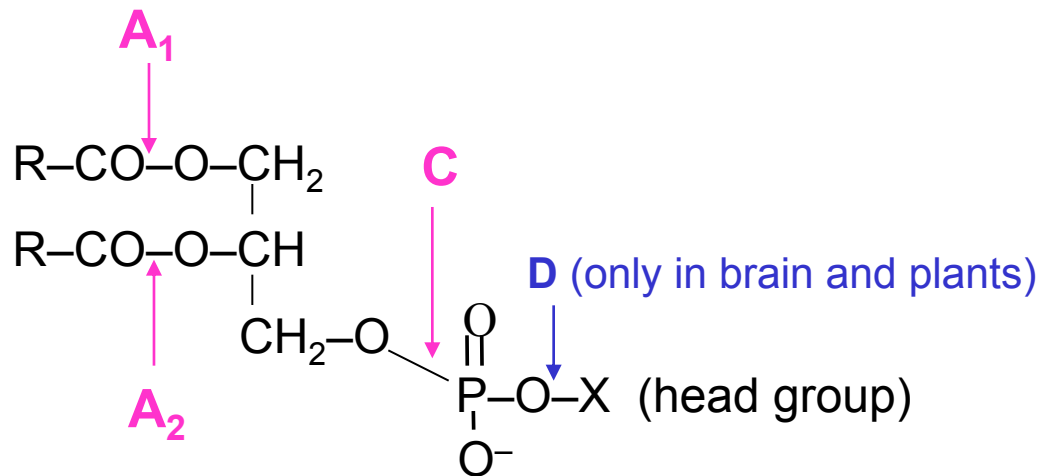
Artificial surfactant is given



Normally, alveoli stay open after each breath. RDS occurs when alveoli collapse after each breath. This means the baby has to work harder to breathe

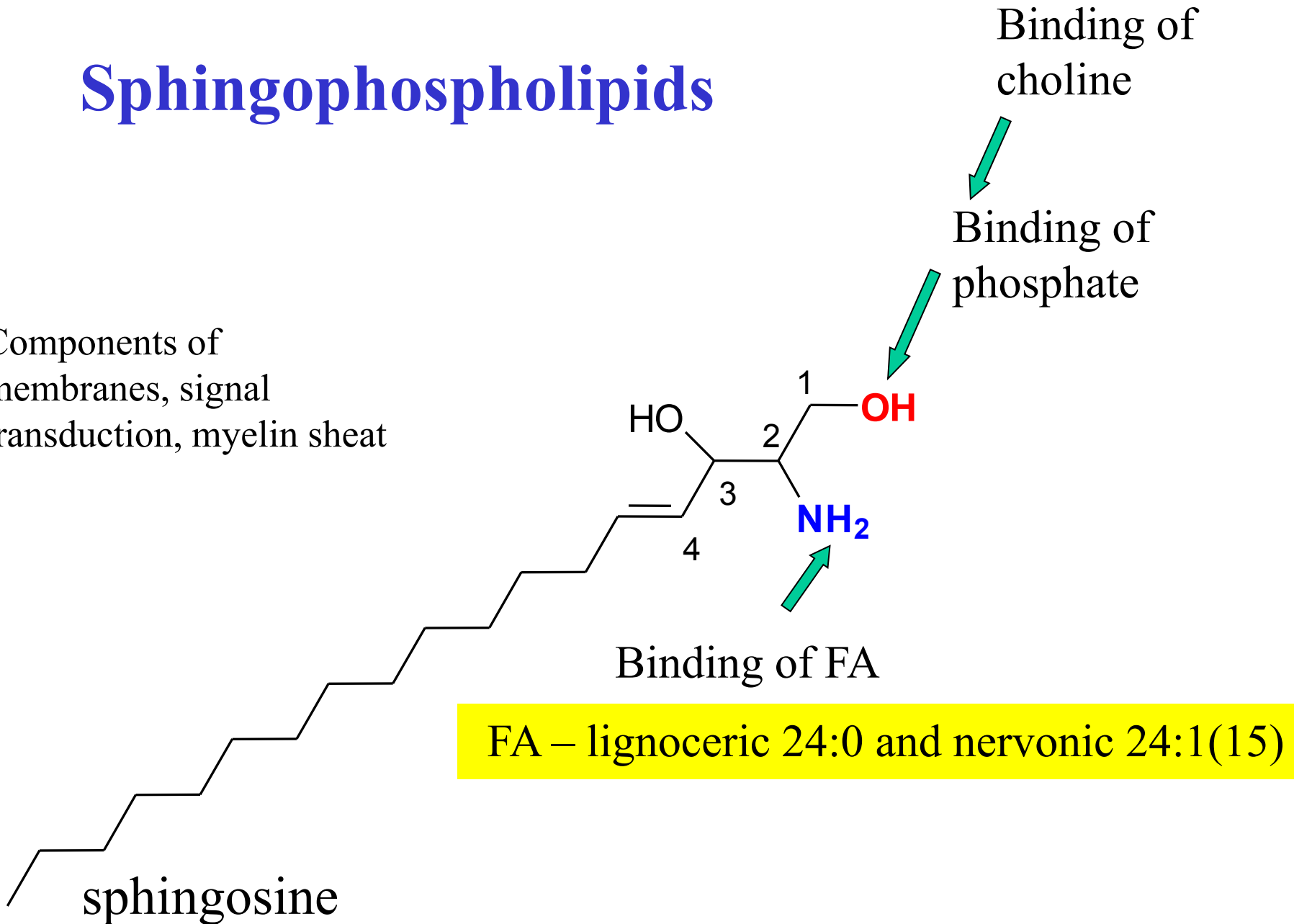
# Catabolism of glycerophospholipids

Enzymes catalysing hydrolysis of glycerophospholipids are called **phospholipases**. Phospholipases are present in cell membranes or in lysosomes. Different types ( $A_1$ ,  $A_2$ , C, D) hydrolyse the substrates at specific ester bonds:



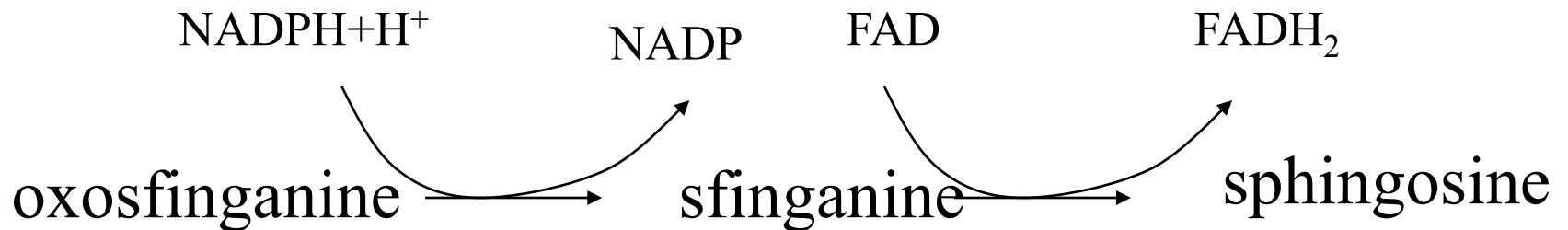
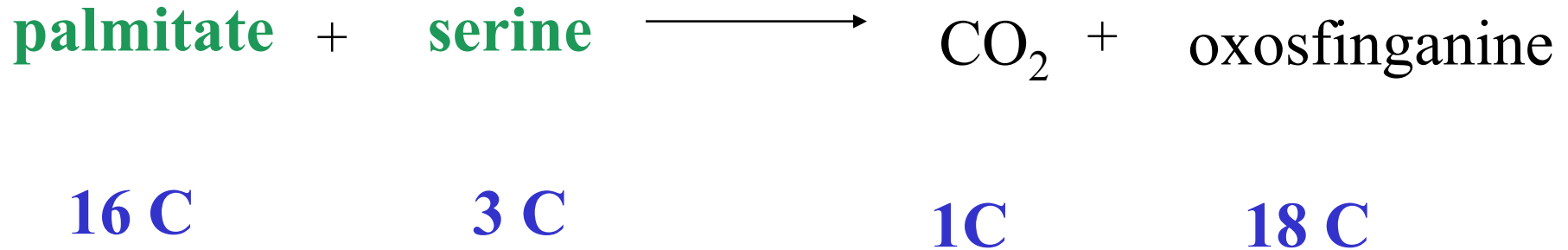
# Sphingophospholipids

Components of membranes, signal transduction, myelin sheat

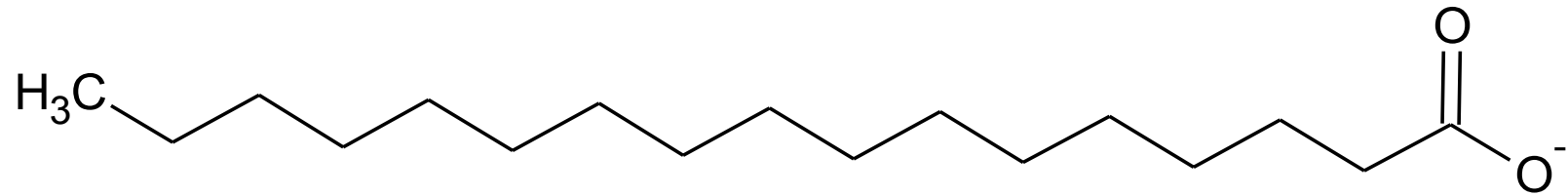


# Biosynthesis of sphingosine

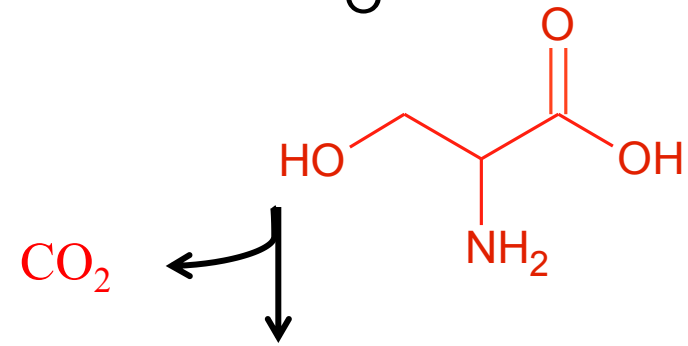
- overall equation



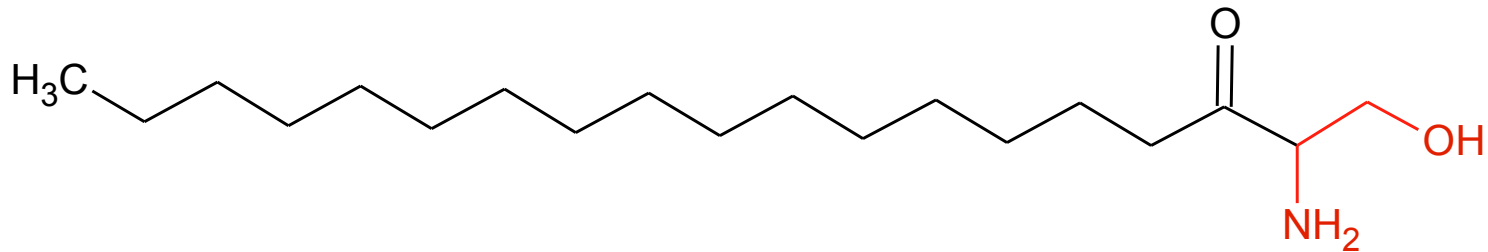
# Biosynthesis of sphingosine



Palmitic acid

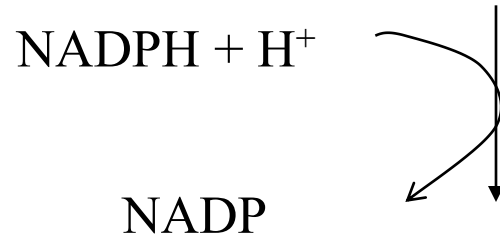
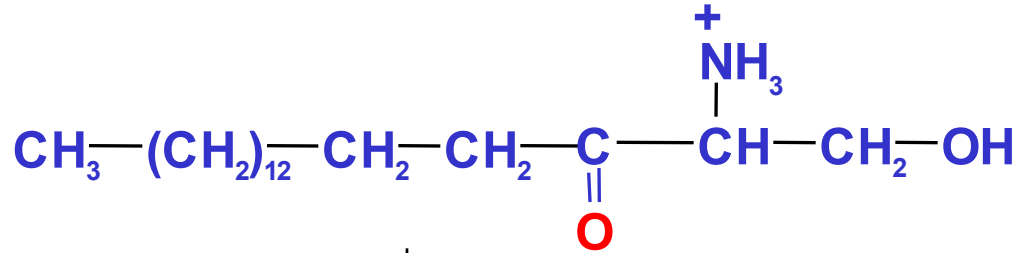


serine

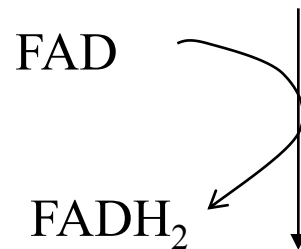
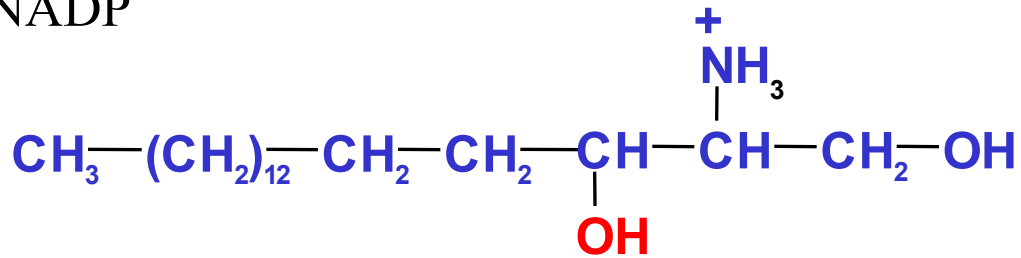


oxosfinganine

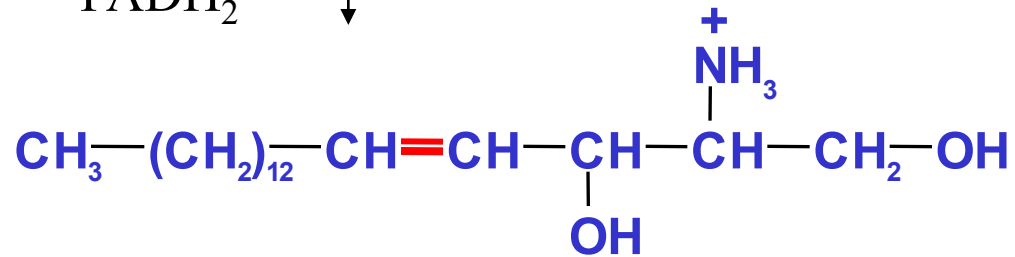
oxosfinganine



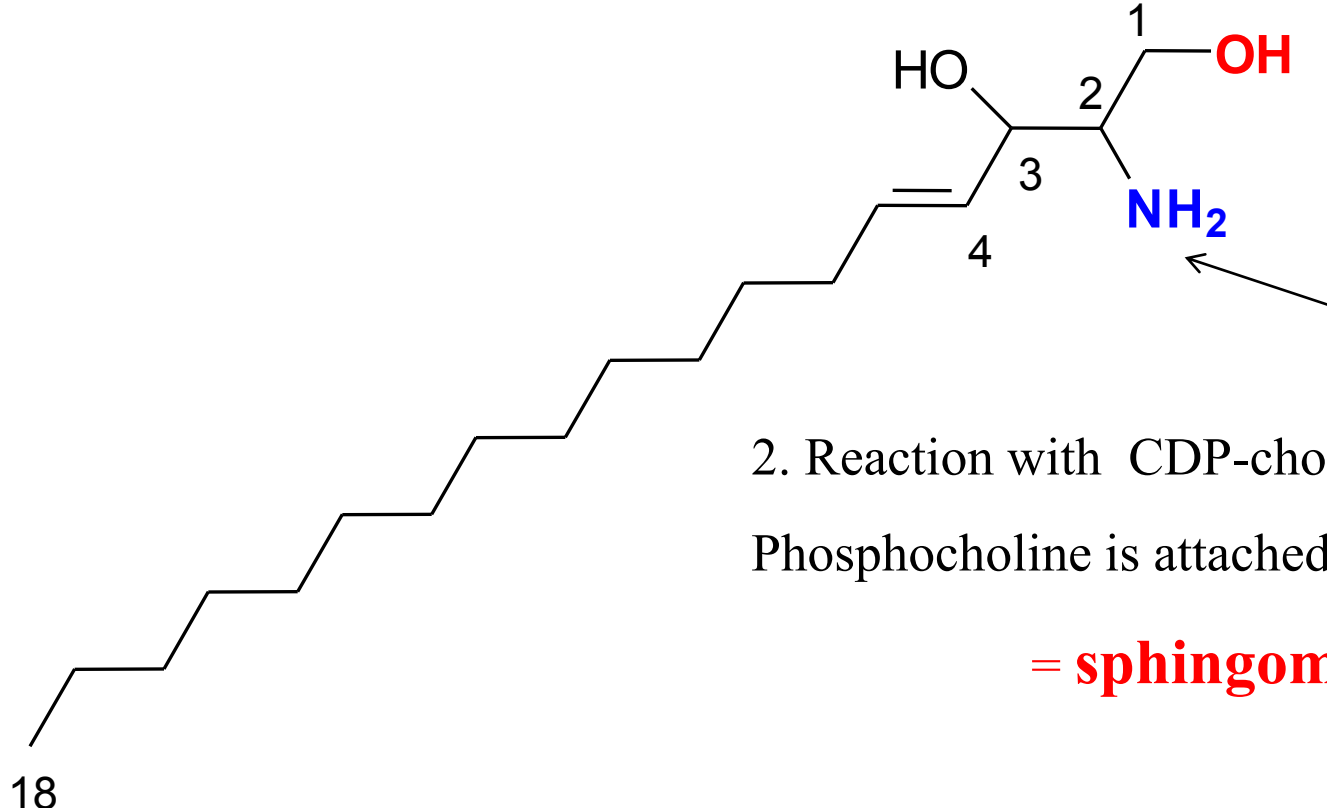
sfinganine



sfingosine



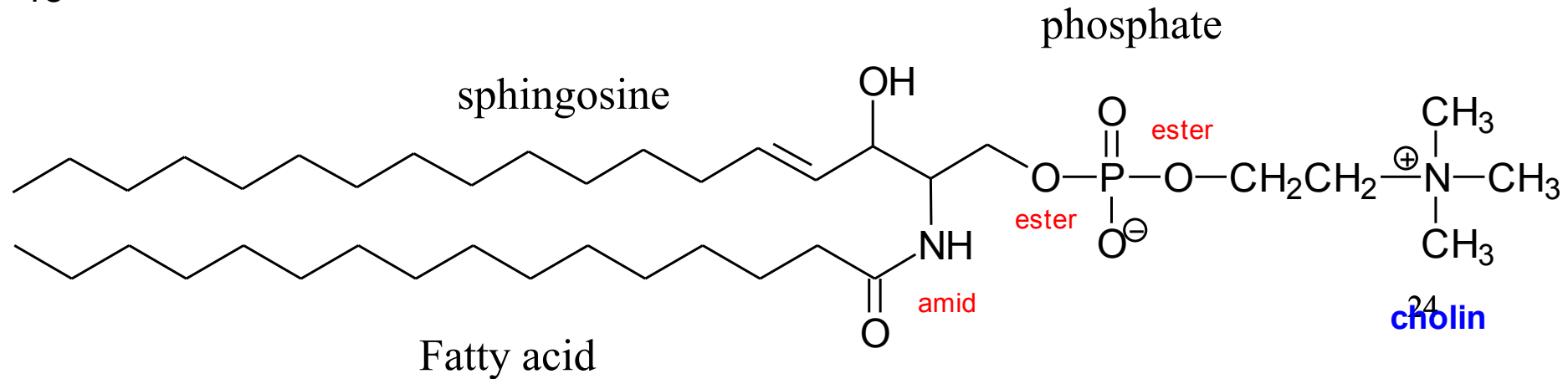
# Biosynthesis of sphingomyelin



1. Attachment of fatty acid by amide bond  
**= ceramide**

2. Reaction with CDP-choline:  
 Phosphocholine is attached to CH<sub>2</sub>OH

**= sphingomyelin**

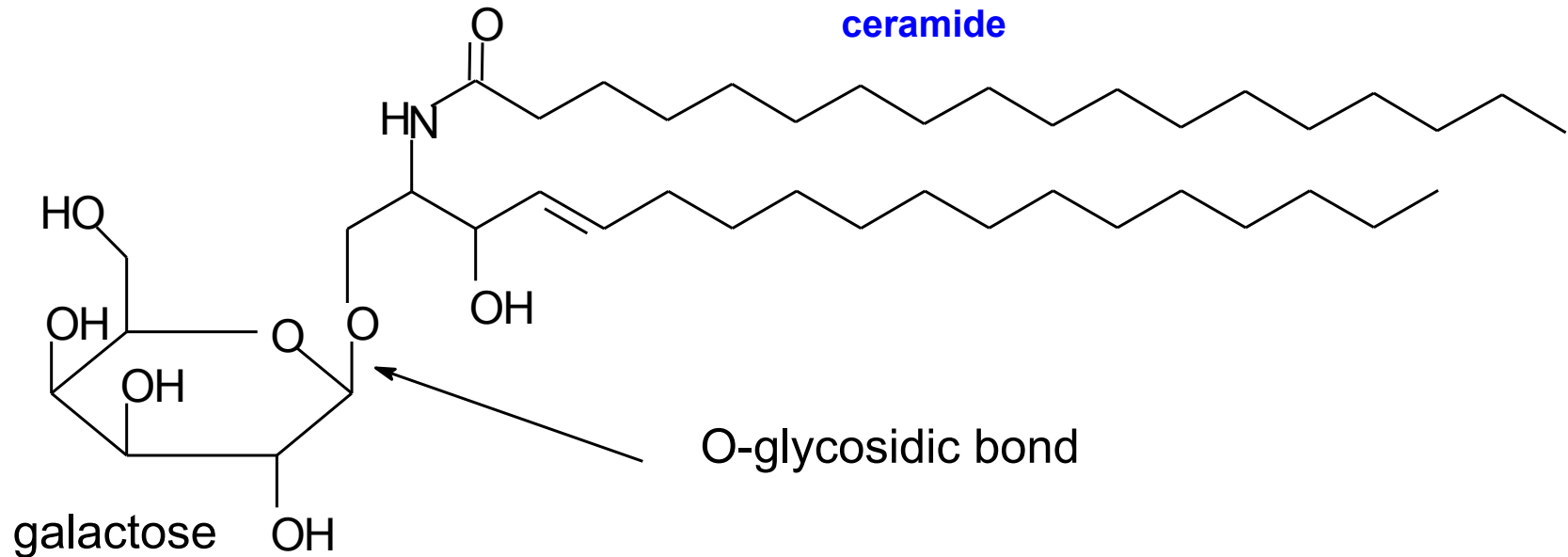




# Glycosphingolipids

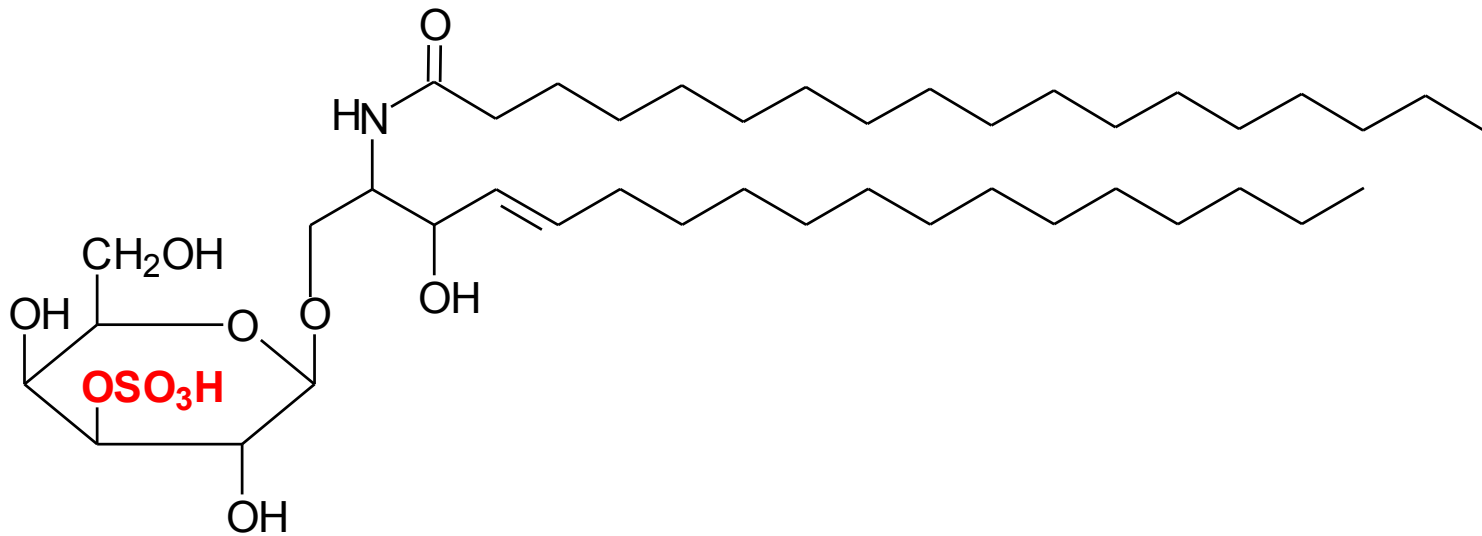
## Cerebrosides (monoglycosylceramides)

ceramide + UDP-galactose



galactosylceramide

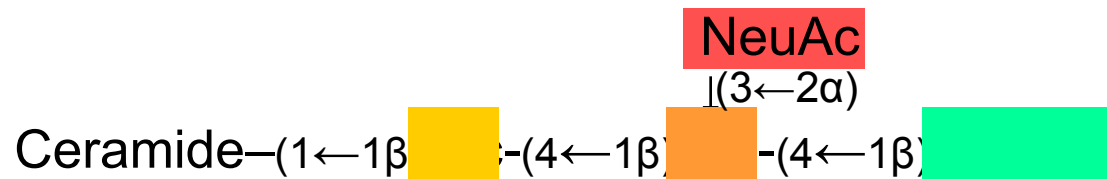
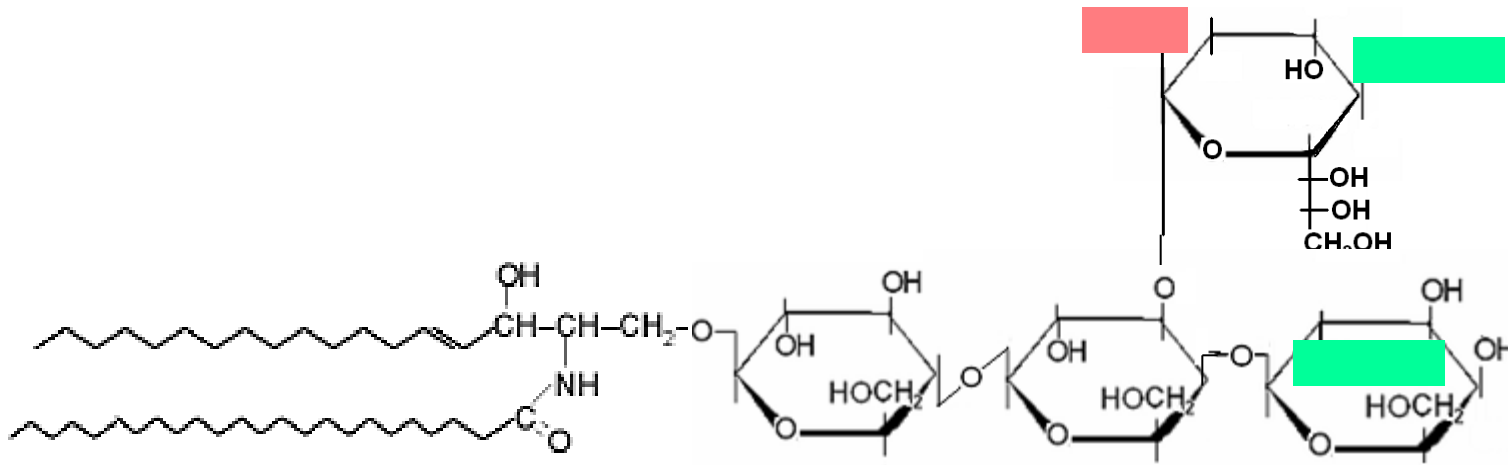
# Sulfoglycolipids are sulfated



Sulfosphingolipids are formed by transfer of sulphate from 3'-phosphoadenosine-5'-phosphosulfate ( PAPS).

# Gangliosides

Sialic acid is attached to oligosaccharide chain



# Biosynthesis of glycosphingolipids

## Synthesis of cerebroside:

**ceramid** + UDP-gal → ceramid -gal + UDP

..... + binding of other UDP-monosacharides

## Synthesis of sulfatides:

Sulfatation of cerebroside by PAPS

## Synthesis of gangliosides:

**ceramide** + UDP -hexoses + CMP-NeuAc

# Degradation of sphingolipids in lysosomes

In lysosomes, a number of specific enzymes catalyse hydrolysis of ester and glycosidic linkages of sphingolipids.

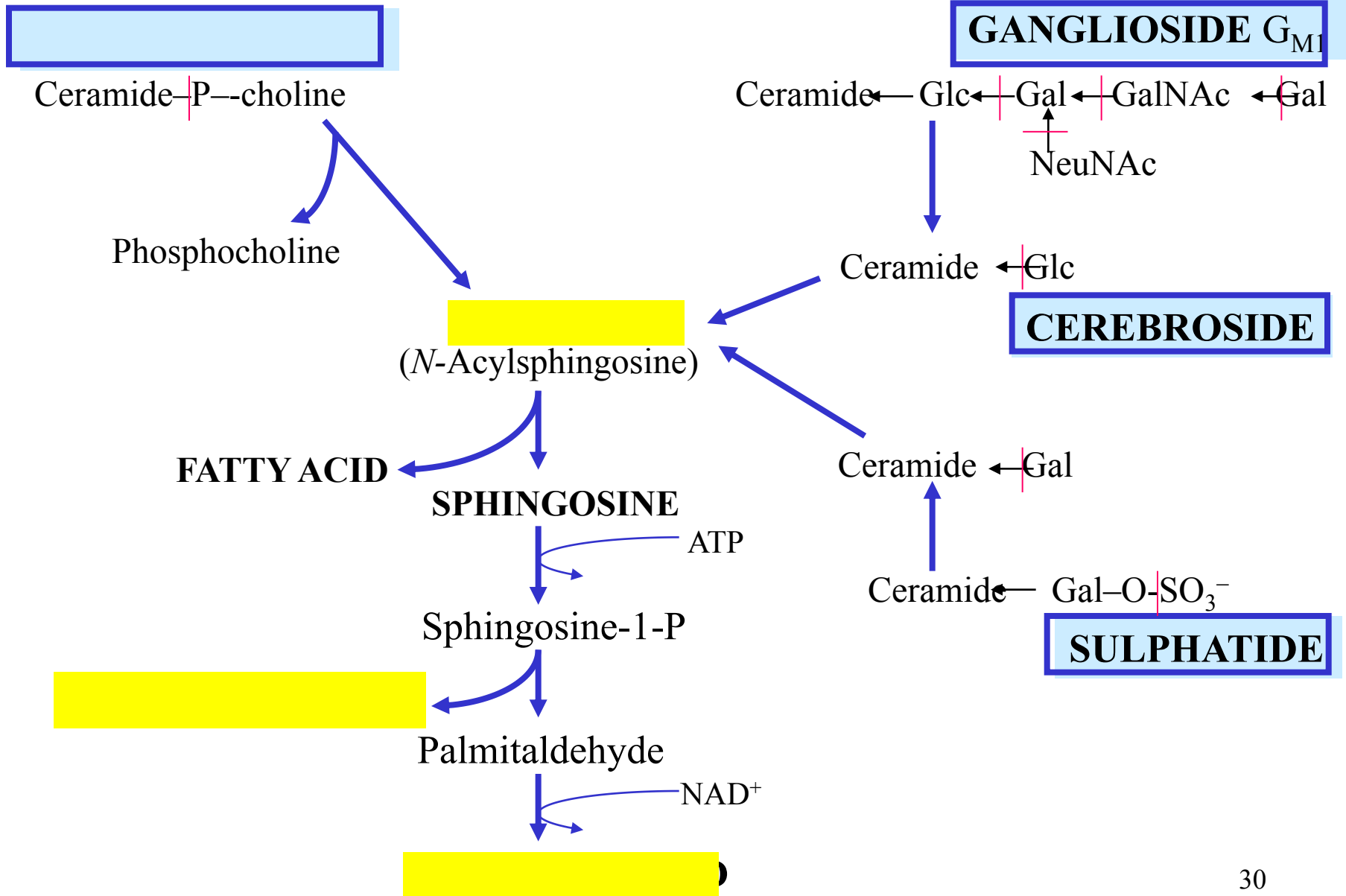
**Sphingomyelins** lose phosphocholine to give **ceramide**.

**Glycolipids** due to the action of various specific glycosidases get rid of the saccharidic component to give **ceramide**.

**Ceramide** is hydrolysed (ceramidase) to **fatty acid** and **sphingosine**.

**Sphingosine** is decomposed in the pathway that looks nearly like the reversal of its biosynthesis from palmitoyl-CoA and serine. After phosphorylation, sphingosine is broken down to **phosphoethanolamine** (decarboxylated serine) and **palmitaldehyde**, that is oxidized to palmitate.

# Degradation of sphingolipids



# Sphingolipidosis

## **Lipid storage disorders**

Inherited defects in production of the enzymes that catabolize sphingolipids.

They result in accumulation of their substrates in lysosomes, leading to lysosomal damage and to disruption of the cell as new lysosomes continue to be formed and their large number interferes with other cellular functions.

In the sphingolipidosis mainly the cells of the central nervous system (including brain and retina) are affected.

# Eicosanoids



# Eicosanoids

## Local hormones

The main types of eicosanoids:

prostaglandins (PG)

thromboxans (TX)

leukotriens (LT)

They are synthesized from polyunsaturated fatty acids with 20 carbons

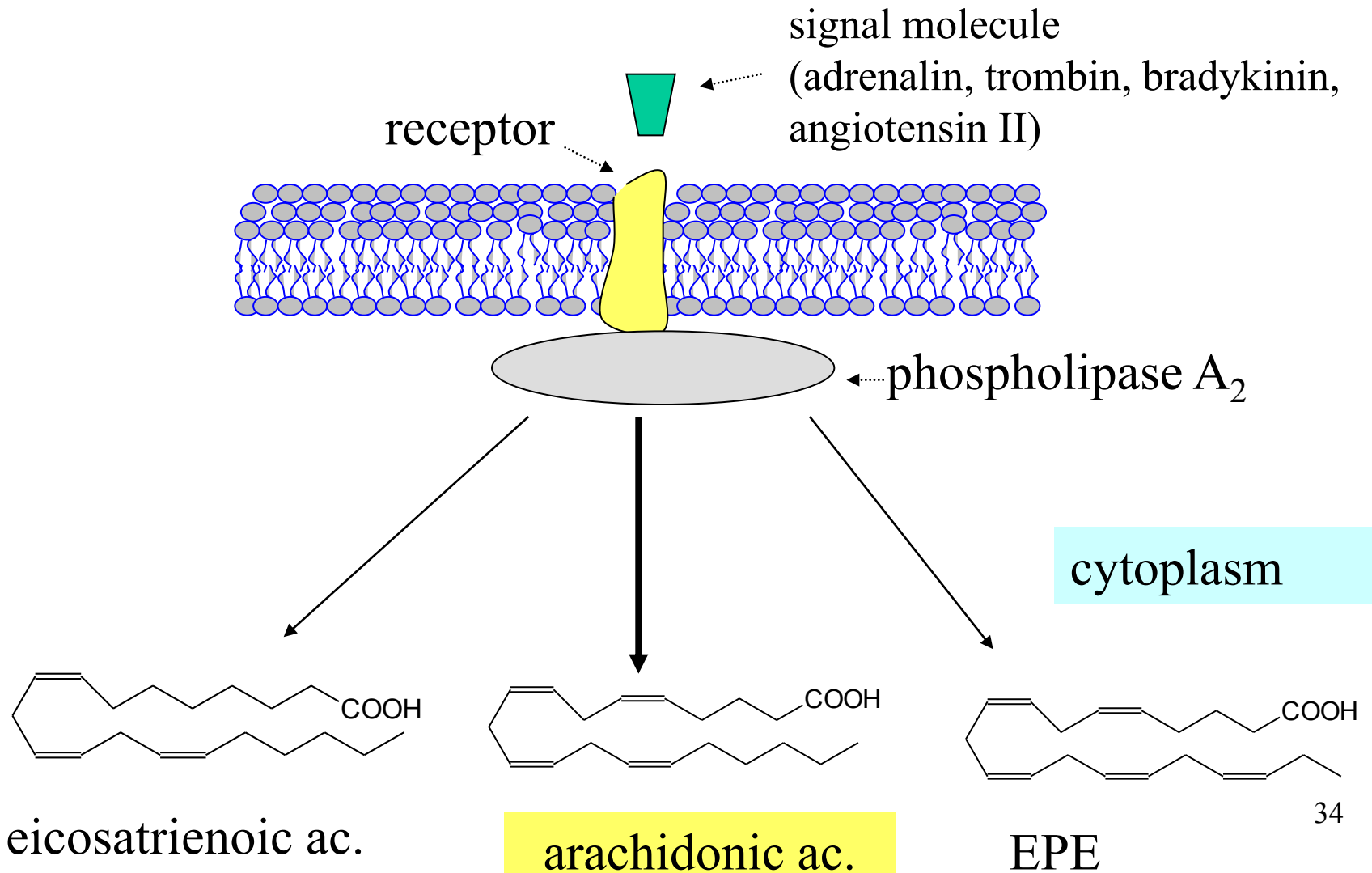
Synthesis of eicosanoids:

PG, TX (prostanoids) – cyclooxygenase pathway

LT (Leukotriens) – lipoxygenase pathway

# Biosynthesis of eicosanoids

## 1. The release of C<sub>20</sub> fatty acids from membrane phospholipids



# Inhibitors of phospholipase A<sub>2</sub>

Membrane phospholipids



phospholipase A<sub>2</sub>

corticoids



lipocortin



PUFA

Steroidal antiphlogistics (hydrocortisone, prednisone) stimulate the synthesis of protein lipocortin which inhibits phospholipase A<sub>2</sub> and block the release of PUFA and eicosanoids formation.

Principle of anti-inflammatory effect of glucocorticoids. They inhibit the two main products of inflammation, prostaglandins and leukotrienes.

# Prostanoids:

## prostaglandins and prostacyclins

- they are produced in nearly all cell types
- endoplasmic reticulum
- the site of their synthesis depends on expression of genes for the enzymes which take part in the synthetic pathways.
- they have various effect (many types of receptors)

# Involvement of prostanoids in physiological processes - examples

TXA<sub>2</sub> (tromboxane A<sub>2</sub>)

It is produced in platelets, it stimulates vasoconstriction and platelet aggregation

Duration of action ~ 30-60 s

PGI<sub>2</sub> (prostacycline A<sub>2</sub>)

It is antagonist of TXA<sub>2</sub>, it is produced by vascular endothelium, it inhibits platelet coagulation and has vasodilatation effects, half-life 3 min.

Their equilibrated effects takes part in platelet coagulation and vasomotor and smooth muscle tone.

$\text{PGE}_2$  is produced by mucose cells of the stomach and inhibits HCl secretion

It reduces the risk of peptic ulcer

$\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  are synthesized in endometrium and induce uterine contractions. Their concentration in amniotic fluid during pregnancy is low, it significantly increases during delivery. Together with oxytocin is involved in the induction of labor.

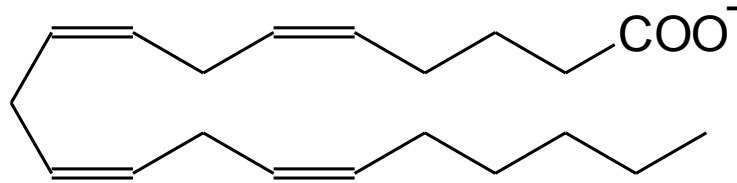
They can be used to induce abortion by intravenous or intravaginal application

# Examples of some biological effects of prostanoids

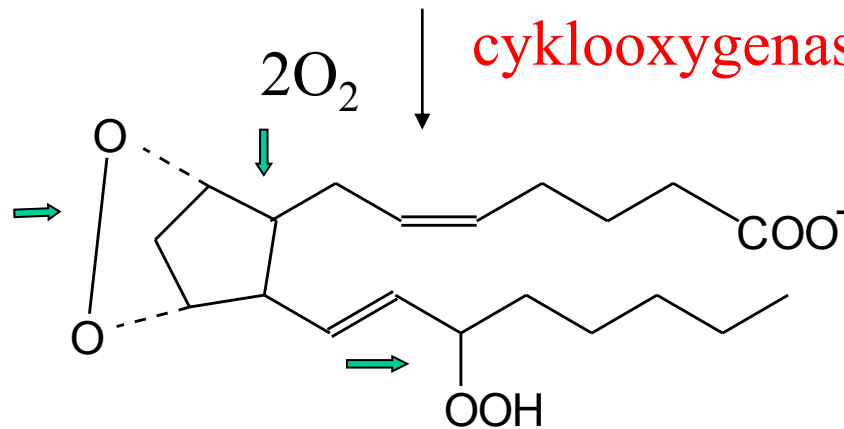
prostanoid	Structural group	Synthesized in	The most remarkable effect
$\text{PGE}_2$	prostaglandin E	nearly all cell types	inflammatory reaction, vasodilation, inhibition of HCl secretion, secretion of mucine, increase of body temperature, increase of intensity and duration of pain, increase of vessel permeability
$\text{PGF}_{2\alpha}$	prostaglandin F	nearly all cell types	vasoconstriction increase of body temp.
$\text{PGI}_2$	prostacyclin	endothelial cells, smooth muscle cells of blood vessels	vasodilation, inhibition of platelet aggregation, increase of intensity and duration of pain,
$\text{TXA}_2$	thromboxane	blood platelets	platelet aggregation, 39 vasoconstriction

# Synthesis of prostanoids (cyclooxygenase pathway)

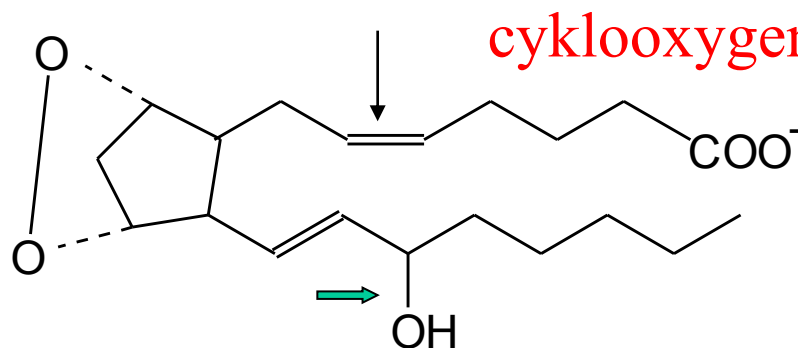
The enzyme **cyclooxygenase** (COX) has two enzyme activities:



Arachidonic acid



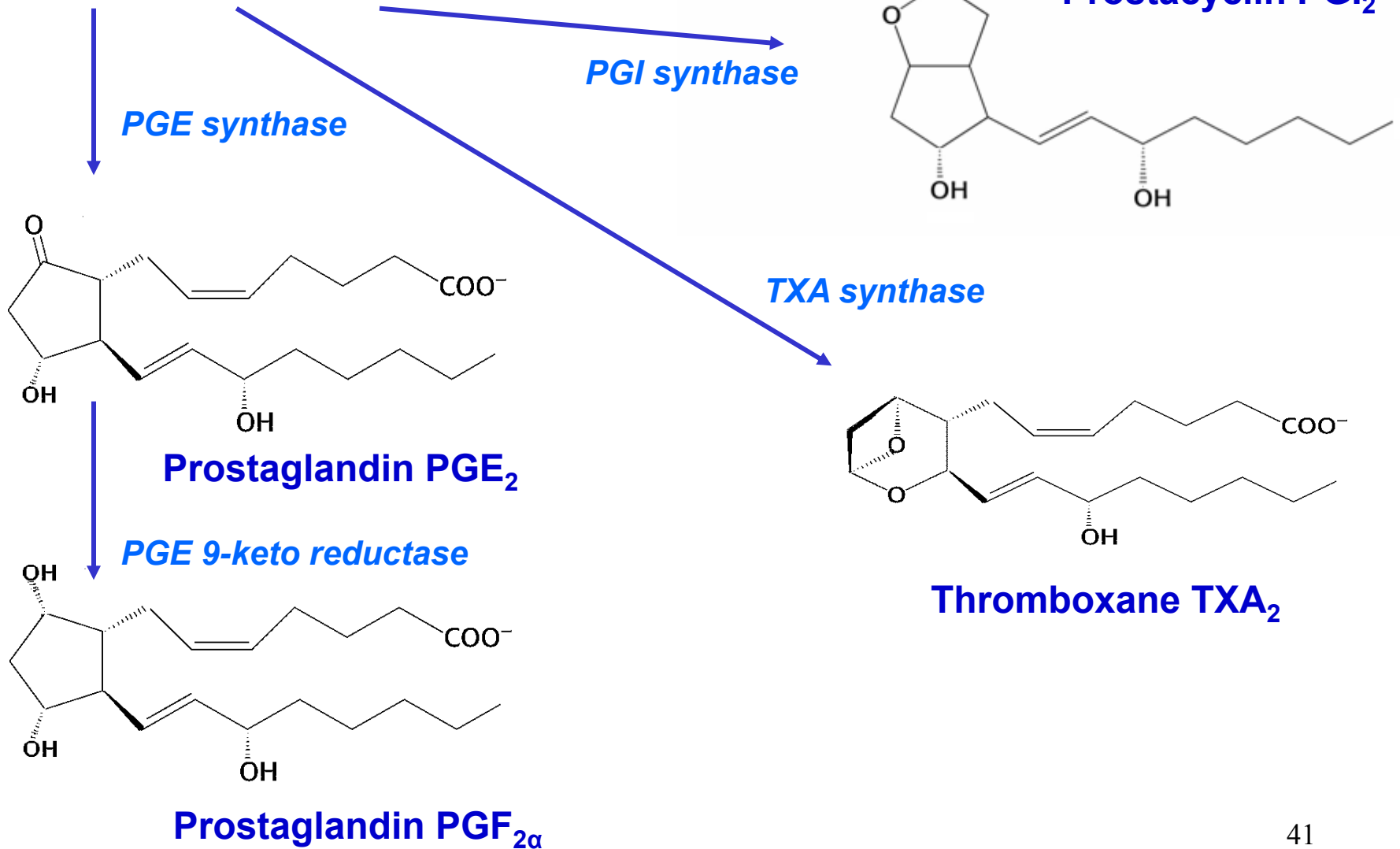
**PGG<sub>2</sub>** (two double bonds)



**PGH<sub>2</sub>** - precursor of all prostanoids of the 2-series



# Prostaglandin H<sub>2</sub>



# The enzyme equipment of various tissues is different

E.g., in **the lung** and **the spleen**, the enzyme equipment enables biosynthesis of all eicosanoid types.

In **blood platelets**, only thromboxan synthase is present.

The **endothelial cells** of blood vessels synthesize only prostacyclins.

The catabolism of prostanoids is very rapid

- Enzyme catalyzed (  $t_{1/2} \sim 0,1-10$  min)
- non-enzymic hydrolysis ( $t_{1/2}$  sec-min)

# Cyclooxygenase (COX) exists in two forms

COX-1: constitutive (still present) – involved into the synthesis of prostanoids at physiological conditions

- COX-2: predominantly inducible – its synthesis is induced during inflammation (stimulation by cytokines, growth factors)



Prostanoids mediate, at least partly, the inflammatory response (they activate inflammatory response, production of pain, and fever)

# Inhibitors of cyclooxygenase

**Because of importance of prostaglandins in mediating the inflammatory response, drugs that blocks prostaglandin production should provide relief from pain**

The main *COX* inhibitors are the **nonsteroidal anti-inflammatory drugs** (NSAIDs, analgetics-antipyretics):

- **acetylsalicylic acid (Aspirin)** – irreversible inhibition
- **acetaminophen (Tylenol), ibuprofen** – reversible inhibition

They inhibit the both forms of COX

# Inhibition of cyclooxygenase suppresses the effects of prostanoids

... it has the positive effects (the anti-inflammatory effect, relief of pain, mitigation of fever. ...)

....on the contrary, there may be some **undesirable effects** of blocked prostanoid production, e.g. decline in blood platelet aggregation, decreased protection of endothelial cells and of gastric mucosa.

Therefore drugs are being developed which would act as selective inhibitors of COX-2 without the adverse gastrointestinal and anti-platelet side effects of non-specific inhibitors of COX.

# COX-2 inhibitors

They are proposed to act as potent anti-inflammatory agents by inhibiting COX-2 activity, without the gastrointestinal (stomach ulcer) and antiplatelet side effects associated with NSAIDs

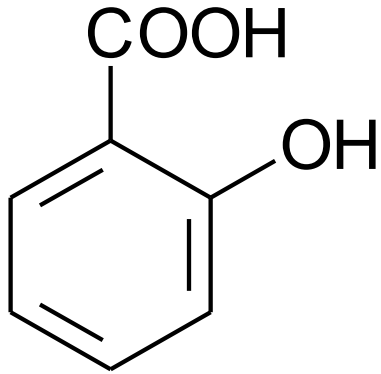
Examples: celecoxib, rofecoxib

However further studies indicated that specific COX-2 inhibitors may have a negative effect on cardiovascular function.

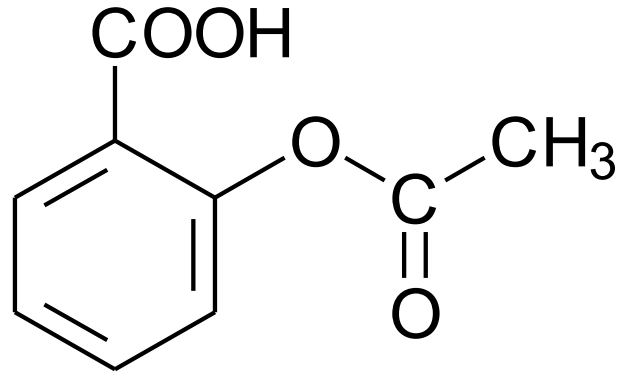
Coxibs were withdrawn from the market by its manufacturer because of negative patients study

Nimesulid (AULIN, COXTRAL), meloxicam (ANTREND, LORMED, MELOBAX) – are still used, they inhibit more COX-2 than COX-1 and must be used with caution

# Acetylsalicylic acid (Aspirin)



salicylic acid



acetylsalicylic acid

It covalently acetylates the active site of cyclo-oxygenase, causing its irreversible inhibition

~ 500 mg	analgetic, anti-pyretic actions
~ 50 mg	anti-thrombotic action (prevention)

## Protective effect of acetylsalicylic acid

Low-doses of aspirin (ASA, 81-325 mg daily) has been shown to be effective in prevention of acute myocardial infarction.

Aspirin blocks the production of  $\text{TXA}_2$  by inhibition of COX

The principal effect of  $\text{TXA}_2$  is the stimulation of platelets aggregation.

It may initiate the formation of thrombus at sites of vascular injury or in the vicinity of ruptured atherosclerotic plaque.

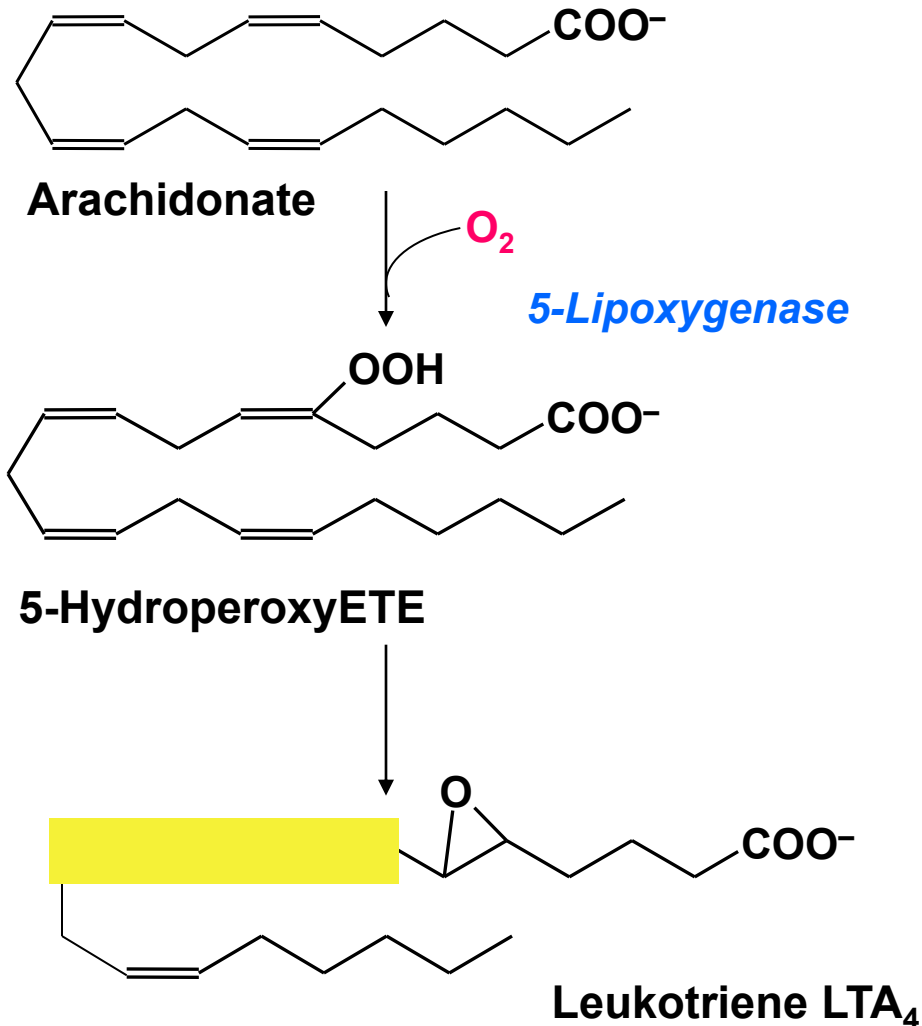
Such thrombi may cause sudden total occlusion of vascular lumen.

By aspirin treatment the effects of thromboxane are attenuated.



# Lipoxygenase pathway

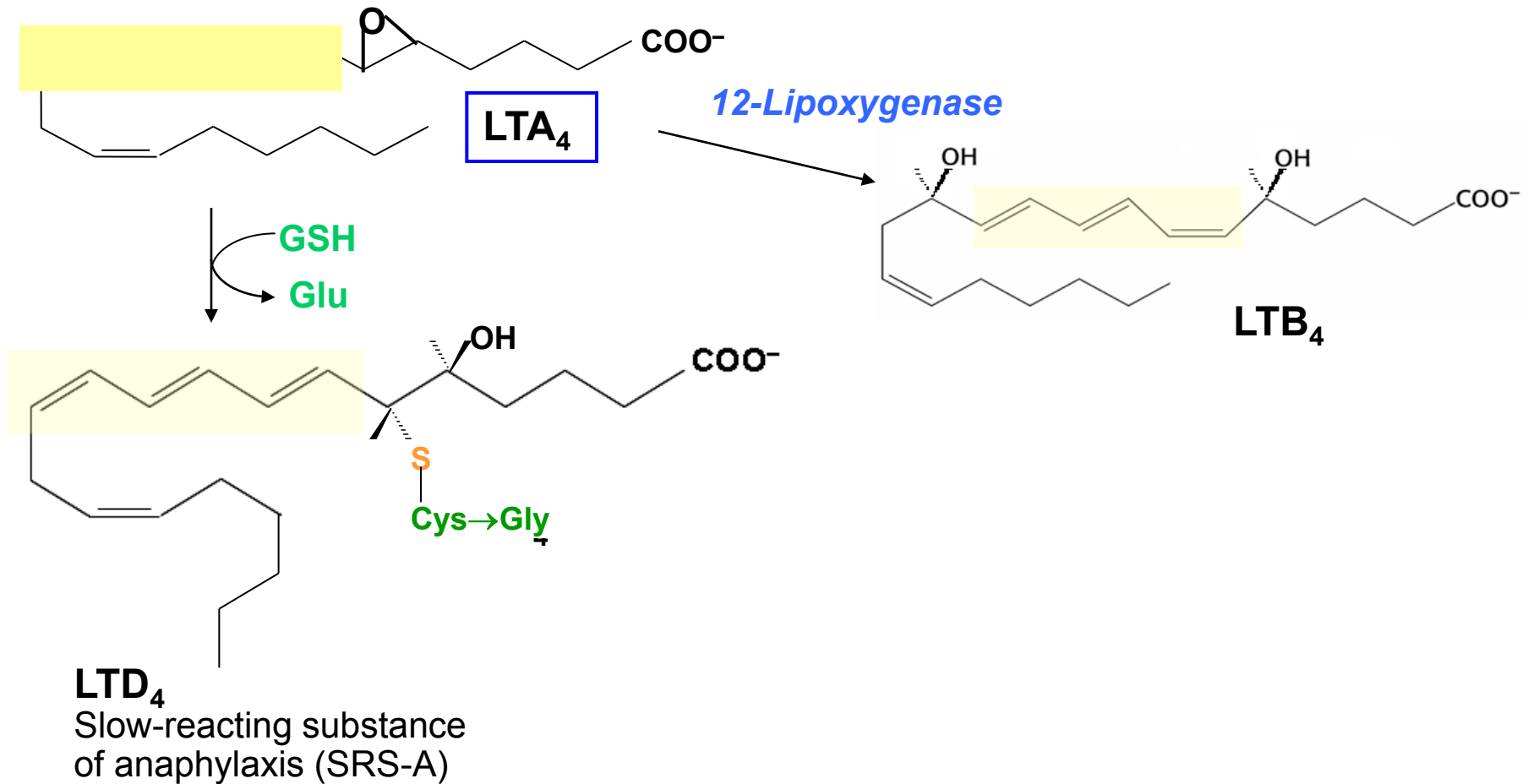
## Synthesis of leukotrienes



all of them have three conjugated double bonds (trienes), the position of which may be different and the configuration either *trans* or *cis*..

Precursor of all leukotrienes of the 4-series

Leukotrienes are produced primarily in leukocytes and mast cells. The classes of LTs are designated by letters A – E, the subscript denotes the total number of double bonds.



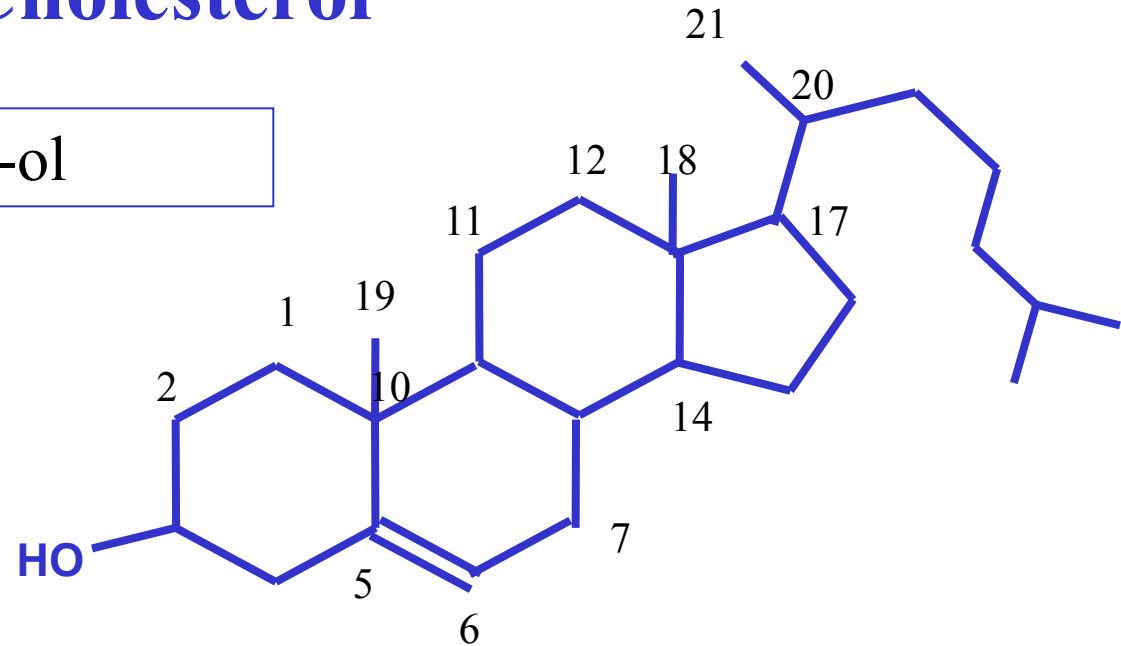
## Eicosanoids

Example	Structural group	Synthesized in	The most remarkable effect
LTD <sub>4</sub>	leukotriene	leukocytes, mast cells	bronchoconstriction, vasoconstriction
LXA <sub>4</sub>	lipoxin	various cell types	bronchoconstriction, vasodilation

# Cholesterol

# Cholesterol

5-cholesten-3 $\beta$ -ol



Essential component of membranes  
Source for synthesis of bile acids, steroids and  
vitamin D3

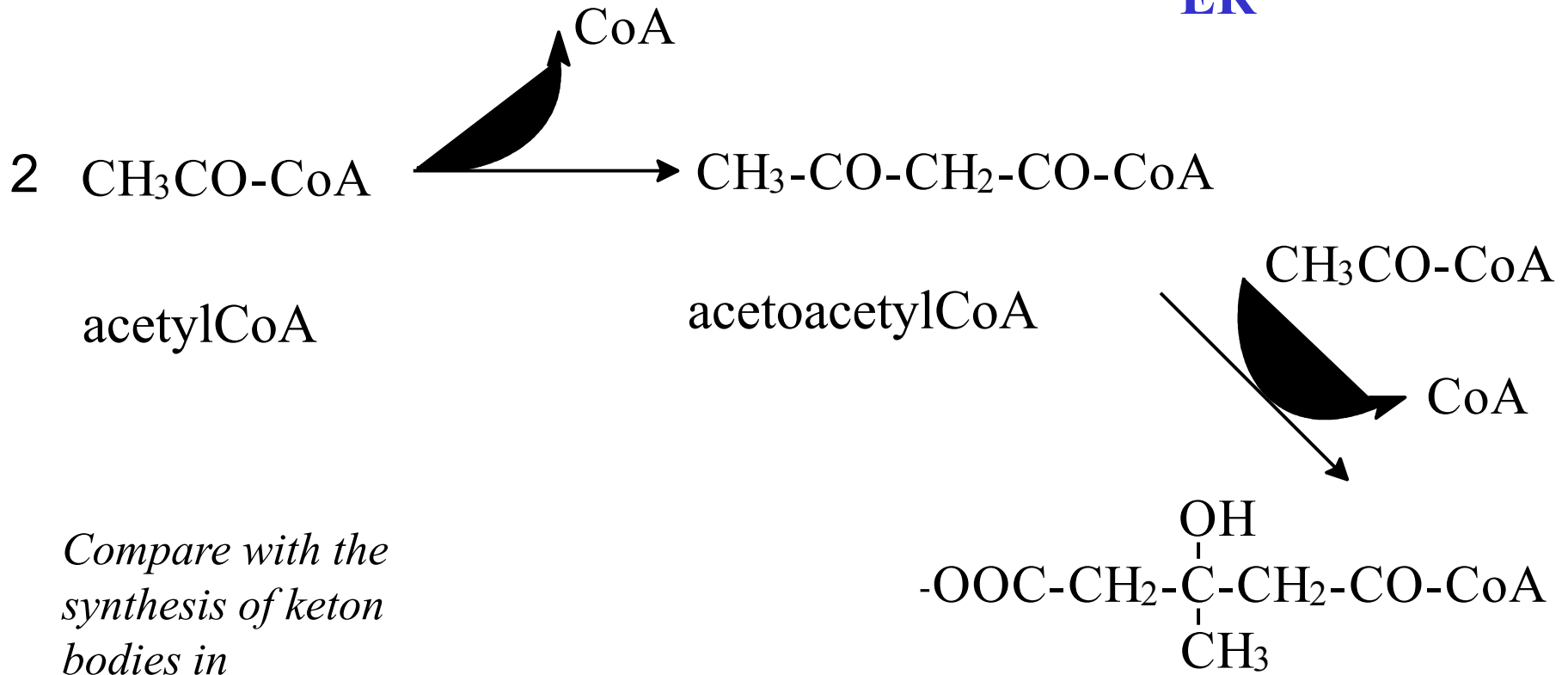
# Biosynthesis of cholesterol

- where: most of cells, **mainly liver**, adrenal cortex, red blood cells, reproductive tissues....
- where in the cell: cytoplasm, some enzymes located on ER
- initial substrate: acetylCoA
- balance of synthesis: 18 acetylCoA, 36 ATP, 16 NADPH

# 1. phase of cholesterol synthesis

## - synthesis of 3-HMG-CoA

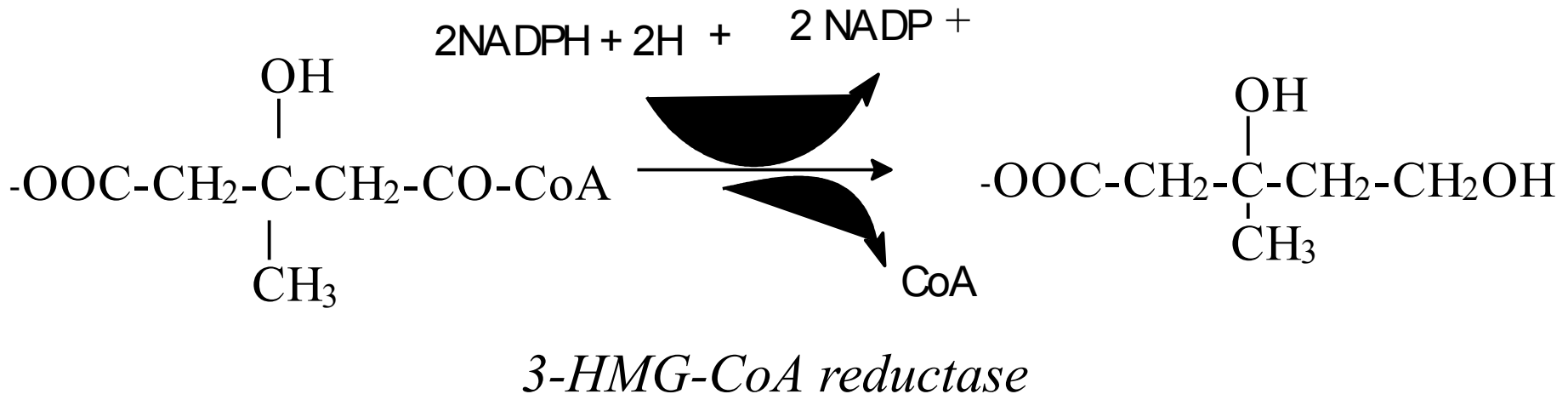
ER



*Compare with the synthesis of keton bodies in mitochondrial matrix*

**3-hydroxy-3-methylglutarylCoA (HMG-CoA)**

## 2. phase - formation of mevalonate



3-HMG-CoA

Mevalonic acid

Double reduction of carboxylic group to primary alcohol group



# Synthesis of mevalonate determines the overall rate of the cholesterol synthesis

## Enzyme **3-HMG-CoA reductase**

- bonded on the ER membrane
- major control point of the synthesis
- inhibited by some drugs

## 3-HMG-CoA reductase

### Kinds of metabolic control

- control of enzyme synthesis by sterol level
- control of enzyme proteolysis by sterol level
- control of enzyme activity by covalent modification (phosphorylation)
- competitive inhibition by drugs – statins (e.g.lovastatin, pravastatin, cerivastatin)

# Control of 3-HMG-CoA reductase synthesis by cholesterol

- affection of gene transcription by transcription factor SREBP  
(sterol regulatory element binding protein)
- SREBP is activated at low level of cholesterol
- SREBP binds DNA at sterol regulatory element (SRE)
- the transcription is accelerated after SREBP binding

# Regulation of HMG-CoA reductase proteolysis by sterols

- Degradation of the enzyme is stimulated by cholesterol, mevalonate and farnesol.
- Enzyme includes transmembrane sterol-sensitive region, that is responsible for ubiquitination of the enzyme at high level of sterols

# Regulation of HMG-CoA reductase by covalent modification

Forms of the enzyme

phosphorylated



inactive

kinase –AMP dependent

dephosphorylated



active

phosphatase

Activation:

Glucagon, intracellular sterols (cholesterol, bile acids), glucocorticoids

Insulin, thyroidal hormones

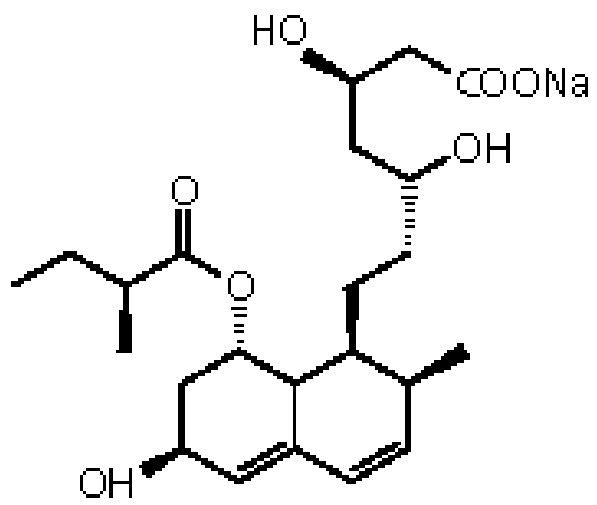
## Inhibition of HMG-CoA-reductase by drugs

The **statin drugs** are reversible competitive inhibitors of HMG-CoA-reductase in liver.

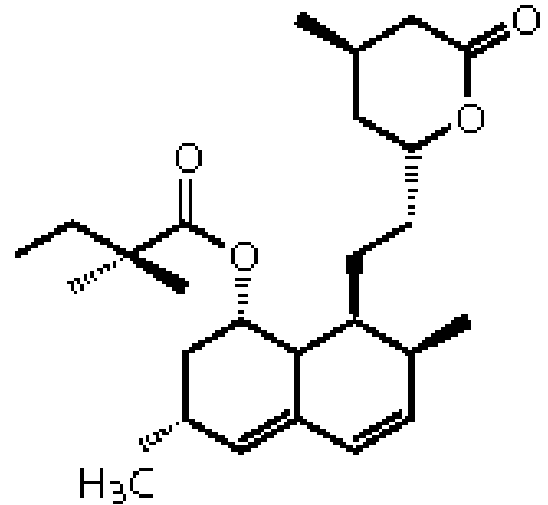
The synthesis of cholesterol in liver is decreased by their action.

Statins – various structures part of their structure resembles to HMGCoA.

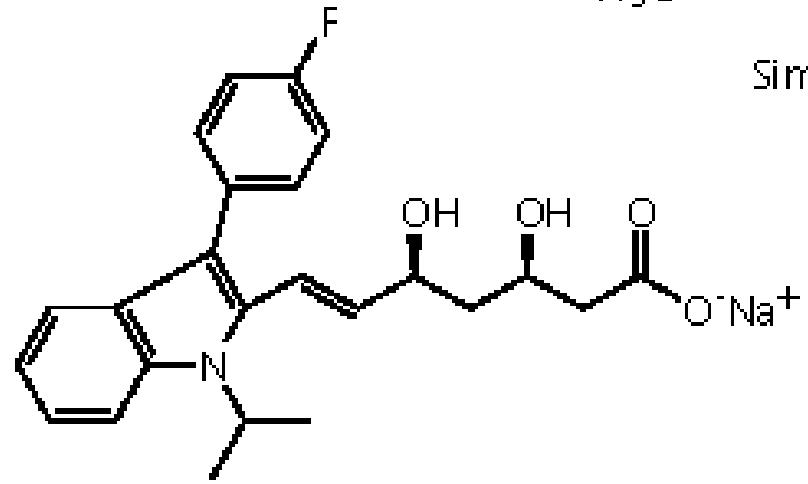
**Simvastatin (Zocor), Lovastatin (Mevacor), Pravastatin (Mevalotin), Pravastatin (Pravachol), Simvastatin (Lipovas), Fluvastatin (Lescol),...**



Pravastatin



Simvastatin

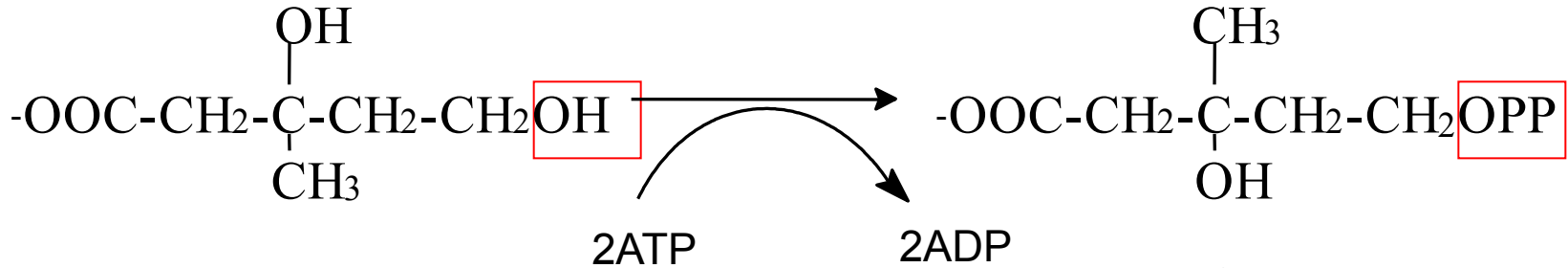


Fluvastatin

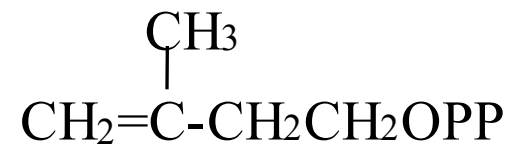
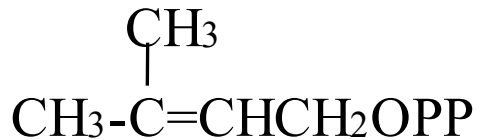
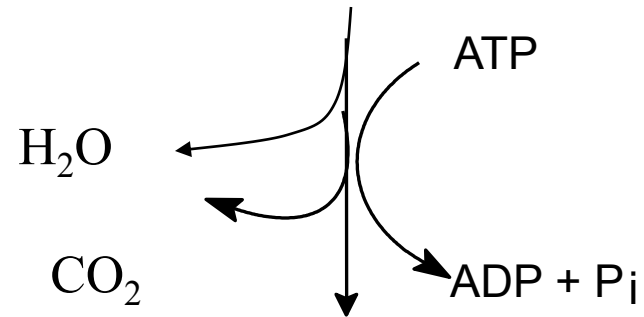
# 3. phase of cholesterol synthesis:

## formation of five carbon units

mevalonyldiphosphate



mevalonate



Dimethylallyl diphosphate

Isopentenyl diphosphate

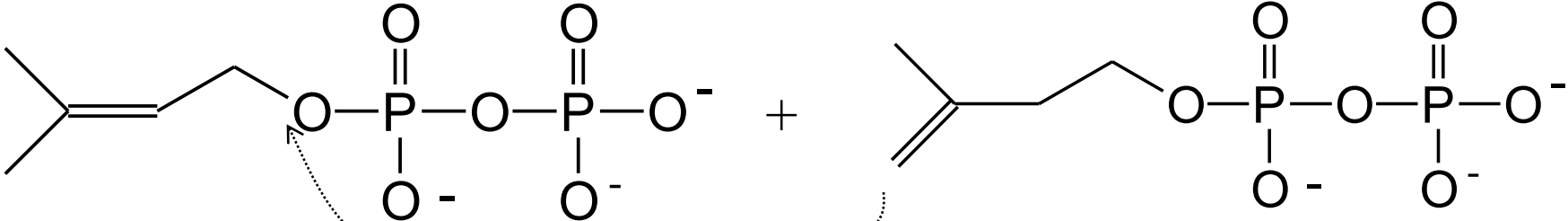
5C

5C



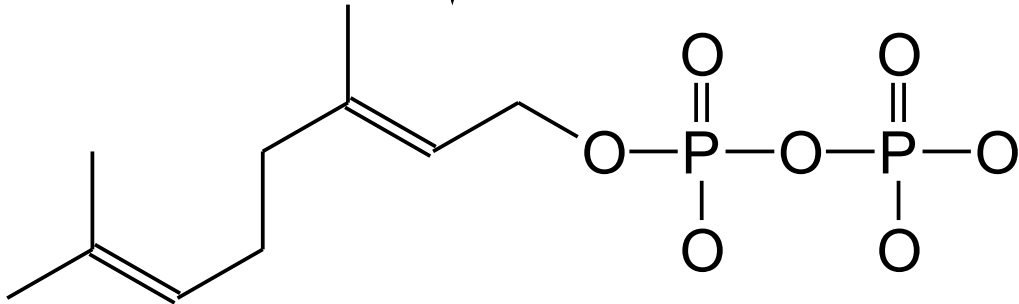
Dimethylallyldiphosphate

isopentenylidiphosphate



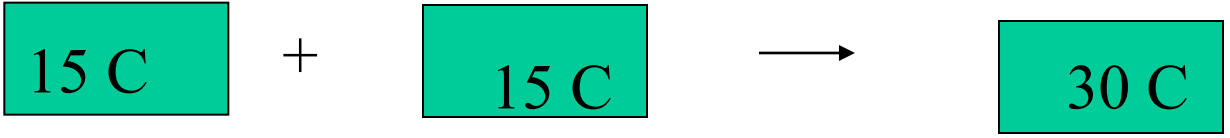
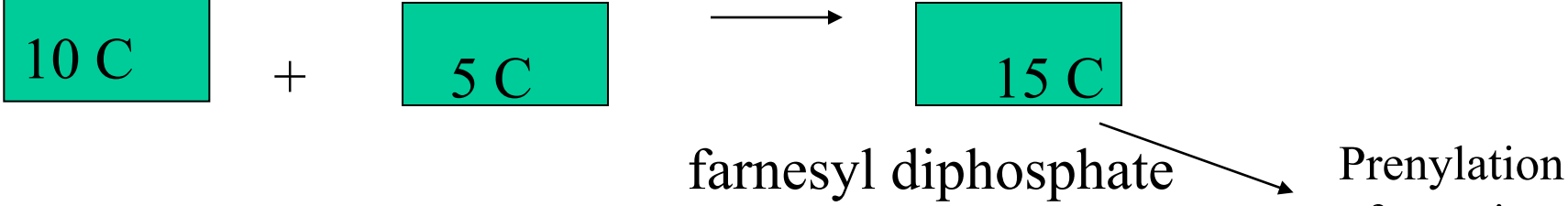
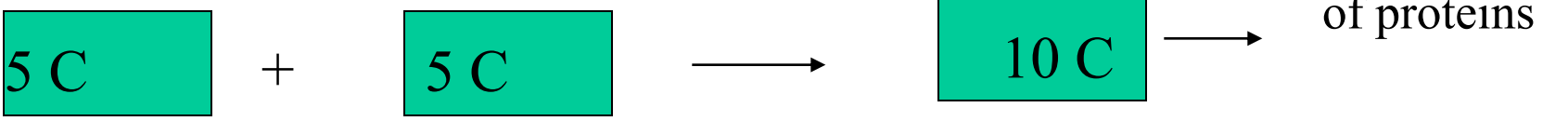
prenyltransferase

PP<sub>i</sub>

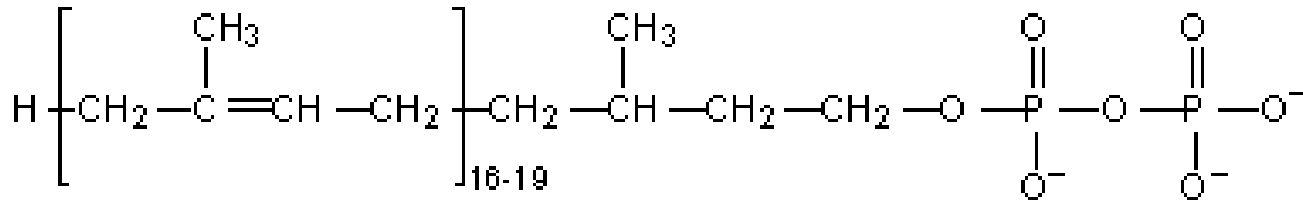


geranyldiphosphate

Dimethylallyldiphosphate + isopentenylidiphosphate → geranyldiphosphate

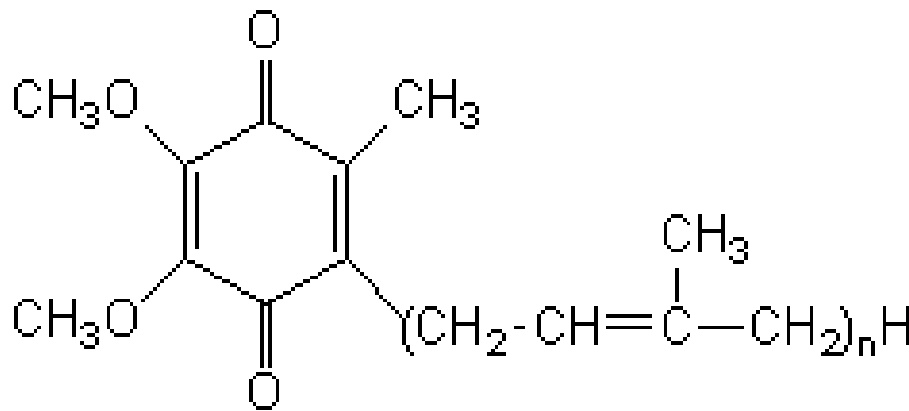


squalene



Dolichol diphosphate

### Synthesis of oligosaccharide chains of glycoproteins

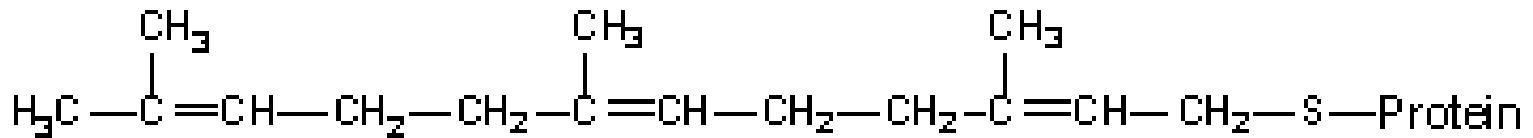
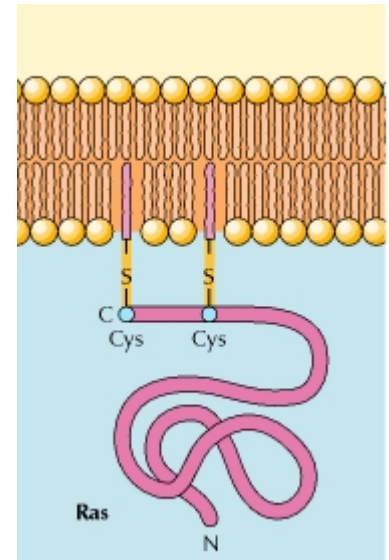


ubiquinon

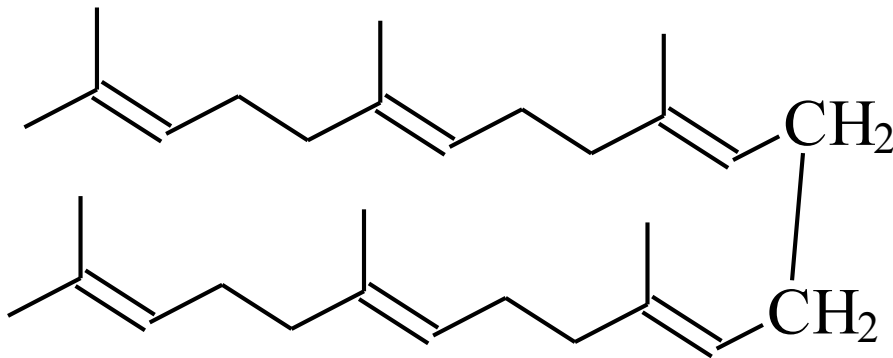
### Respiratory chain

# Prenylation of proteins

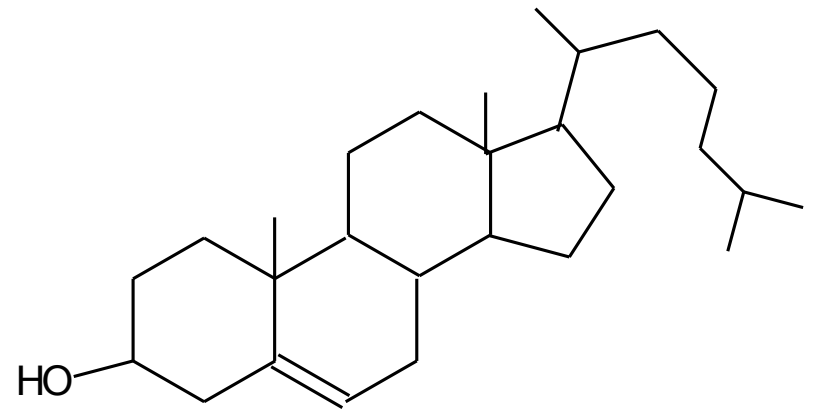
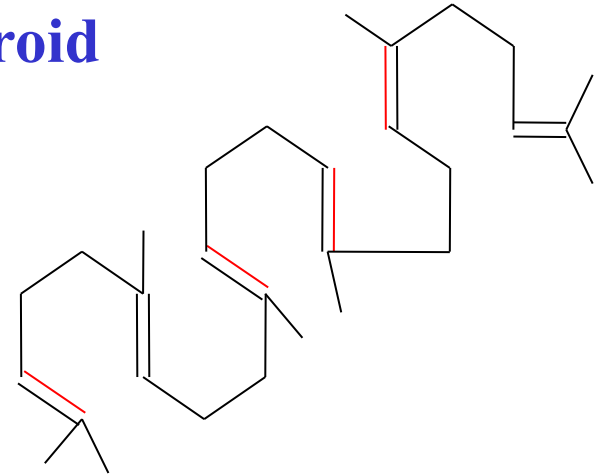
- Covalent modification of proteins
- Binding farnesyl or geranyl-geranyl to SH- group of cystein
- Mediates the interaction of proteins with membrane (anchoring) or protein –protein interaction or membrane –associated protein trafficking.
- modifies some proteins affecting cell proliferation (GTP-binding proteins, eg. Ras, Rac, Rho)
- inhibition of prenylation inhibits cell proliferation
- inhibitors of prenylation – drugs at treatment of osteoporosis, cancer, cardiovascular diseases



**Squalene is linear molecule that can fold into a structure that closely resembles the steroid structure**



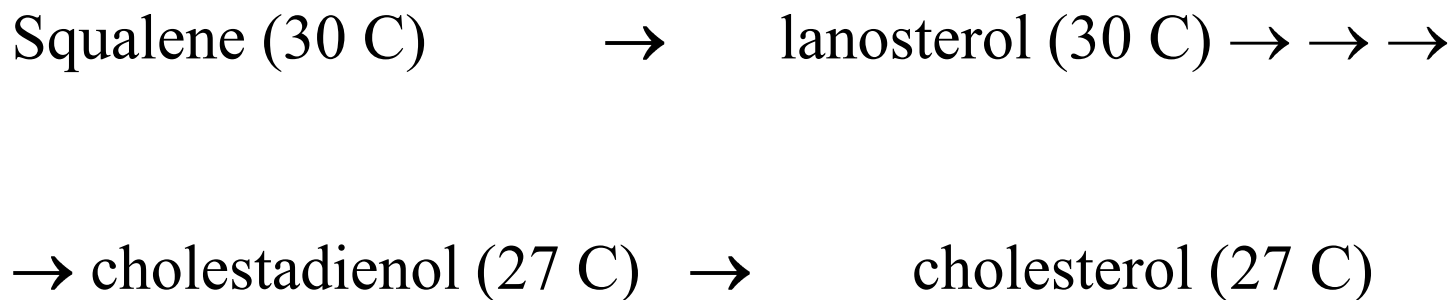
squalen



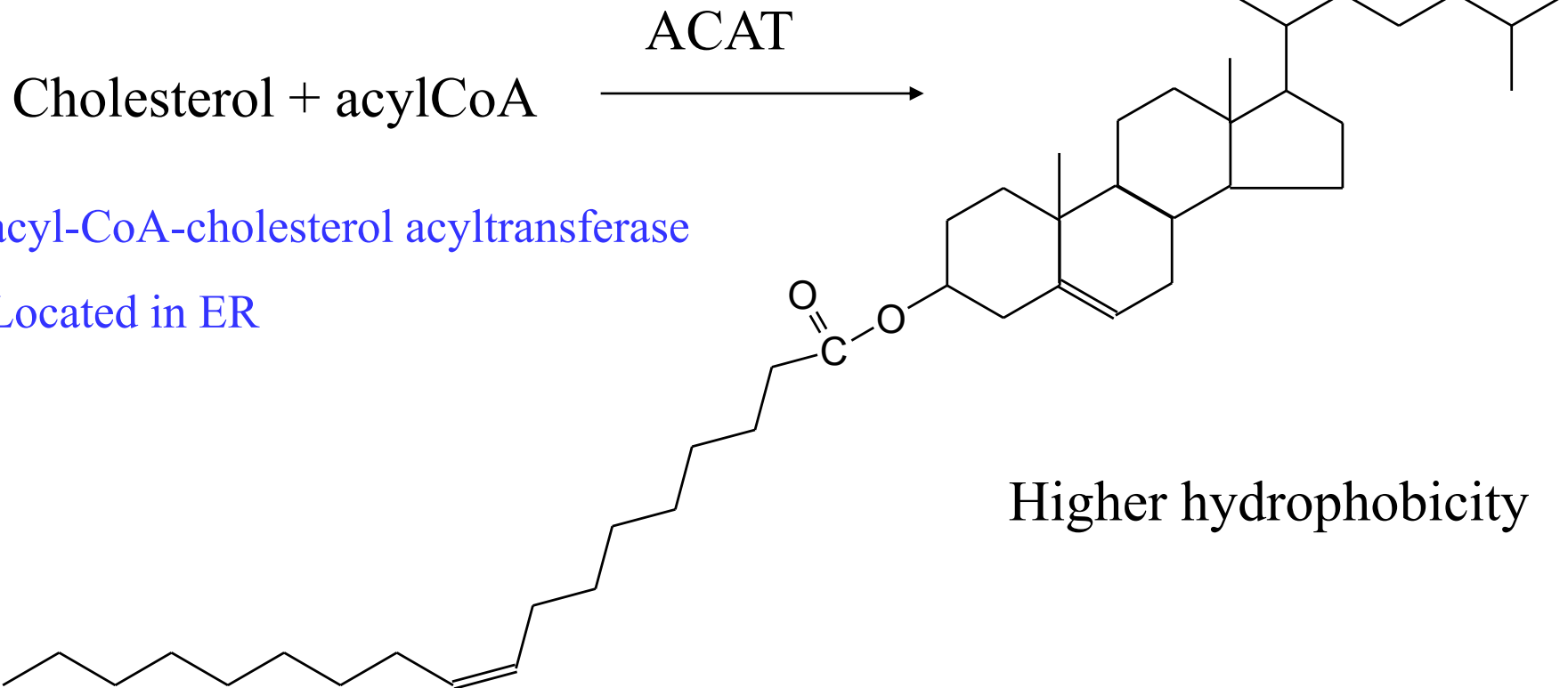
cholesterol

## Conversion of squalen to cholesterol is a process involving about 19 steps in ER :

- cyclisation
- shortening carbon chain from 30 to 27 C
- movement of double bonds
- reduction of double bond



# Esterification of cholesterol



Most often linoleic and linolenic acid

# Transport of cholesterol in blood in form of lipoproteins

From liver transported in form of VLDL

Most of VLDL is converted to LDL after the utilization of main part of TG contained in them

LDL transfers cholesterol into the periferal tissues

Reverse transport of cholesterol to the liver - HDL

25-40% - esterified cholesterol



# Cholesterol in blood

Recommended value  $< 5$  mmol/l

When the total cholesterol level exceeds 5 mmol/l further investigation of lipid metabolism is necessary, especially the finding of the cholesterol distribution in the lipoprotein fractions

LDL-cholesterol = „bad“ cholesterol

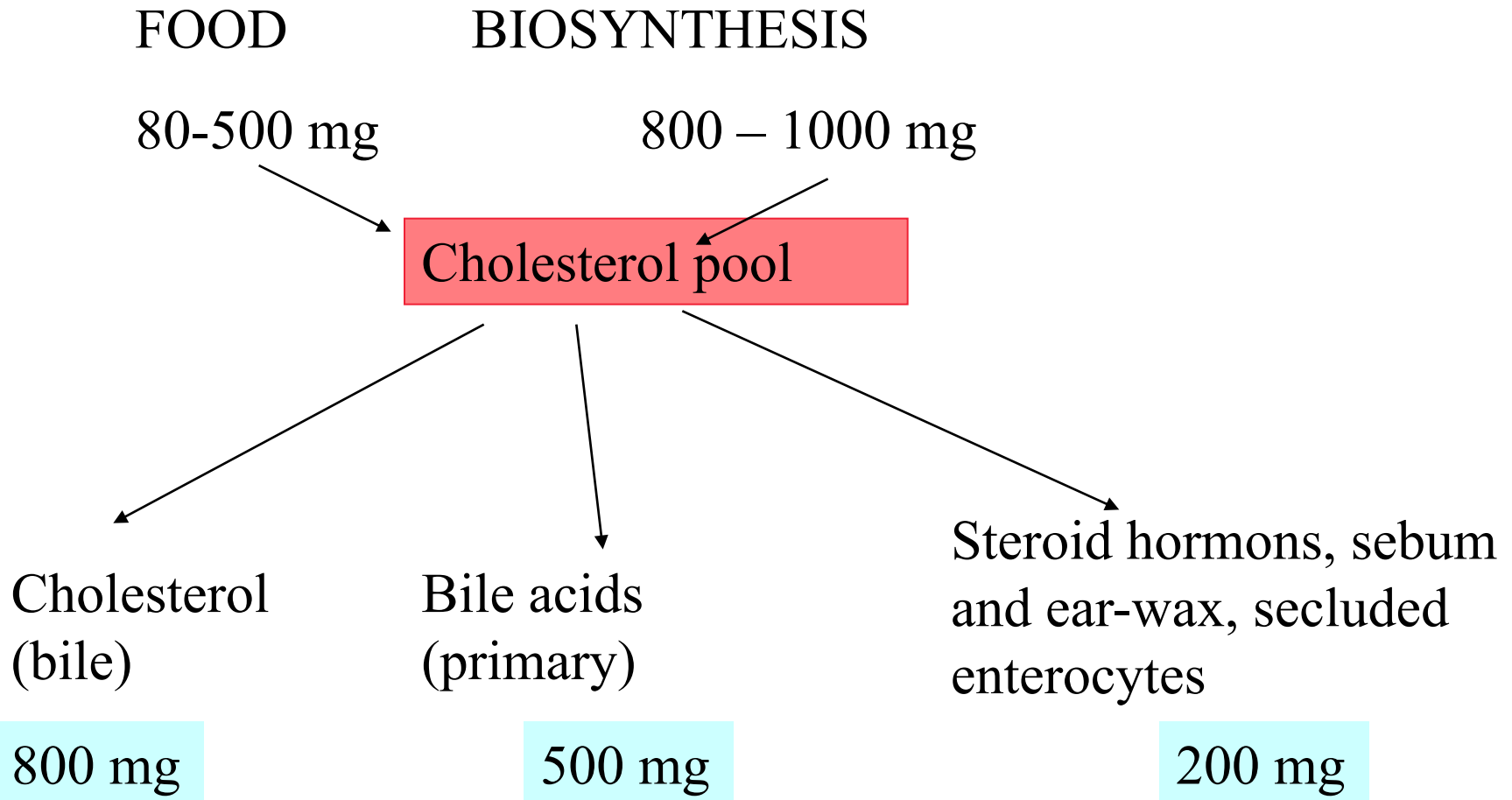
HDL-cholesterol = „good“ cholesterol

A high proportion of serum total cholesterol incorporated in HDL is considered as a sign of the satisfactory ability of an organism to eliminate undesirable excess cholesterol. On the contrary, an increased concentration of LDL-cholesterol represents the high coronary risk involved in hypercholesterolaemia.

## „Degradation of cholesterol“

- in higher animals steroid nucleus of cholesterol is **neither decomposed** to simple products **nor oxidized** to  $\text{CO}_2$  a  $\text{H}_2\text{O}$
- only liver have ability to eliminate cholesterol
- two ways of cholesterol elimination:
  - conversion to bile acids and their excretion
  - excretion of free cholesterol in bile
- small amount is used for synthesis of steroid hormones and vitamin D
- minimum amount of cholesterol is lost by sebum and ear-wax, in secluded enterocytes

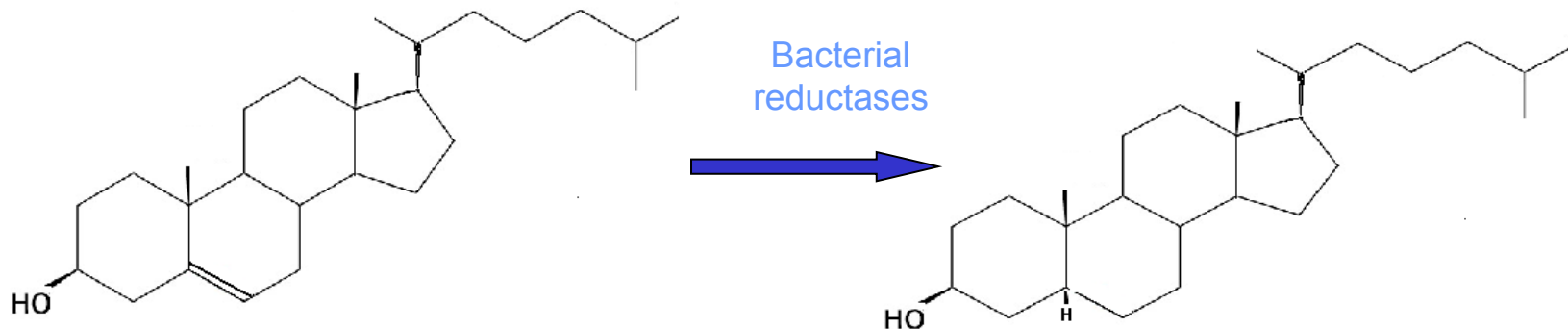
# Cholesterol balance per 24 h



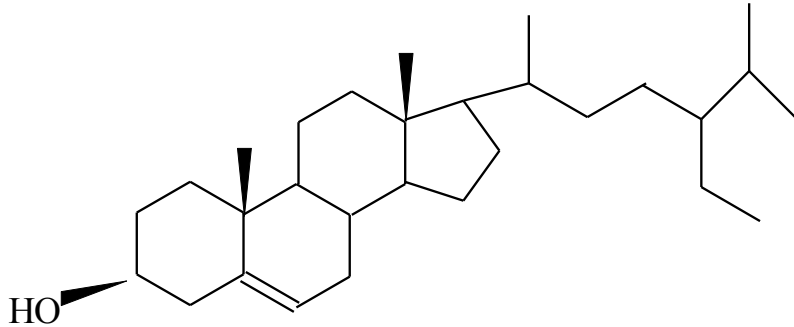
1000-1500 mg/day is excreted

# Cholesterol in the gut

- cholesterol that enters gut lumen is mixed with dietary cholesterol
- about 55% of this cholesterol is resorbed by enterocytes
- remaining part is reduced by bacterial enzymes to coprostanol and excreted in feces



# Phytosterols - sterols of plant origin



$\beta$ -sitosterol

Structurally related to cholesterol; only the side chain on C-17 is changed

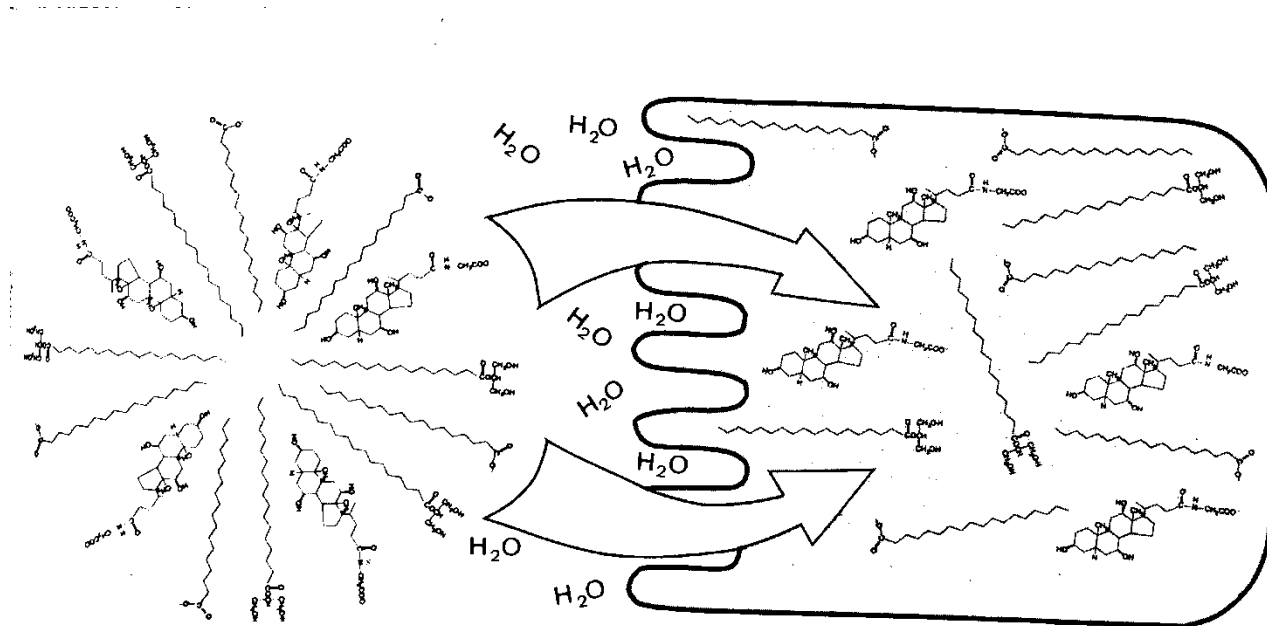
Consumption of phytosterols reduces the resorption of cholesterol.

Plant oils (corn, rapeseed, soya, sunflower, walnut) contain up to 0.9 % phytosterols.

Recommended intake for people with increased level of cholesterol - 2g/day

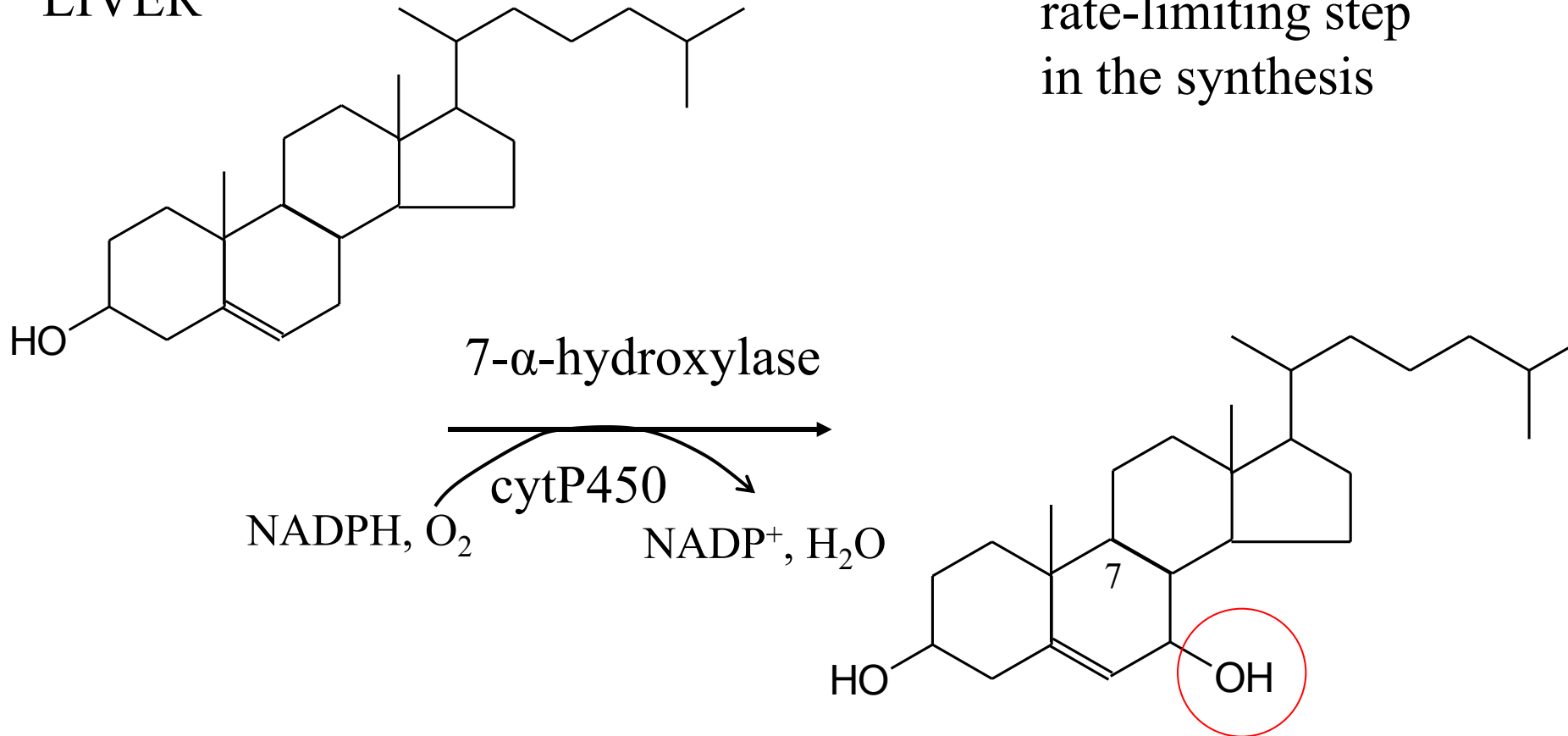
# How do phytosterols function?

They penetrate into the mixed micelles that are in contact with intestine mucosa, they compete with cholesterol in resorption into the enterocytes.



# Synthesis of bile acids

LIVER

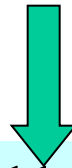


rate-limiting step  
in the synthesis

Located in ER (monooxygenase reaction)



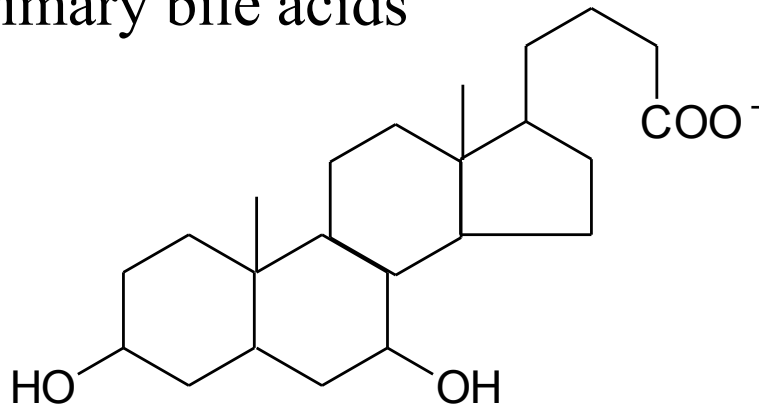
# LIVER



In subsequent steps, the double bond in the B ring is reduced and additional hydroxylation may occur. Two different sets of compounds are produced. One set has  $\alpha$ -hydroxyl groups at position 3, 7, and 12, the second only at positions 3 and 7. Three carbons from the side chain are removed by an oxidation reaction.

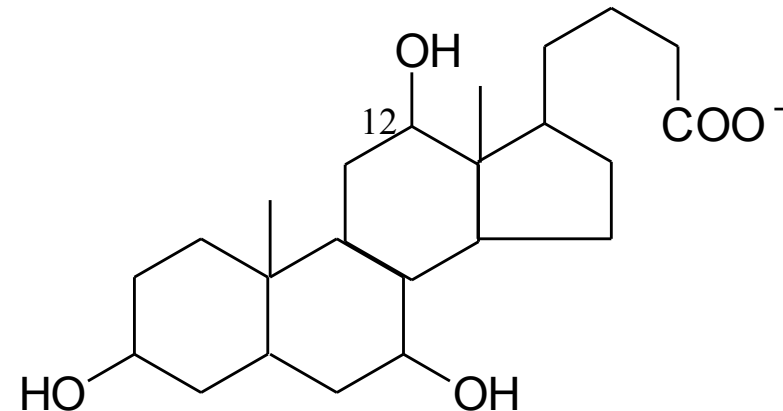
24 C

Primary bile acids



chenodeoxycholate

$pK_A \approx 6$



cholate

$pK_A \approx 6$



LIVER

Conjugation with glycine and taurine (ER)

BILE

ABC-transporter

INTESTINE

deconjugation and partial reduction

bacterias

lithocholate

chenodeoxycholate

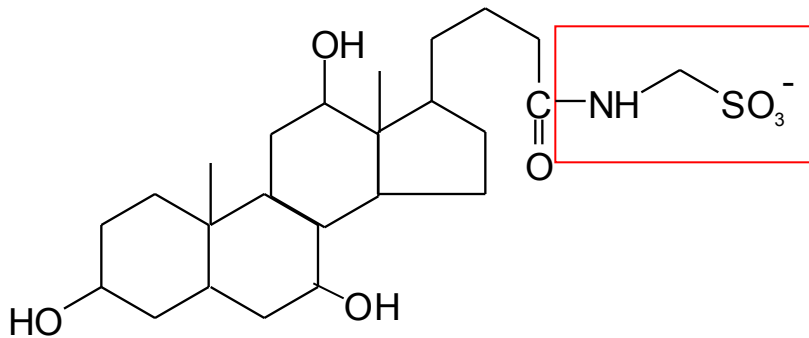
cholate

deoxycholate

feces

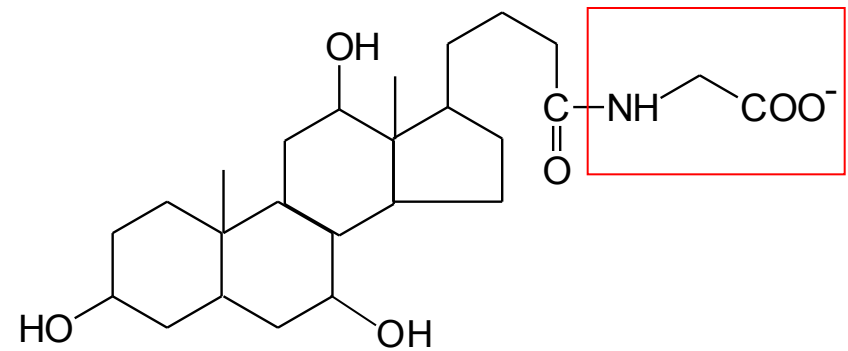
enterohepatal  
circulation

# Conjugated bile acids



taurocholic

$$\text{pK}_A \approx 2$$

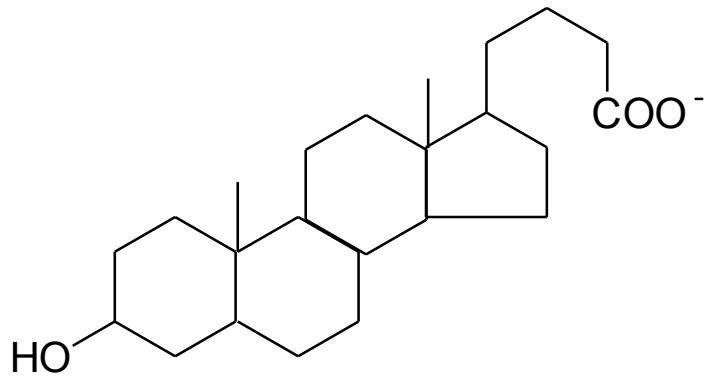


glycocholic

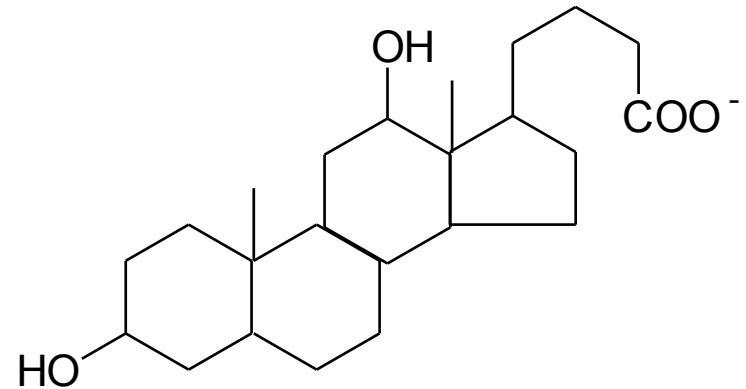
$$\text{pK}_A \approx 4$$

Conjugation increase  $\text{pK}_a$  values, increases detergent efficiency

## Secondary bile acids – do not have OH on C-7



lithocholate



deoxycholate

Less soluble, excreted by feces

# Enterohepatal circulation of bile acids

