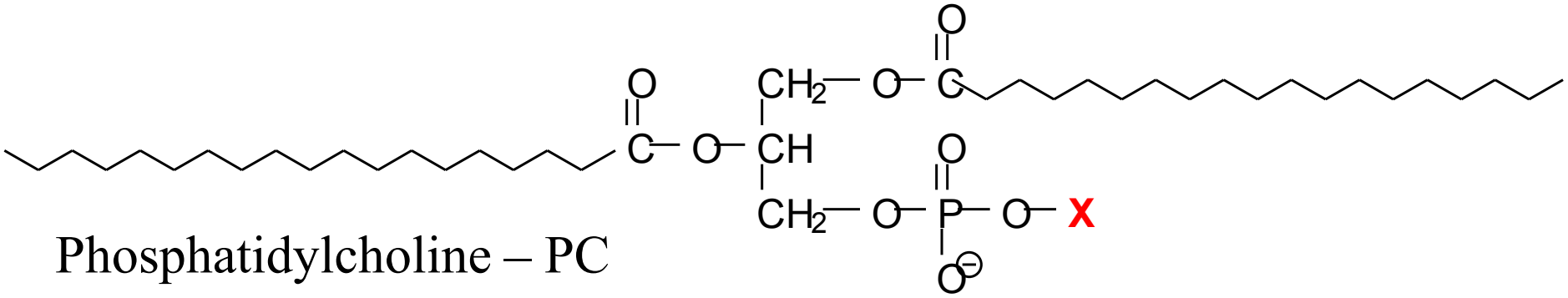
Lipid metabolism II

Phospholipids and glycolipids Eicosanoids. Synthesis and metabolism of cholesterol and bille acids

Biochemistry I
Lecture 9

2012 (E.T.)

Glycerophospholipids



Phosphatidylcholine – PC

Phosphatidylethanolamine – PE

Phosphatidylserine – PS

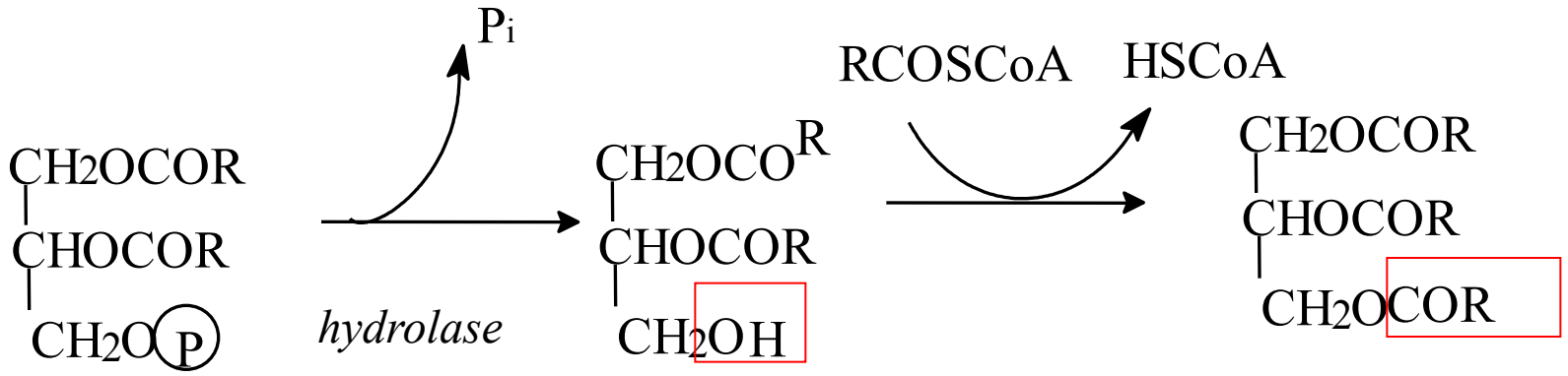
Phosphatidylinositol – PI

Cardiolipin - CL

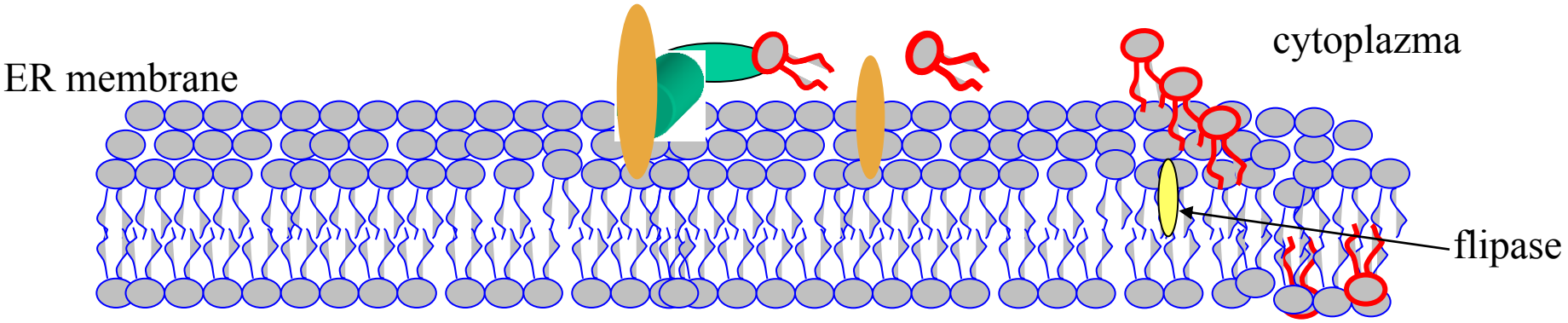
Biosynthesis of glycerophospholipids

- located in all cells with exception of erytrocytes
- 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 cytoplasma
- Newly synthesised phospfolipids are built in the inner layer of the mebrane
- By the action of flippases are transfered 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.

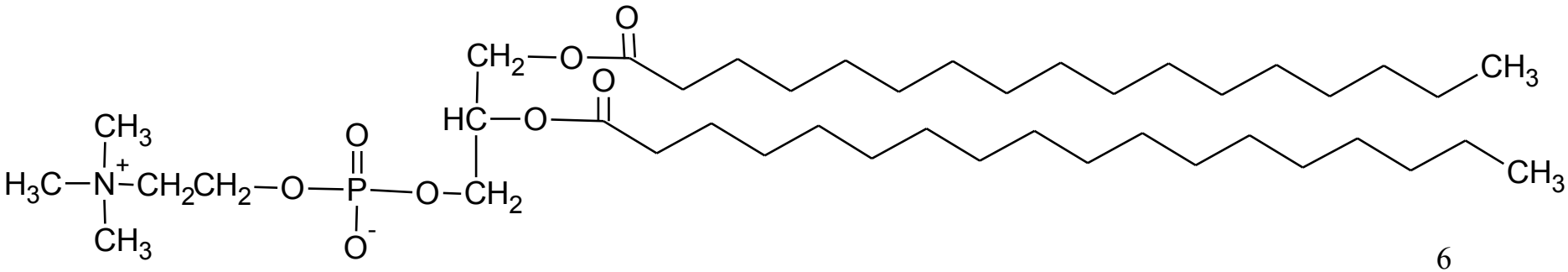
Synthesis of phosphatidyl choline

Choline must be activavated before the synthesis

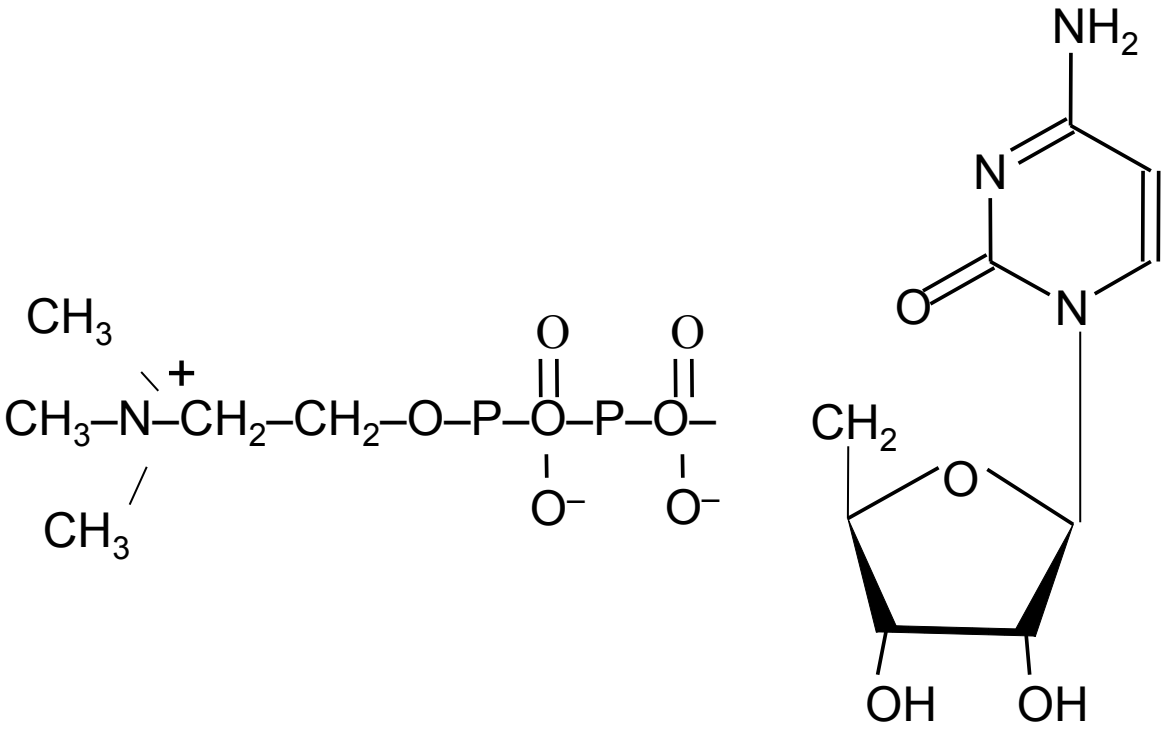
1) Choline + ATP $\rightarrow$ Choline-P + ADP

2) choline-P + CTP $\rightarrow$ CDP-choline + PP_i

3) CDP-choline + 1,2-DG $\rightarrow$ fosfatidylcholine + CMP



CDP-choline

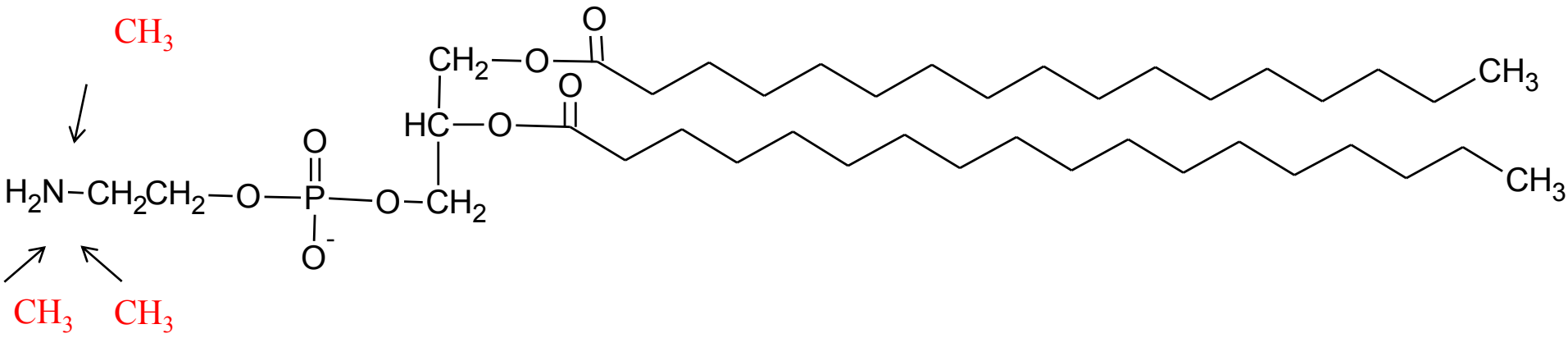


CDP-choline plays a part formally similar to that of UDP-glucose in the synthesis of glycogen.

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

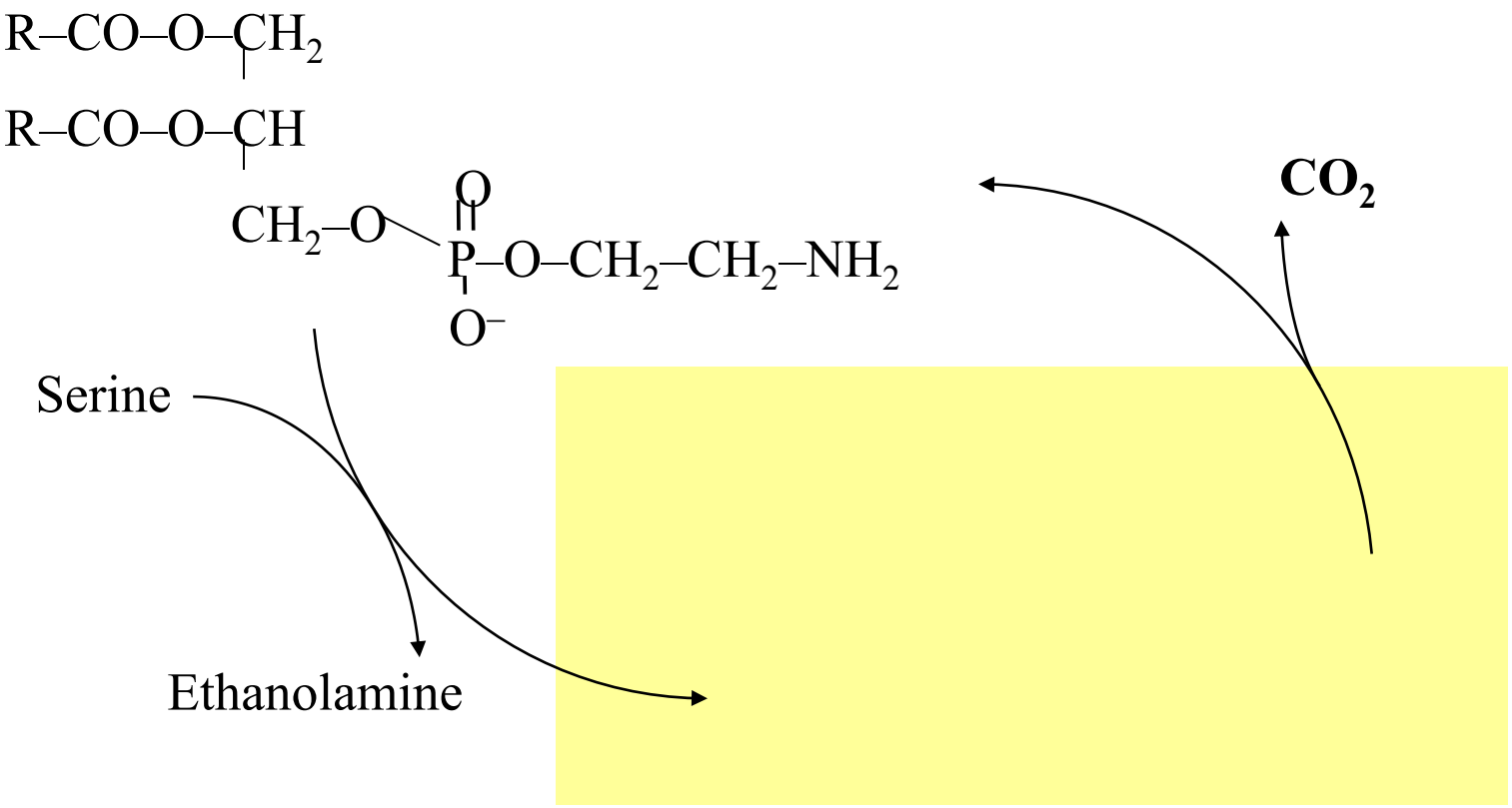
Conversion of phosphatidyl ethanolamine to phosphatidyl choline

N-methylation of phosphatidyl ethanolamine by SAM



Biosynthesis of phosphatidyl serine (PS)

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

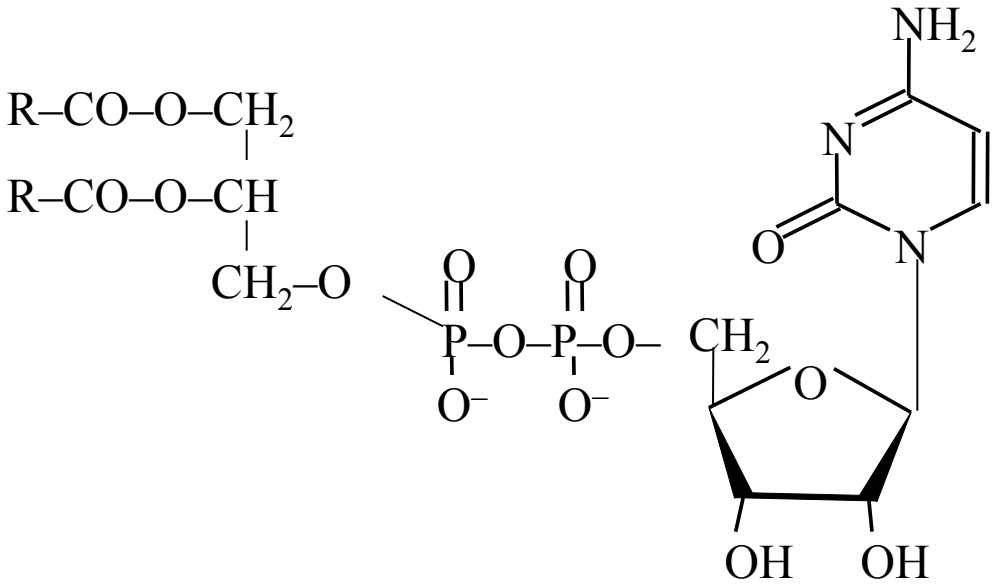


Phosphatidyl serine can be also **decarboxylated** to form **PE**.

2 Synthesis of phosphatidyl inositol

1. Activation of phosphatidic acid

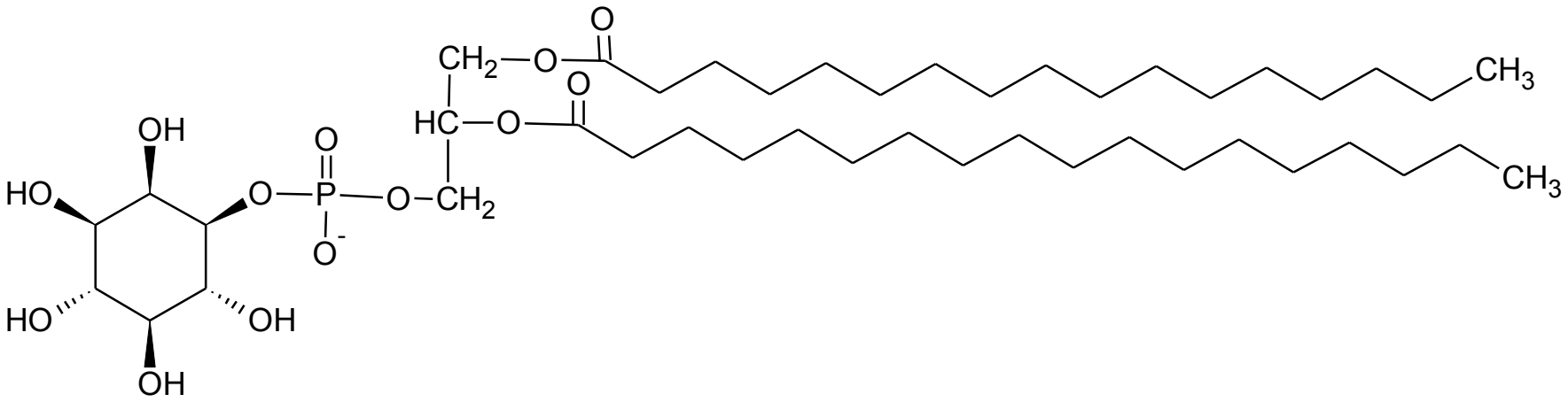
Phosphatidic acid + CTP → **CDP-diacylglycerol** + PP_i



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)

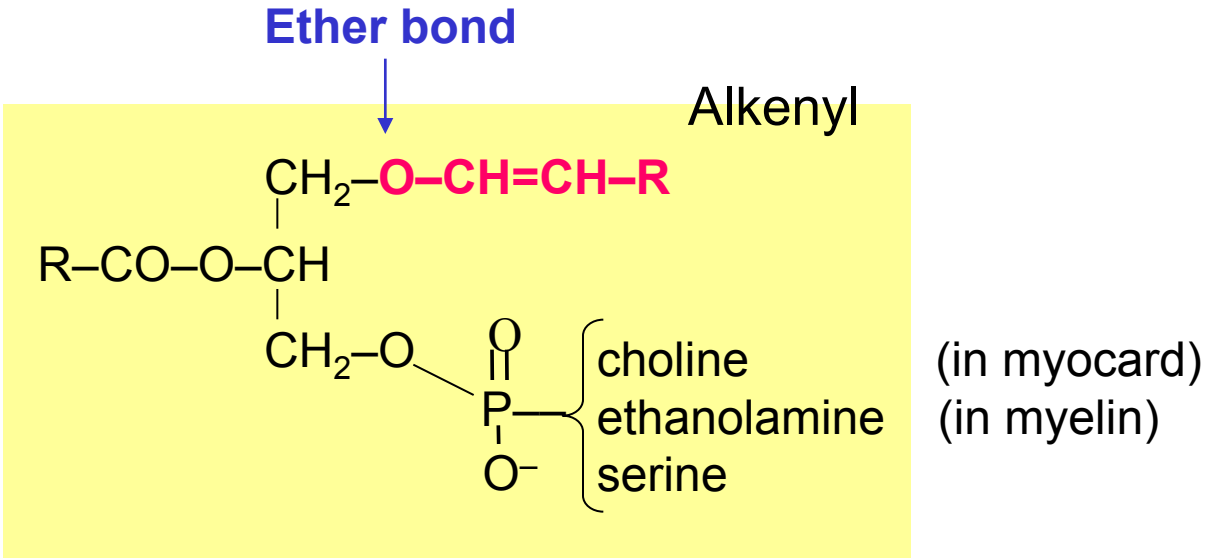


Products of lipid remodelation

Plasmalogens

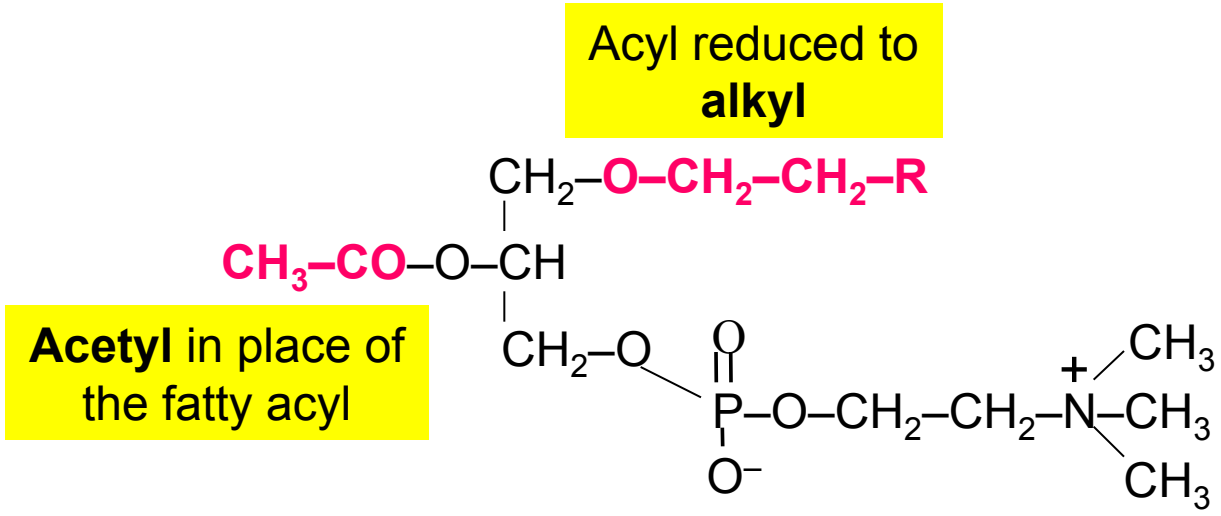
modified glycerophospholipids (alkoxylipids or ether glycerophospholipids).

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 phosholipids:

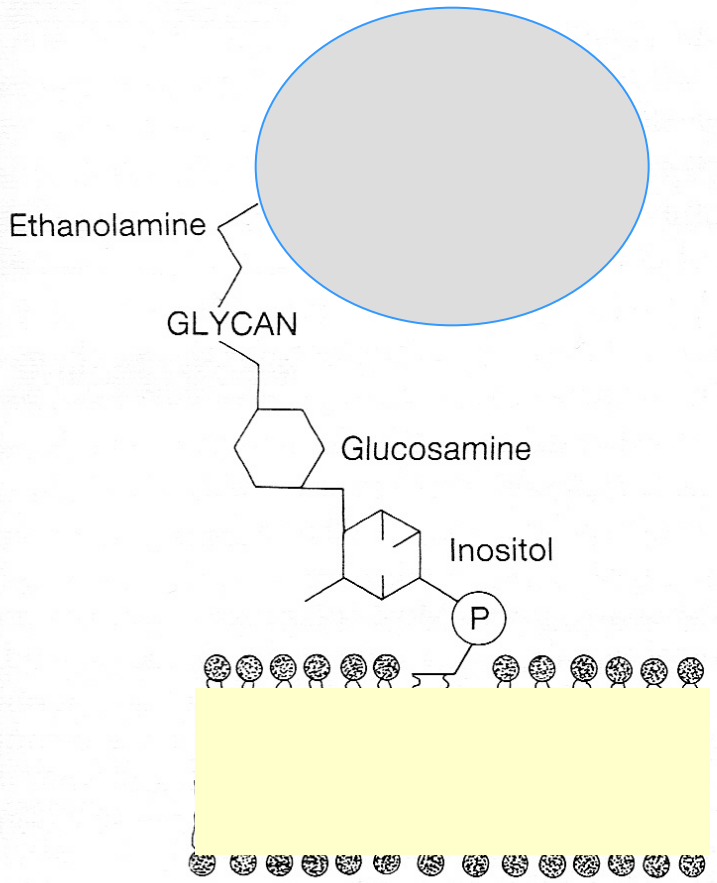
diacylglycerols:	oleic acid on the C-2
phospholipids:	polyunsaturated FA (often arachidonic acid) on the C-2

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_2, DG)

Anchoring of proteins to membrane

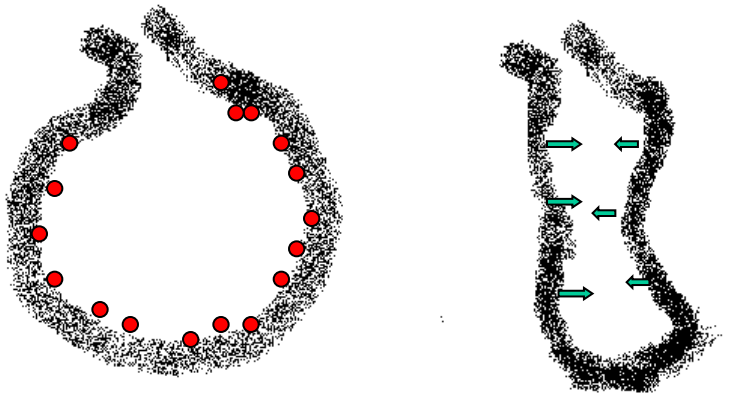
The linkage between the COOH-terminus of a protein and phosphatidylinositol fixed in the membrane lipidic bilayer exist in several ectoenzymes (alkaline phosphatase, acetylcholinesterase, some antigens).



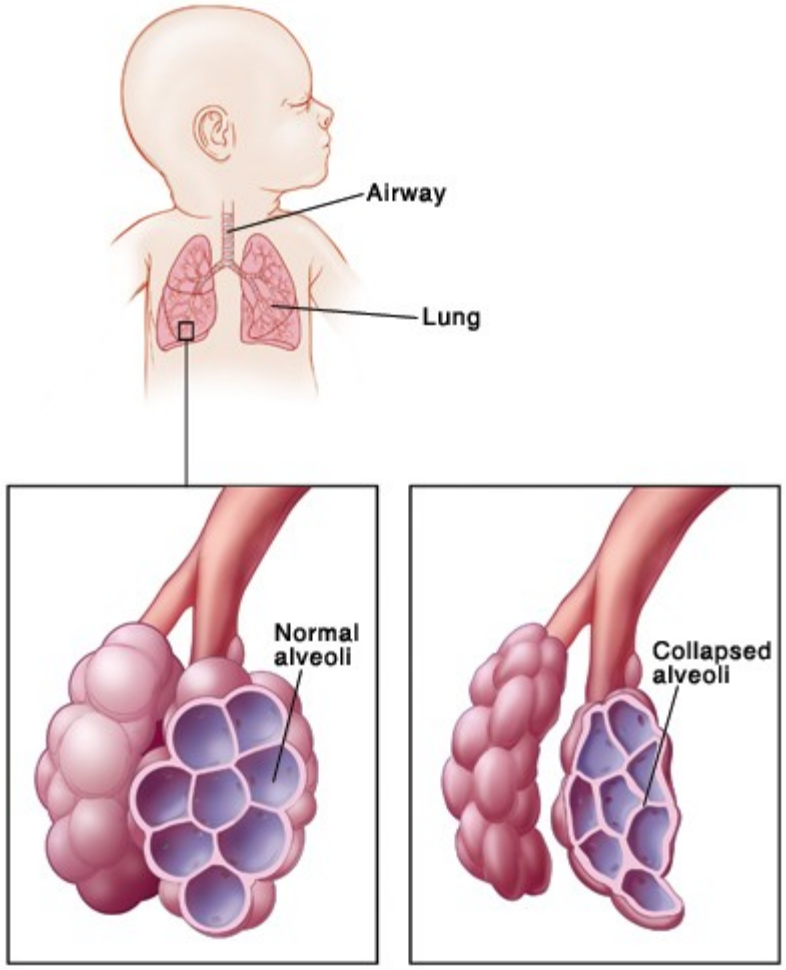
Lung surfactant

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 syndrom

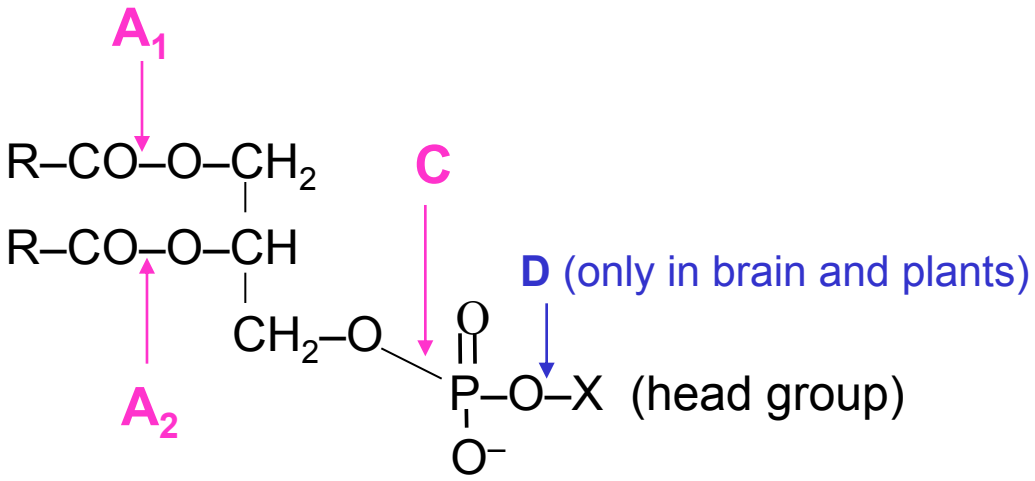
Treatment:

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

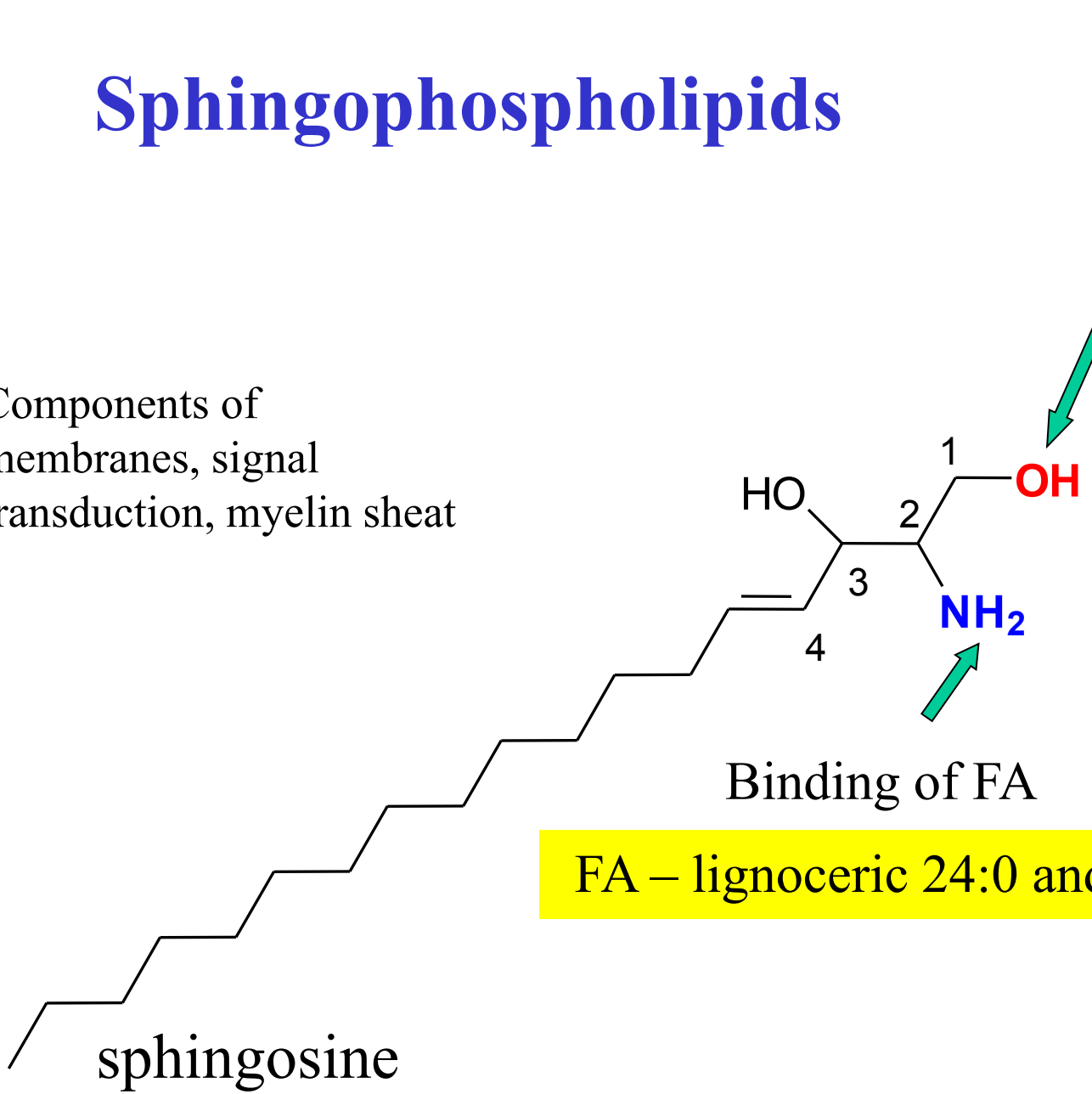
Catabolism of glycerophospholipids

Enzymes catalysing hydrolysis of glycerophosholipids 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

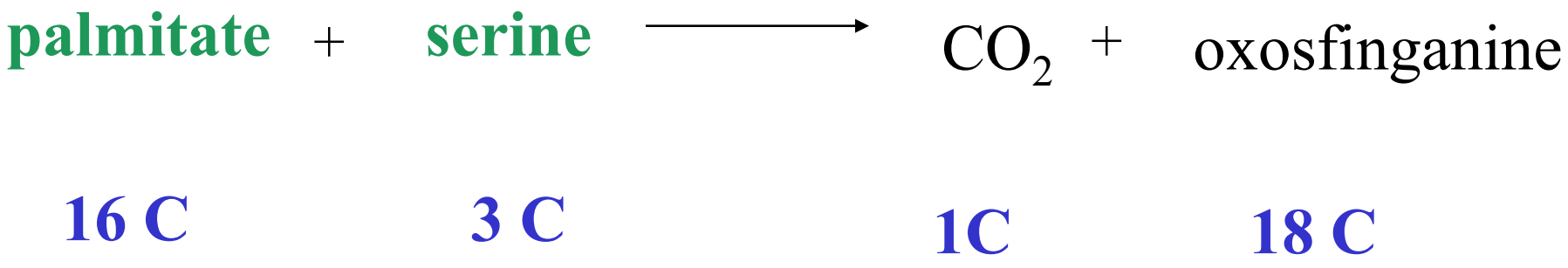
Components of membranes, signal transduction, myelin sheat

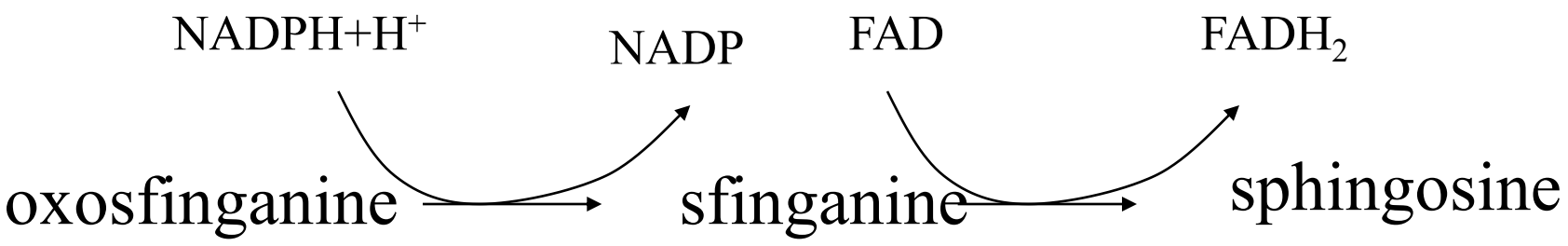


FA – lignoceric 24:0 and nervonic 24:1(15)

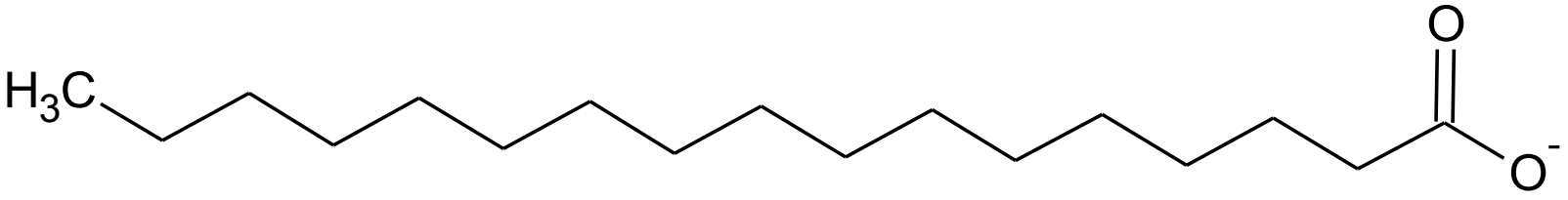
Biosynthesis of sphingosine

- overall equation

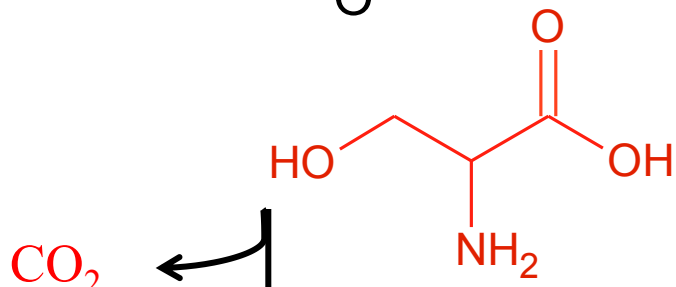




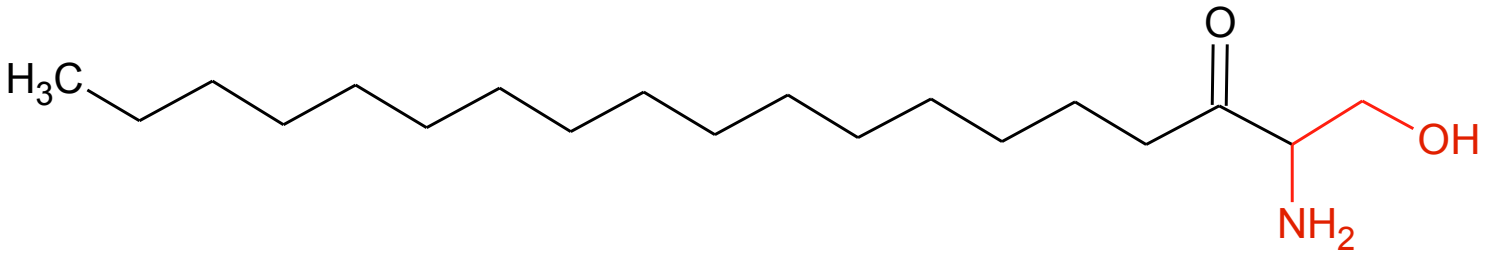
Biosynthesis of sphingosine



Palmitic acid

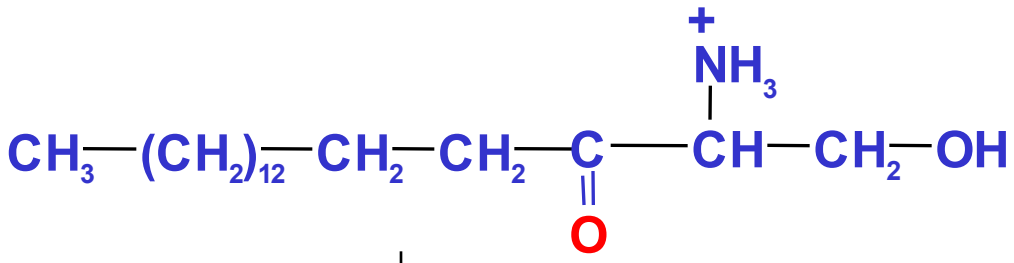


serine



oxosfinganine

oxosfinganine

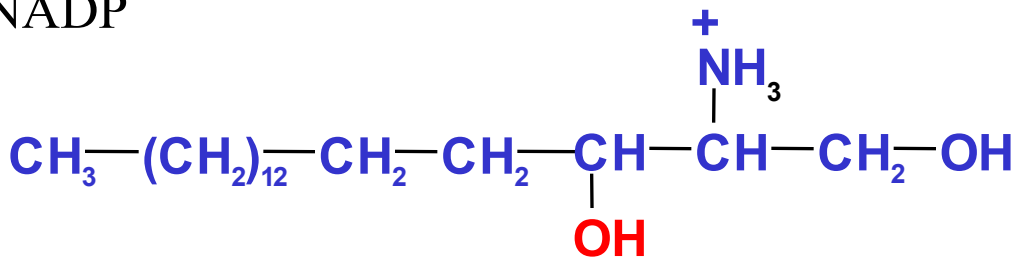


2.

NADPH + H^+

NADP

sfinganine

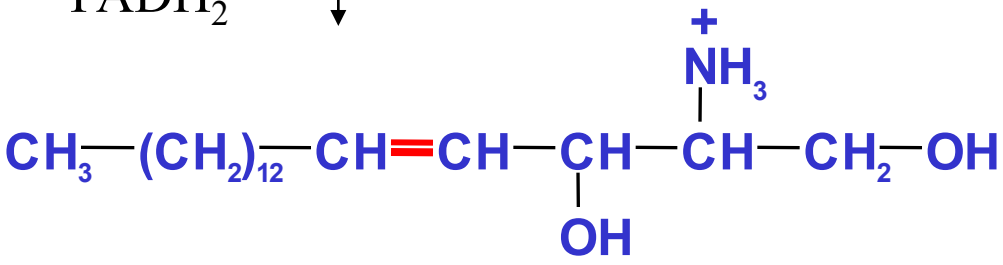


3.

FAD

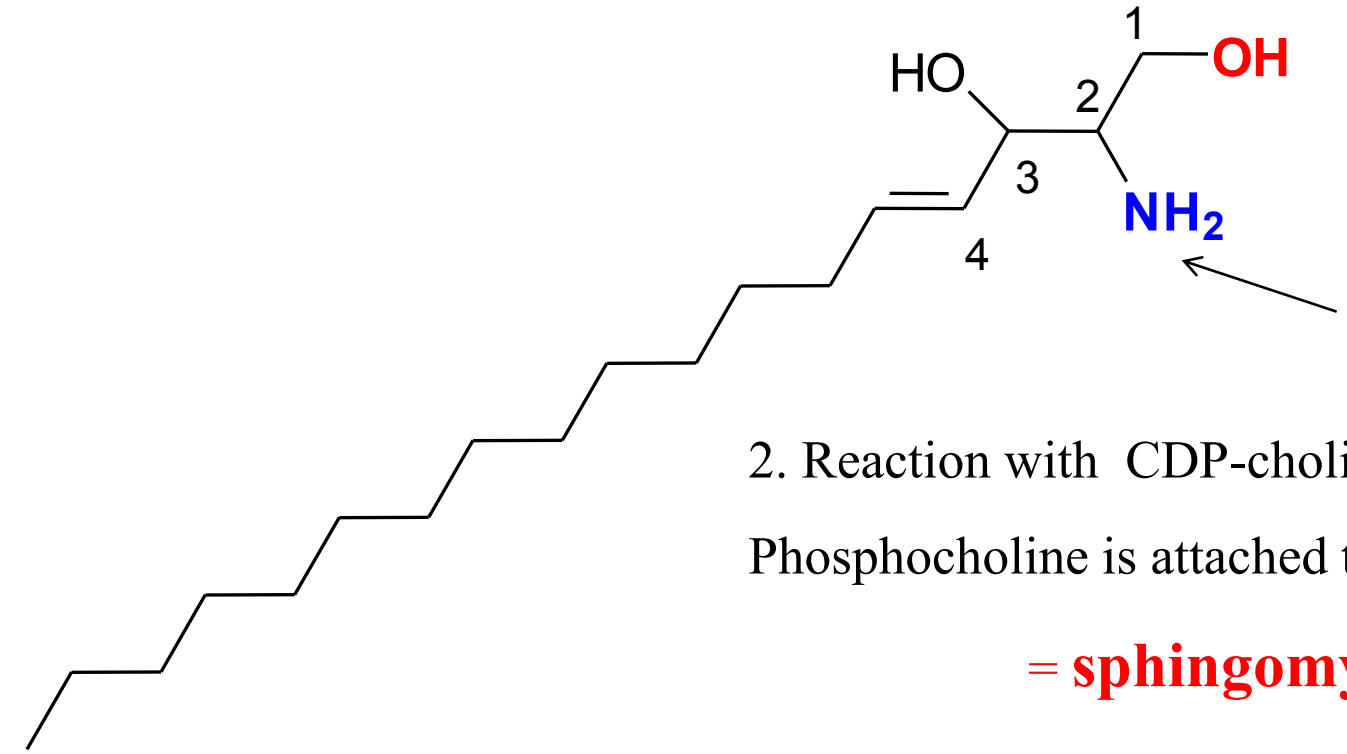
$FADH_2$

sfingosine



4.

Biosynthesis of sphingomyeline



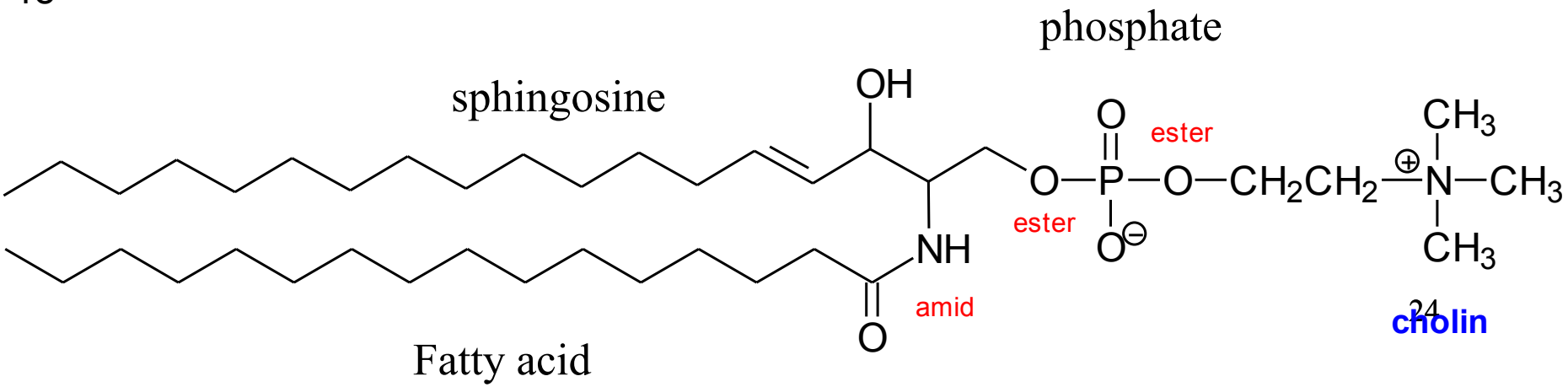
1.Attachment of fatty acid by amide bond

= ceramide

2. Reaction with CDP-choline:

Phosphocholine is attached to CH_2OH

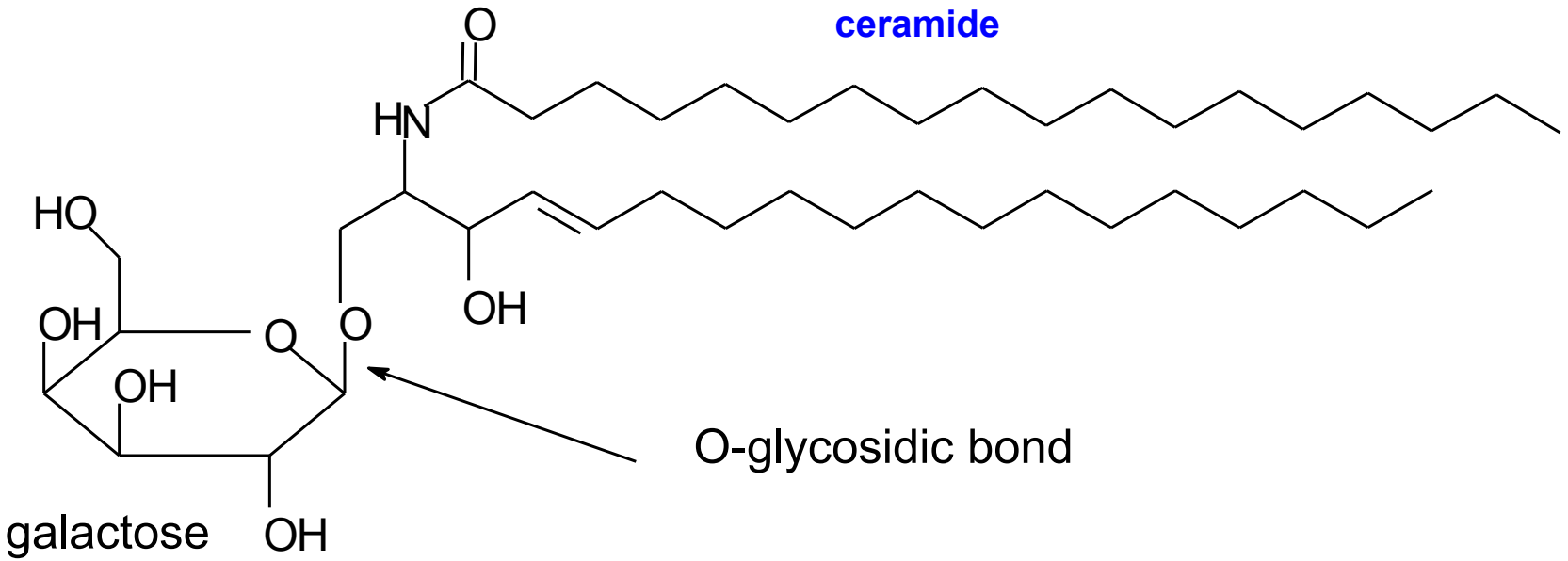
= sphingomyelin



Glycosphingolipids

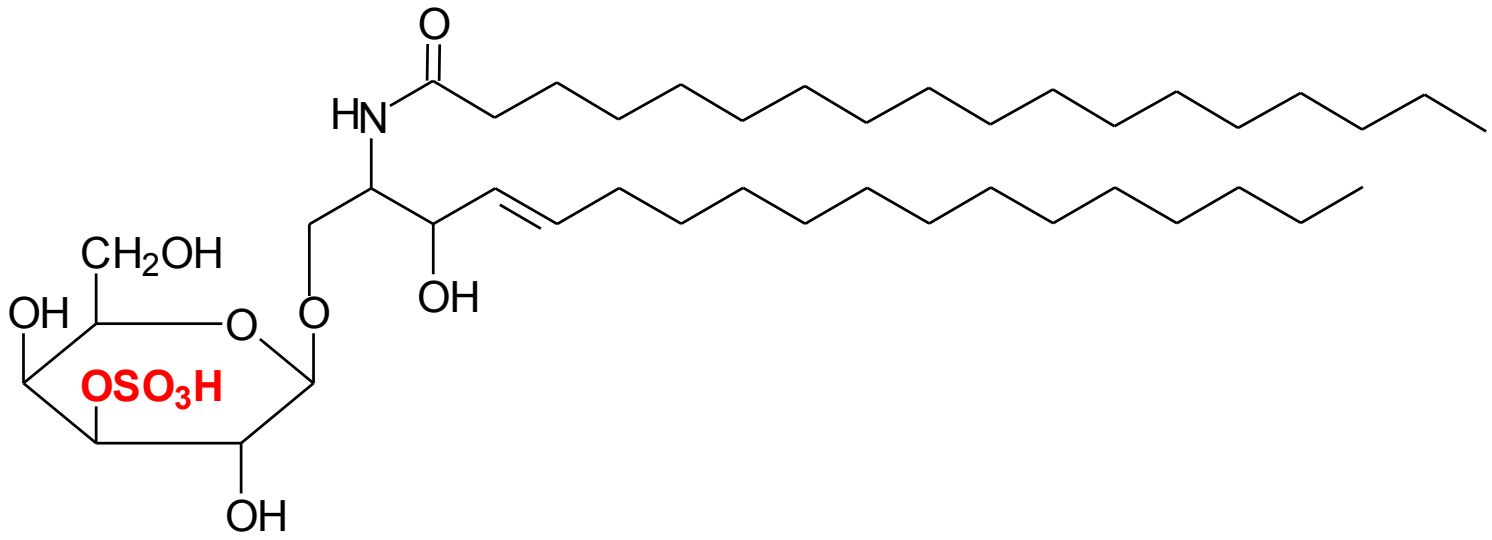
Cerebrosides (monoglycosylceramides)

ceramide + UDP-galactose



galactosylceramide

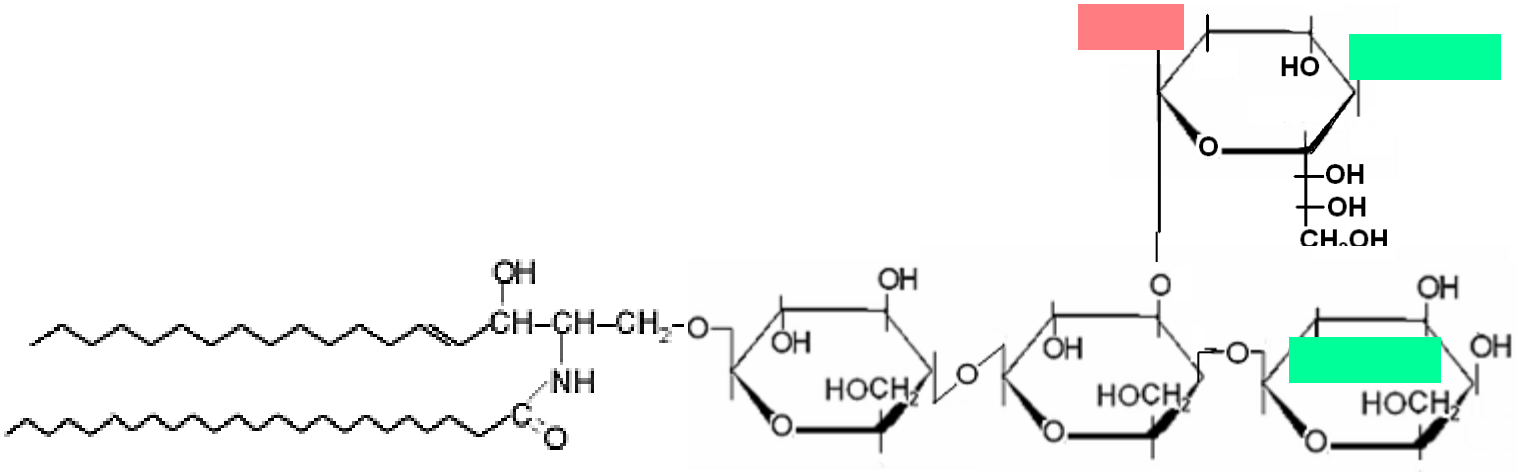
Sulfoglycolipids are sulfated



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

Gangliosides

Sialic acid is attached to oligosaccharide chain



NeuAc
|(3←2α)
Ceramide–(1←1β -(4←1β -(4←1β

Biosynthesis of glycosphingolipids

Synthesis of cerebrosides:

ceramid + UDP-gal → ceramid -gal + UDP

…… + binding of other UDP-monosacharides

Synthesis of sulfatides:

Sulfatation of cerebrosides 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.

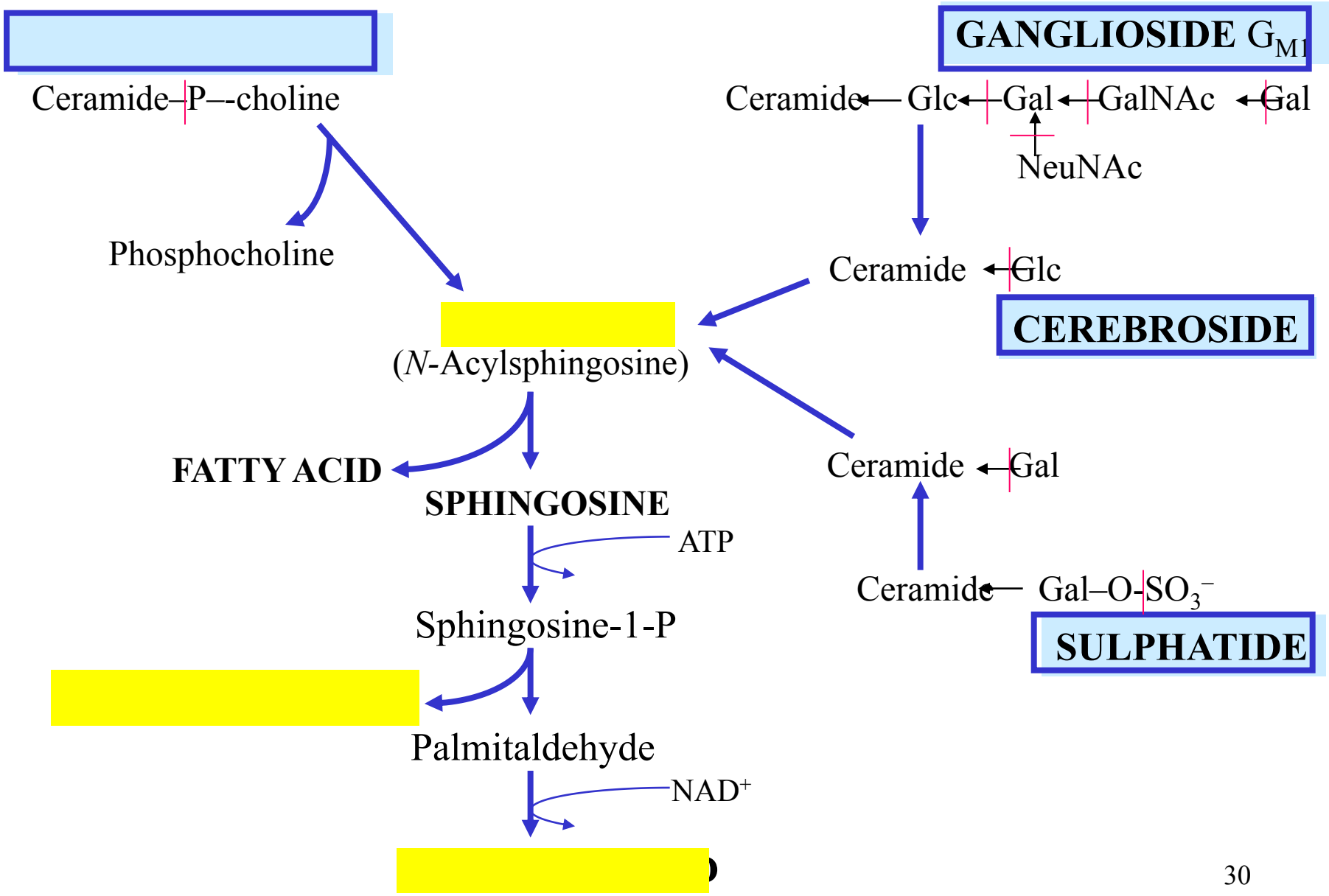
Sphingomyelins loose phosphocholine to give **ceramide**.

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 hormons

The main types of eicosanoids:

prostaglandins (PG)

tromboxans (TX)

leukotriens (LT)

They are synthesized from polyunsatureted fatty acids with 20 carbons

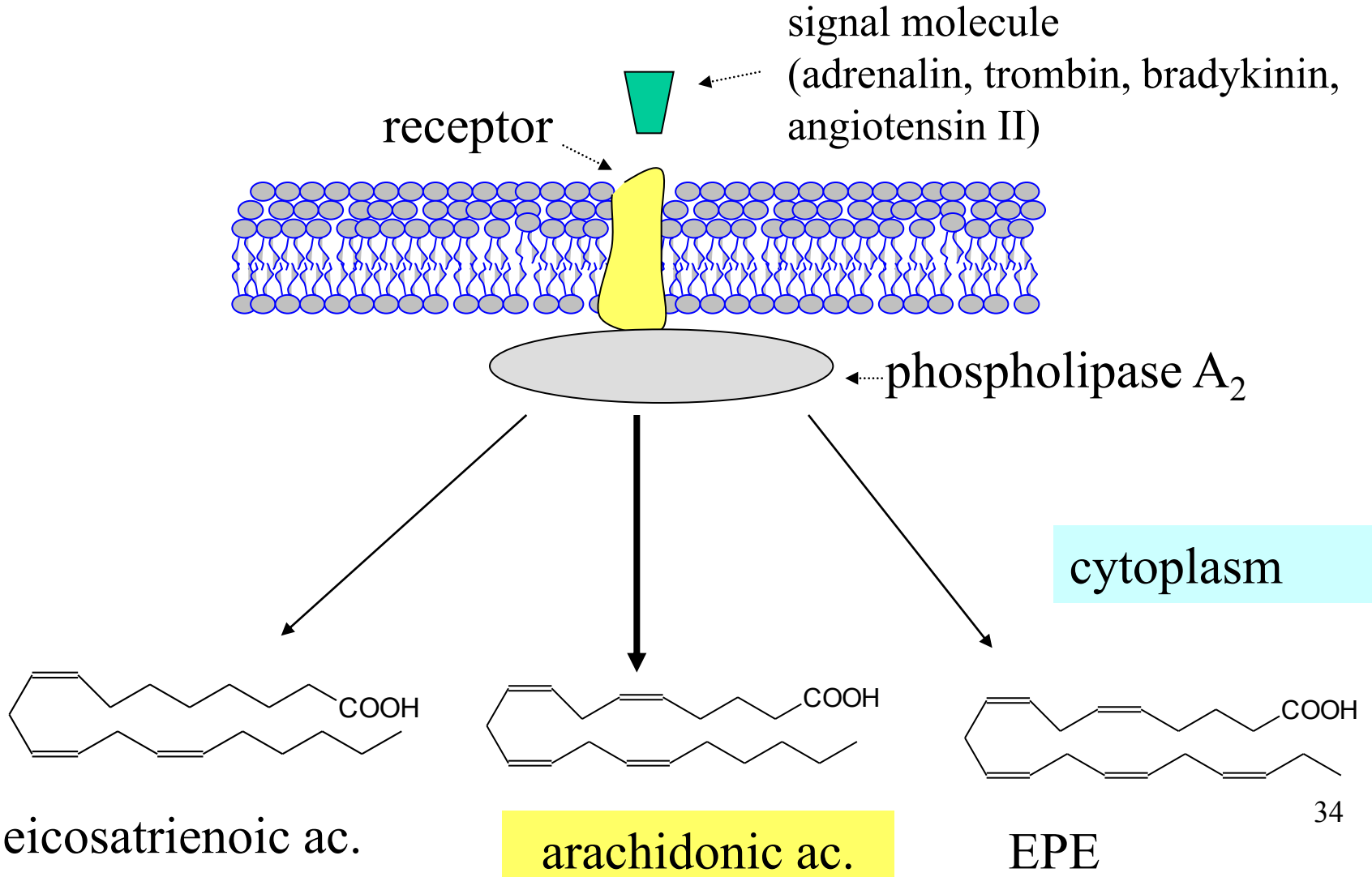
Synthesis of eicosanoids:

PG, TX (prostanoids) – cyclooxygenase pathway

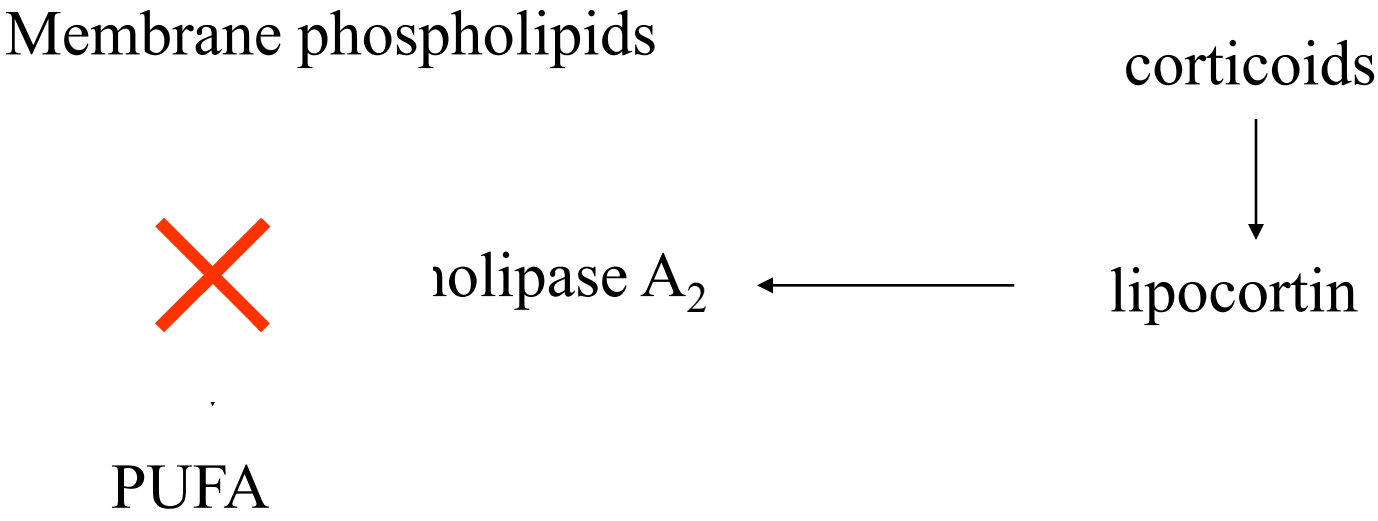
LT (Leukotriens) – lipoxygenase pathway

Biosynthesis of eicosanoids

1. The release of C_{20} fatty acids from membrane phospholids



Inhibitors of phospholipase A_2



Steroidal antiphlogistics (hydrocortisone, prednisone) stimulate the synthesis of protein lipocortin which inhibits phospholipase A_2 and block the release of PUFA and eicosanoids formation.

Principle of anti-inflammatory effect of glucocortikoids. They inhibit the two main products of inflammation, prostglandins 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_2 (tromboxane A_2)

It is produced in platelts, it stimulates vasoconstriction and and platelet agregation

Duration of action ~ 30-60 s

PGI_2 (prostacycline A_2)

It is antagonist of TXA_2, , 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.

PGE_2 is produced by mucose cells of the stomach and inhibits HCl secretion

It reduces the risk of peptic ulcer

PGE_2 and $PGF_{2\alpha}$ 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.

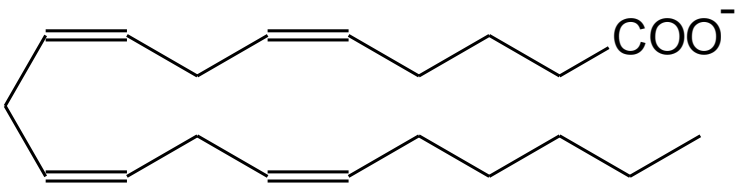
They can be used to induce abortion by inttravenous or intravaginal application

Examples of some biological effects of prostanoids

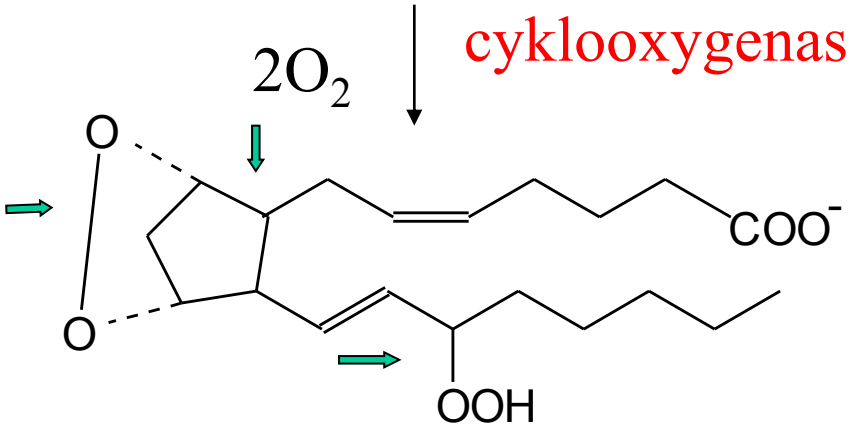
prostanoid	Structural group	Synthesized in	The most remarkable effect
PGE_2	prostaglandin E	nearly all cell types	inflammatory reaction, vasodilation, inhibition of HCl secretion, secretion of mucine, increase of body temperature, increase of intensity and duration of pain, increase of vessel permeability
$PGF_{2\alpha}$	prostaglandin F	nearly all cell types	vasoconstriction increase of body temp.
PGI_2	prostacyclin	endothelial cells, smooth muscle cells of blood vessels	vasodilation, inhibition of platelet aggregation, increase of intensity and duration of pain,
TXA_2	thromboxane	blood platelets	platelet aggregation, vasoconstriction

Synthesis of prostanoids (cycloxygenase pathway)

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

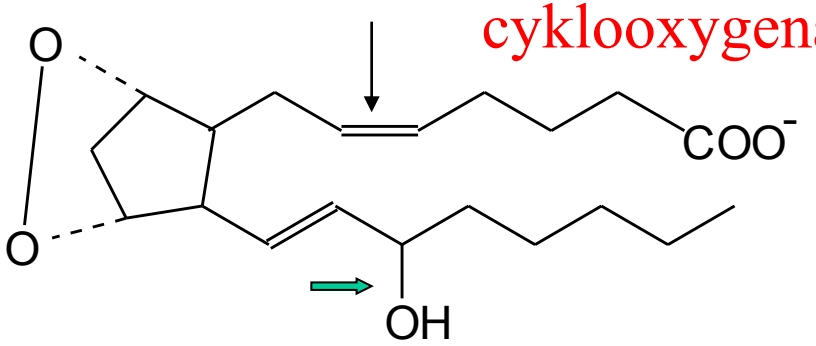


Arachidonic acid



cyklooxygenase (cyklooxygenase activity)

PGG_2 (two double bonds)

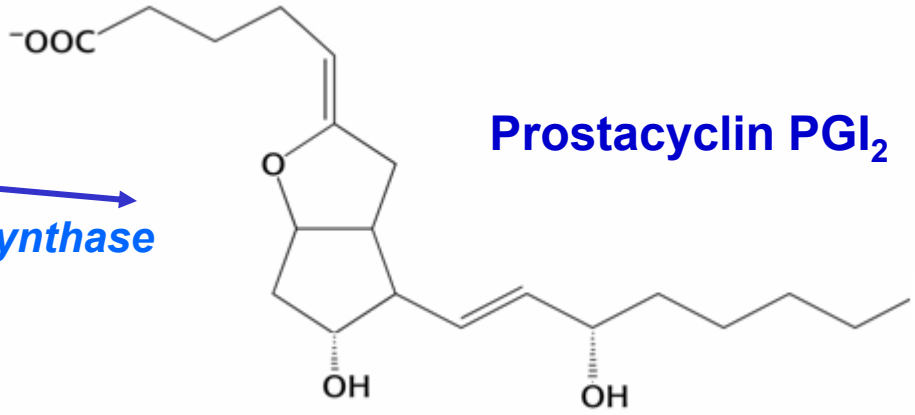


cyklooxygenase (peroxidase activity)

PGH_2 - precursor of all prostanoids of the 2-series

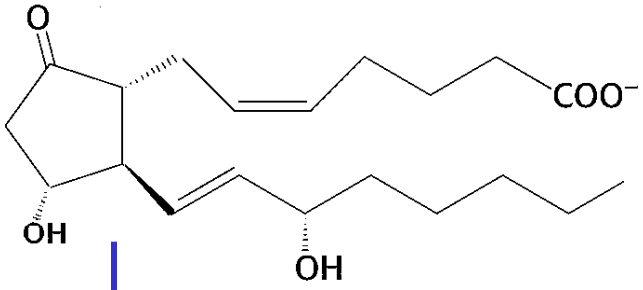
Prostaglandin H_2

PGI synthase



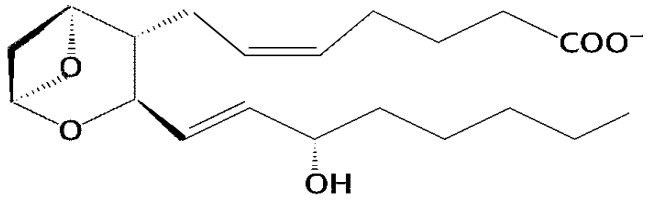
Prostacyclin PGI_2

PGE synthase



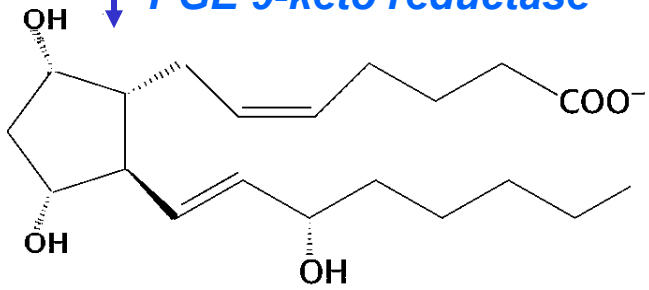
Prostaglandin PGE_2

TXA synthase



Thromboxane TXA_2

PGE 9-keto reductase



Prostaglandin $PGF_{2\alpha}$

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 ($t_{1/2}$ ~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: predomintly 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

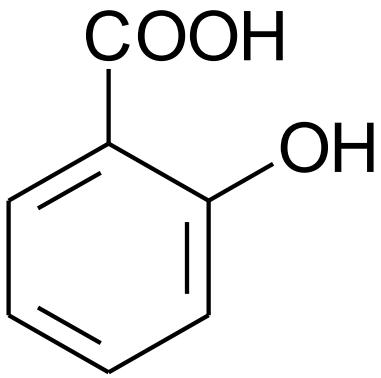
Examples: celecoxib, rofecoxib

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

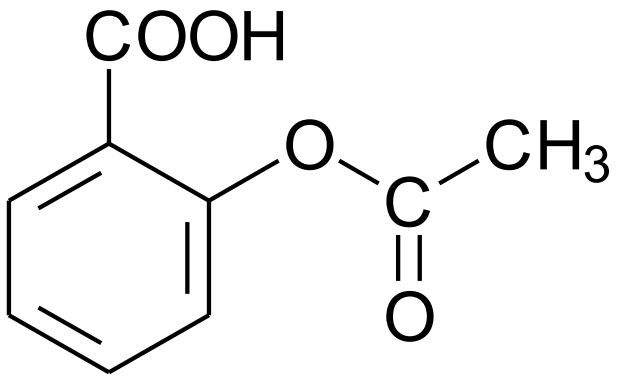
Coxibs were withdrawn from the market by its manufacturer because of negative patients study

Nimesulid (AULIN, COXTRAL), meloxikam (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 TXA_2 by inhibition of COX

The principal effect of TXA_2 is the stimulation of platelets aggregation.

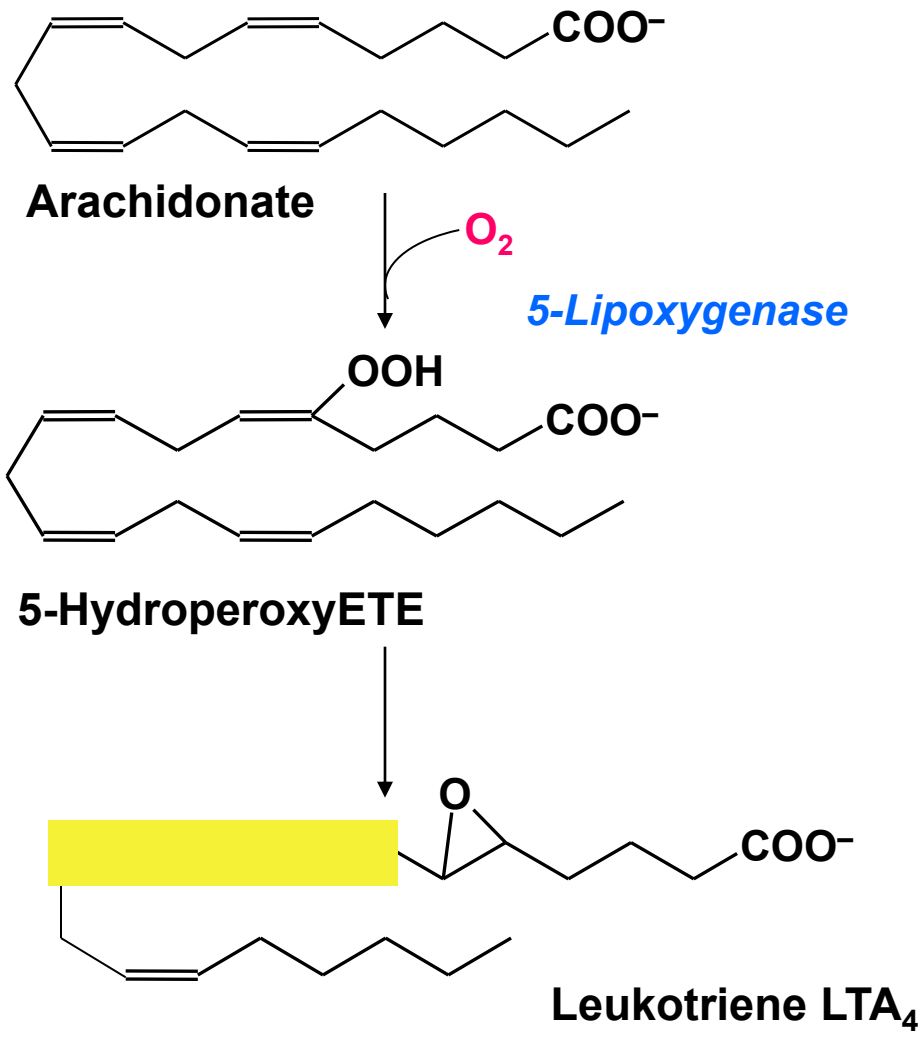
It may initiate the formation of trombus at sites of vascular injury or in the vicinity of ruptured atherosclerotic plaque.

Such thrombi may cause sudden total occlusion of vascular lumen.

By aspirin treatment the effect the effects of thromboxane are attenuated.

Lipoxygenase pathway

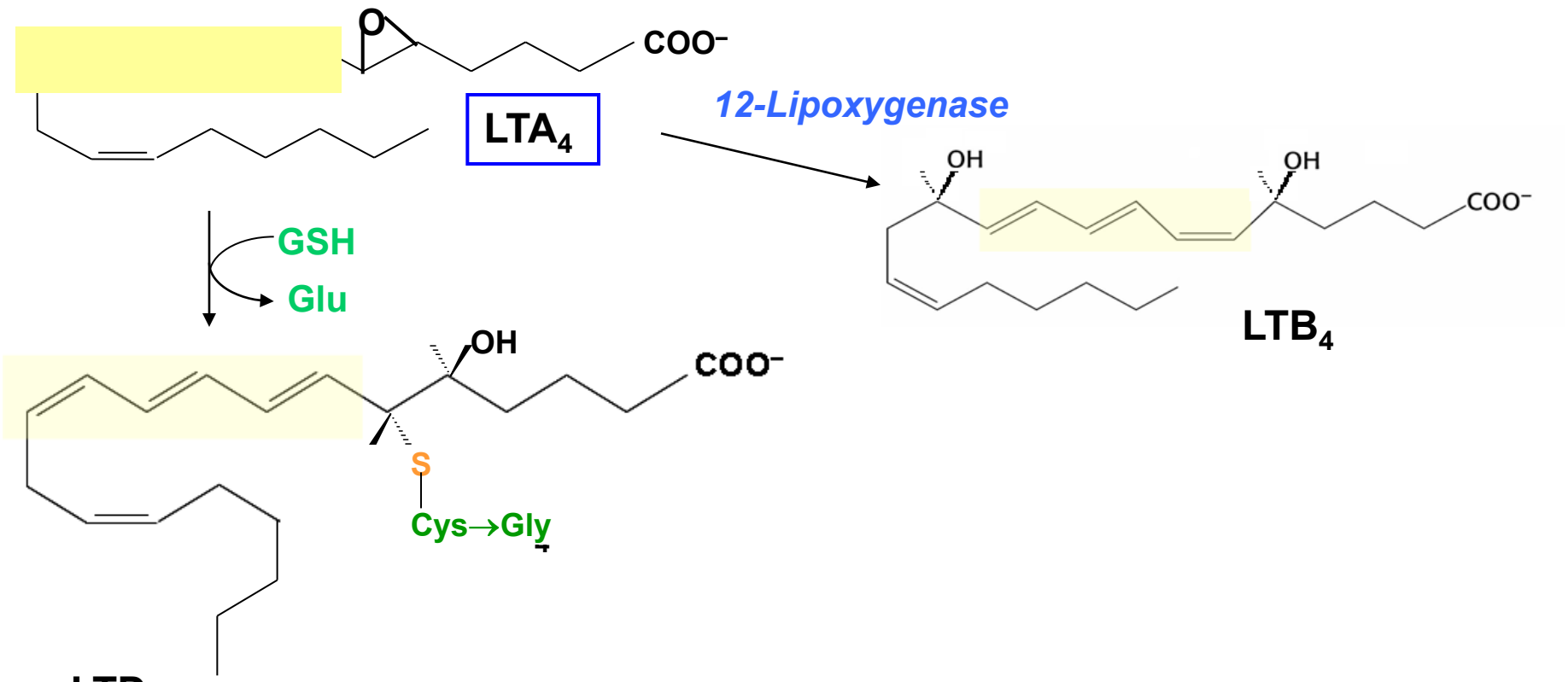
Synthesis of leukotrienes



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.



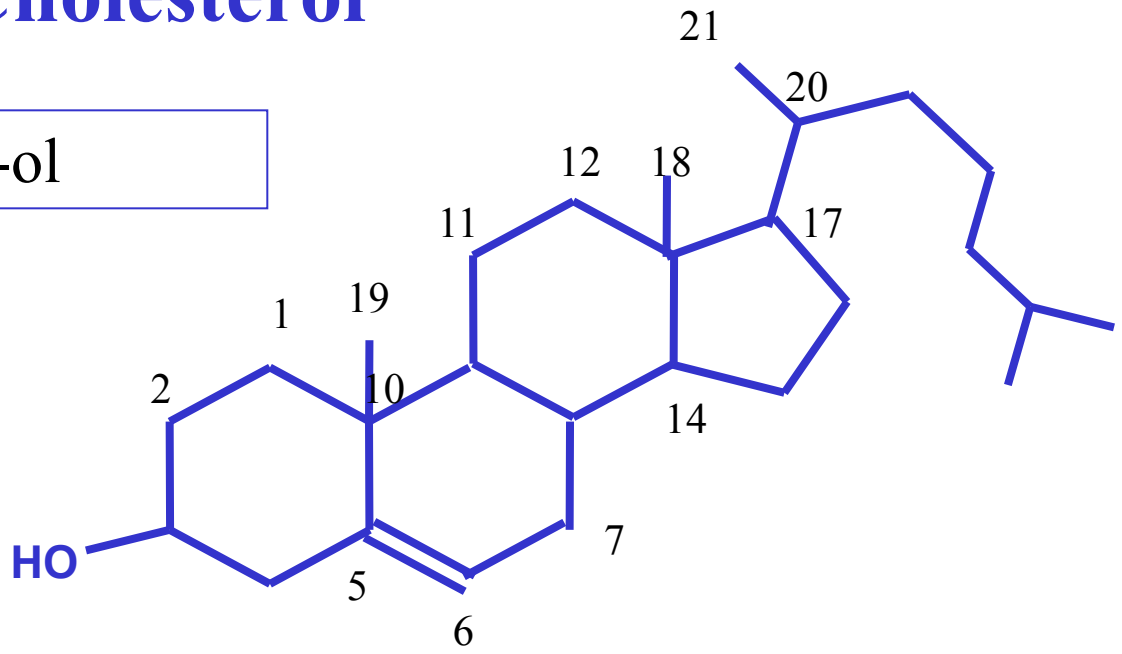
Eicosanoids

Example	Structural group	Synthesized in	The most remarkable effect
LTD_4	leukotriene	leukocytes, mast cells	bronchoconstriction, vasoconstriction
LXA_4	lipoxin	various cell types	bronchoconstriction, vasodilation

Cholesterol

Cholesterol

5-cholesten-3β-ol



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

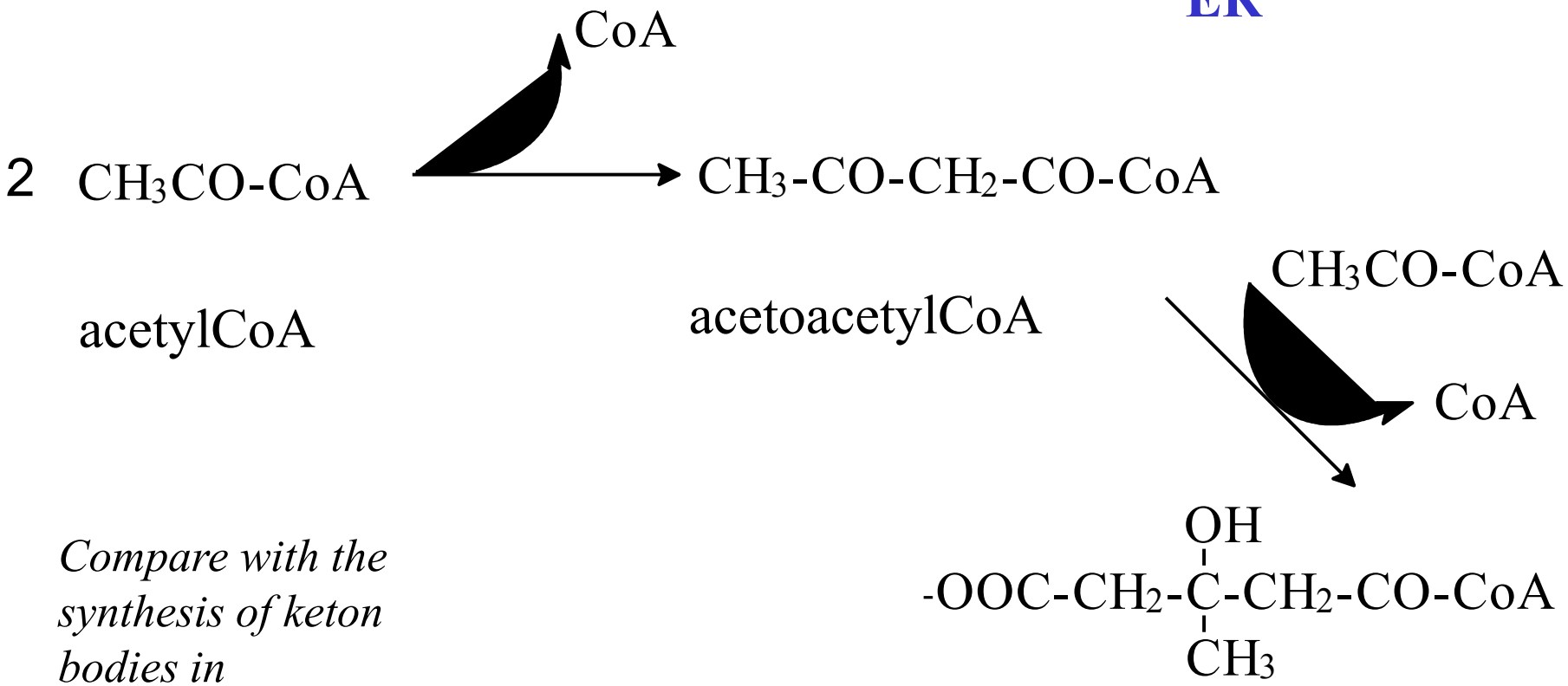
Biosynthesis of cholesterol

- where: most of cells, **mainly liver**, adrenal cortex, red blood cells, reproductive tissues….
- where in the cell: cytoplasma, 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

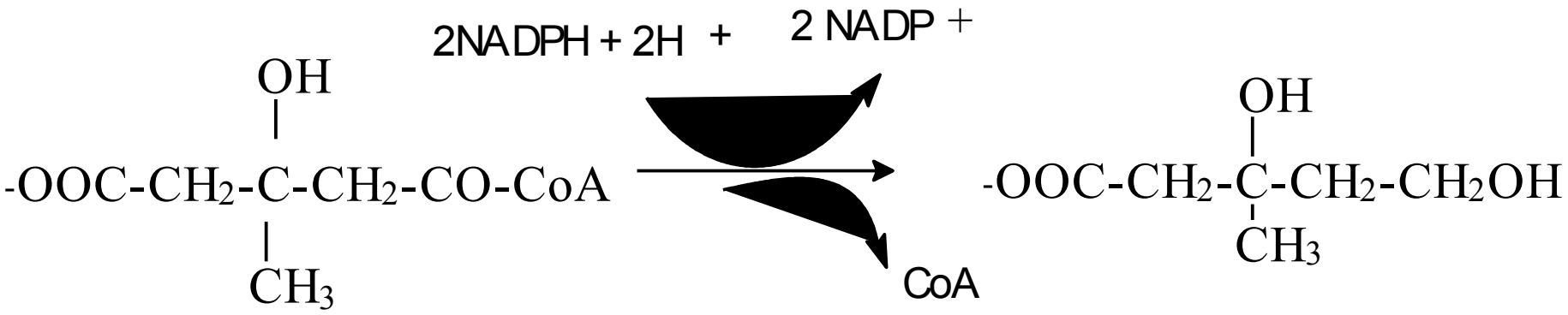
ER



Compare with the synthesis of keton bodies in mitochondrial matrix

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

2. phase - formation of mevalonate



3-HMG-CoA reductase

3-HMG-CoA

Mevalonic acid

Double reduction of carboxylic group to primary alcohol group

Synthesis of mevalonate determines the overal 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 resposible for ubiquitination of the enzyme at high level of sterols

Regulation of HMG-CoA reductase by covalent modification

Forms of the enzyme

phosphorylated	dephosphorylated
↓	↓
inactive	active
kinase –AMP dependent	phosphatase
Activation:	
Glucagon, intracelular sterols (cholesterol, bill acids), glucocorticoids	Insulin, thyroidal hormons

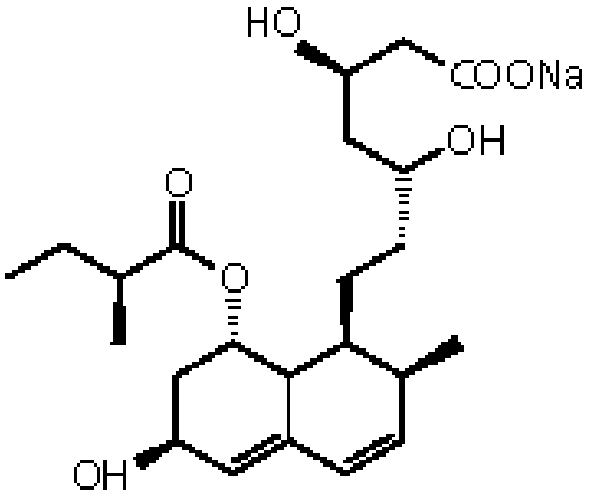
Inhibition of HMG-CoA-reductase by drugs

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

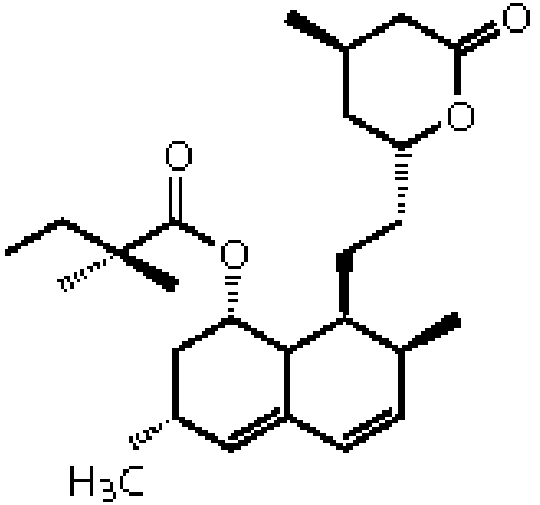
The synthesis of cholesterol in liver is decreased by their action.

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

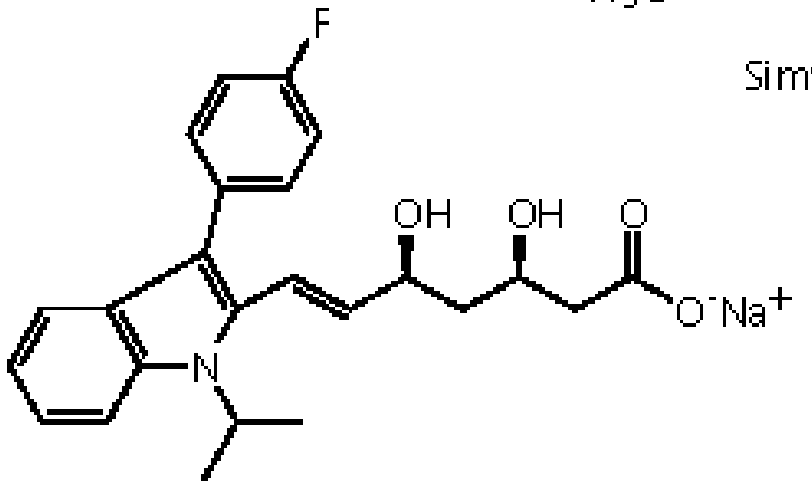
Simvastatin (Zocor), Lovastatin (Mevacor), Pravastatin (Mevalotin), Pravastatin (Pravachol), Simvastatin (Lipovas), Fluvastatin (Lescol),…



Pravastatin



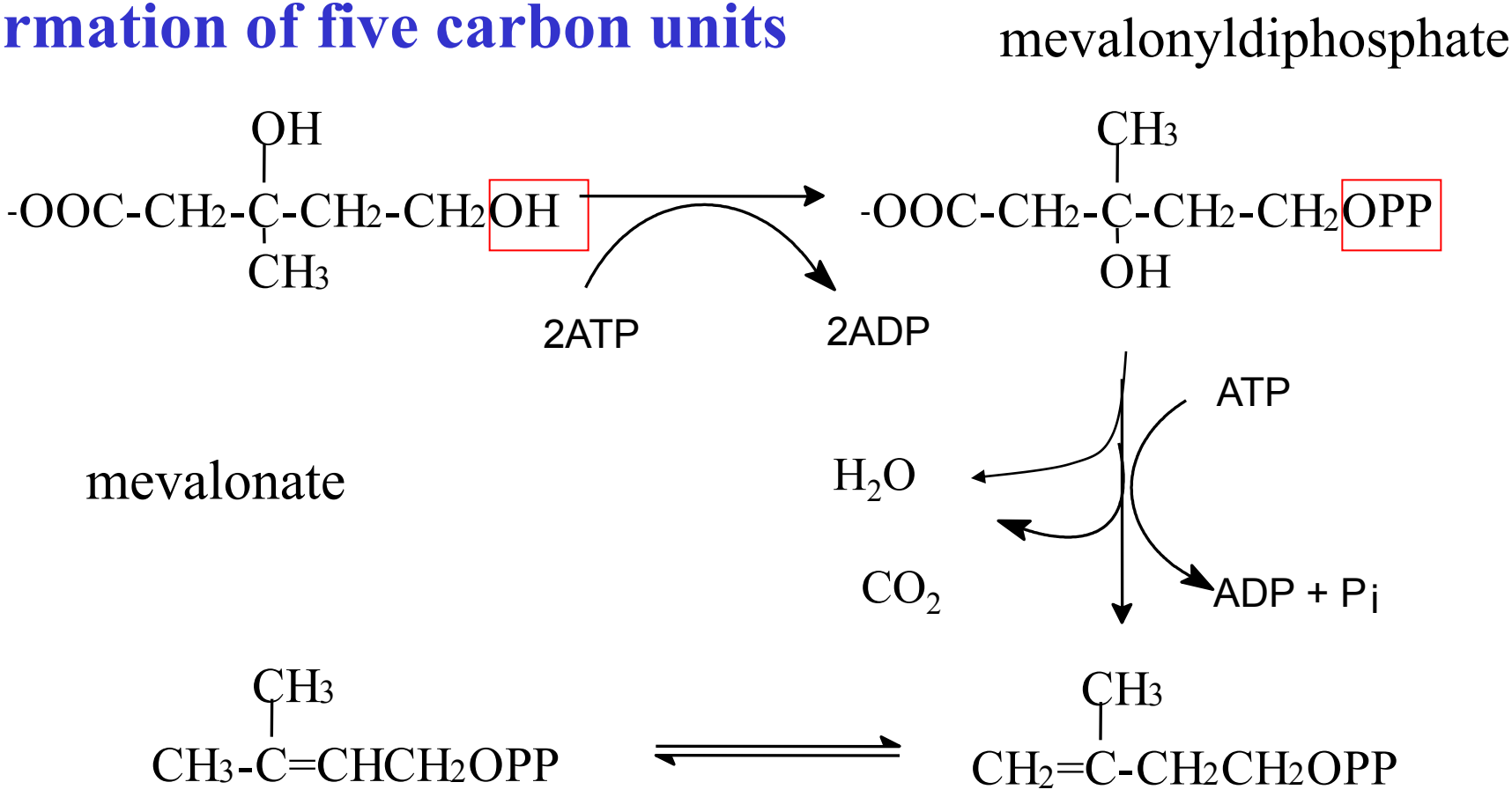
Simvastatin



Fluvastatin

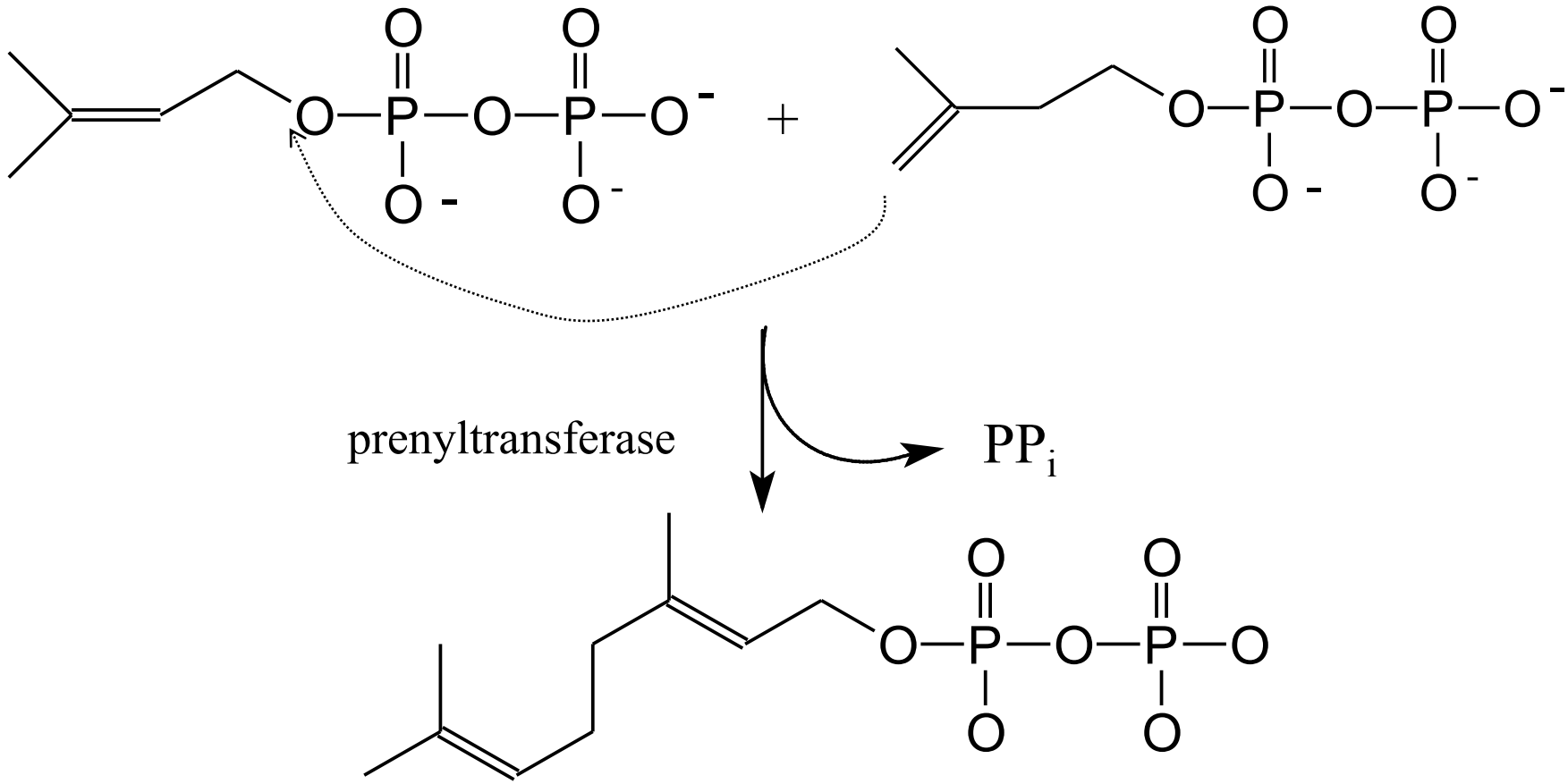
3.phase of cholesterol synthesis:

formation of five carbon units



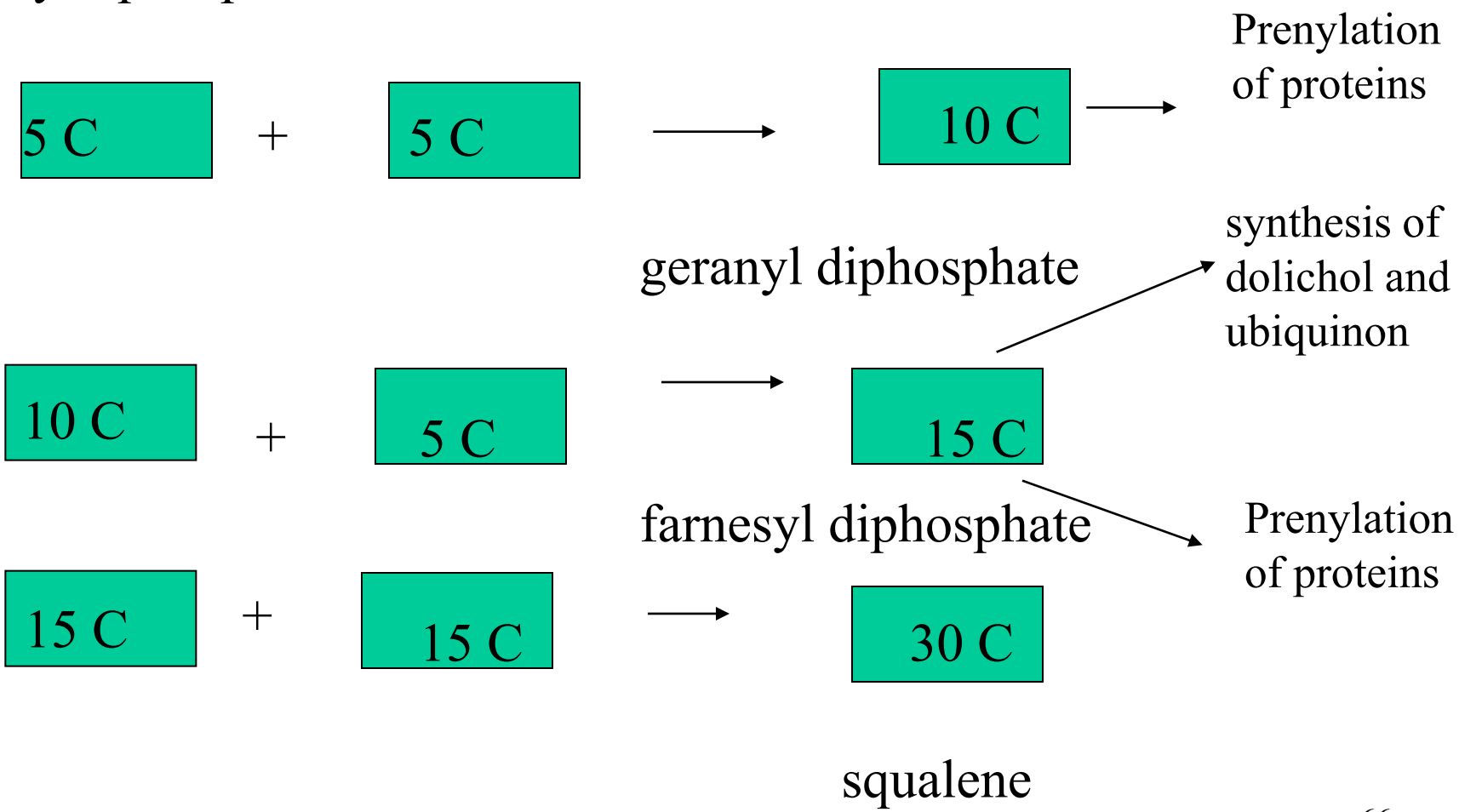
Dimethylallyldiphosphate

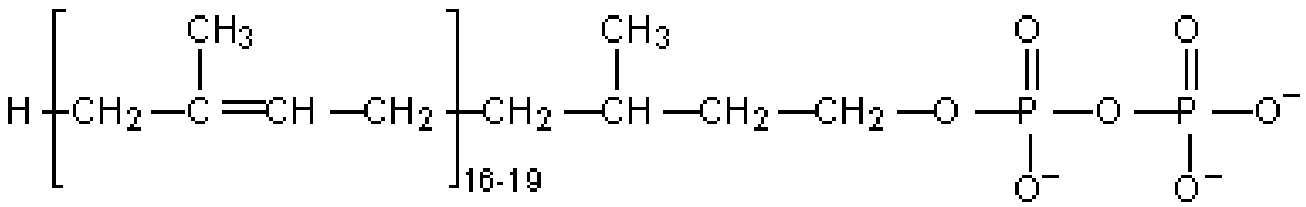
isopentenyldiphosphate



geranyldiphosphate

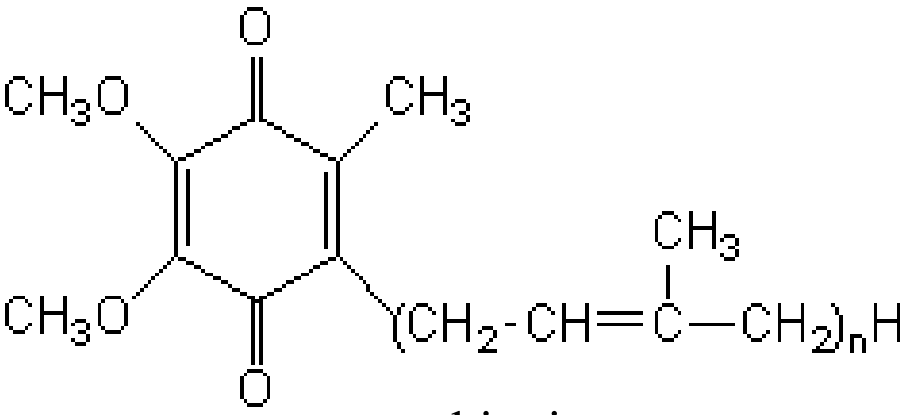
Dimethylallyldiphosphate + isopentenyldiphosphate → geranyldiphosphate





Dolichol diphosphate

Synthesis of oligosaccharide chains of glycoproteins



ubiquinon

Respiratory chain

Prenylation of proteins

•Covalent modification of proteins

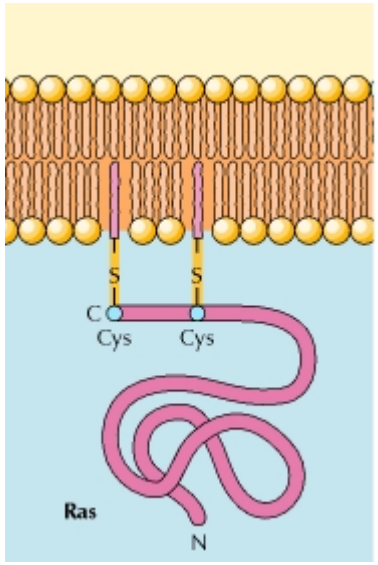
• Binding farnesyl or geranyl-geranyl to SH- group of cystein

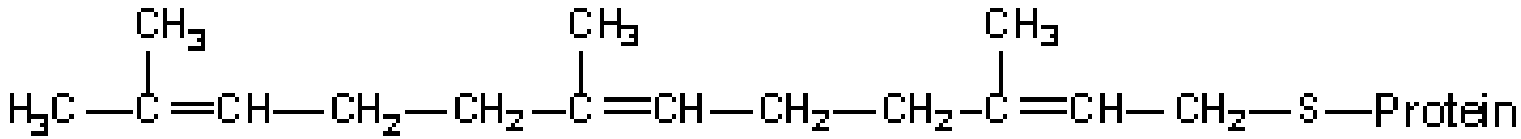
•Mediates the interaction of proteins with membrane (anchoring) or protein –protein interaction or membrane –associated protein trafficking.

• modifies some proteins affecting cell proliferation (GTP-binding proteins, eg. Ras, Rac, Rho)

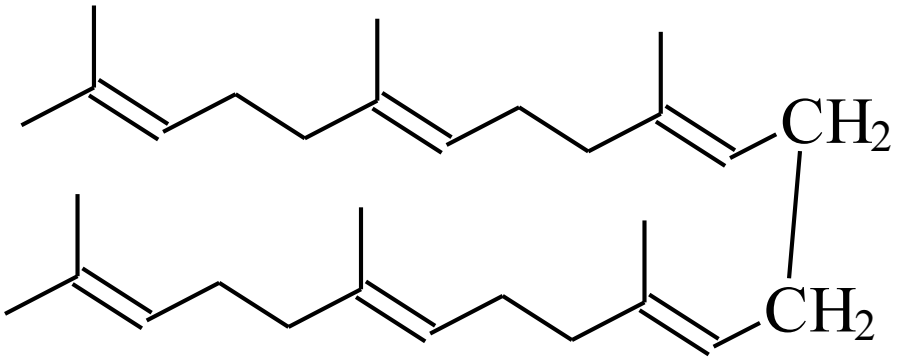
• inhibition of prenylation inhibits cell proliferation

• inhibitors of prenylation – drugs at treatment of osteoporosis, cancer, cardiovascular diseases

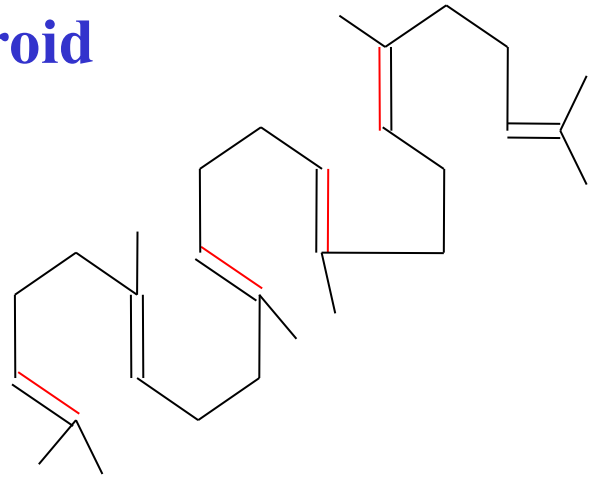


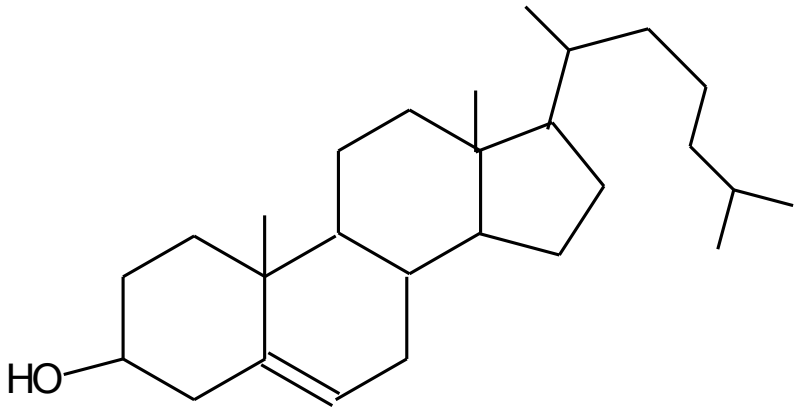


Squalene is linear molecul that can fold into a structure that closely resembles the steroid structure



squalen





cholesterol

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

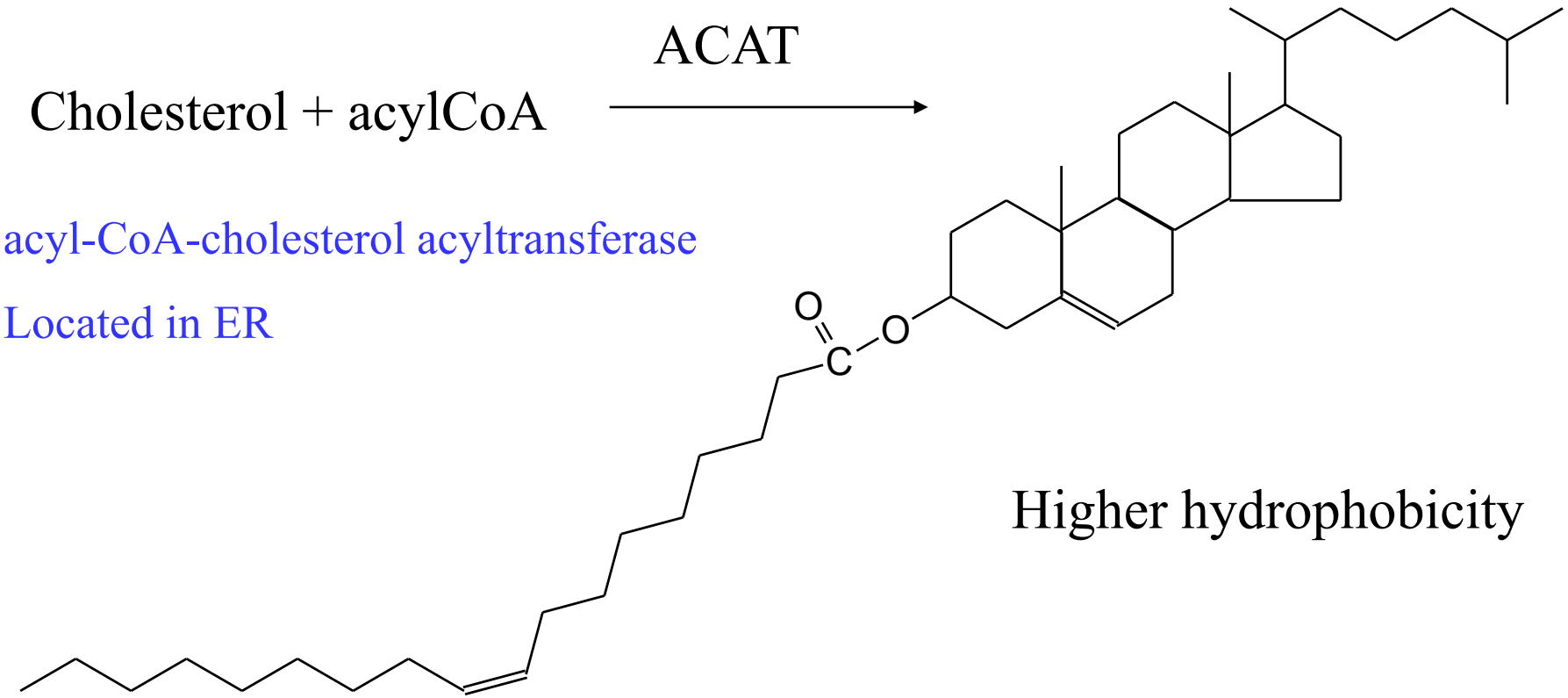
- cyclisation
- shortening carbon chain from 30 to 27 C
- movement of double bonds
- reduction of double bond

Squalene (30 C) → lanosterol (30 C) → → →

→ cholestadienol (27 C) → cholesterol (27 C)

Esterification of cholesterol

Cholesterol + acylCoA $\xrightarrow{\text{ACAT}}$



acyl-CoA-cholesterol acyltransferase

Located in ER

Higher hydrophobicity

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.

See Biochemistry II – 4.semestr

„Degradation of cholesterol“

• in higher animals steroid nucleus of cholesterol is **neither decomposed** to simple products **nor oxidized** to CO_2 a H_2O

• only liver have ability to eliminate cholesterol

• two ways of cholesterol elimination:

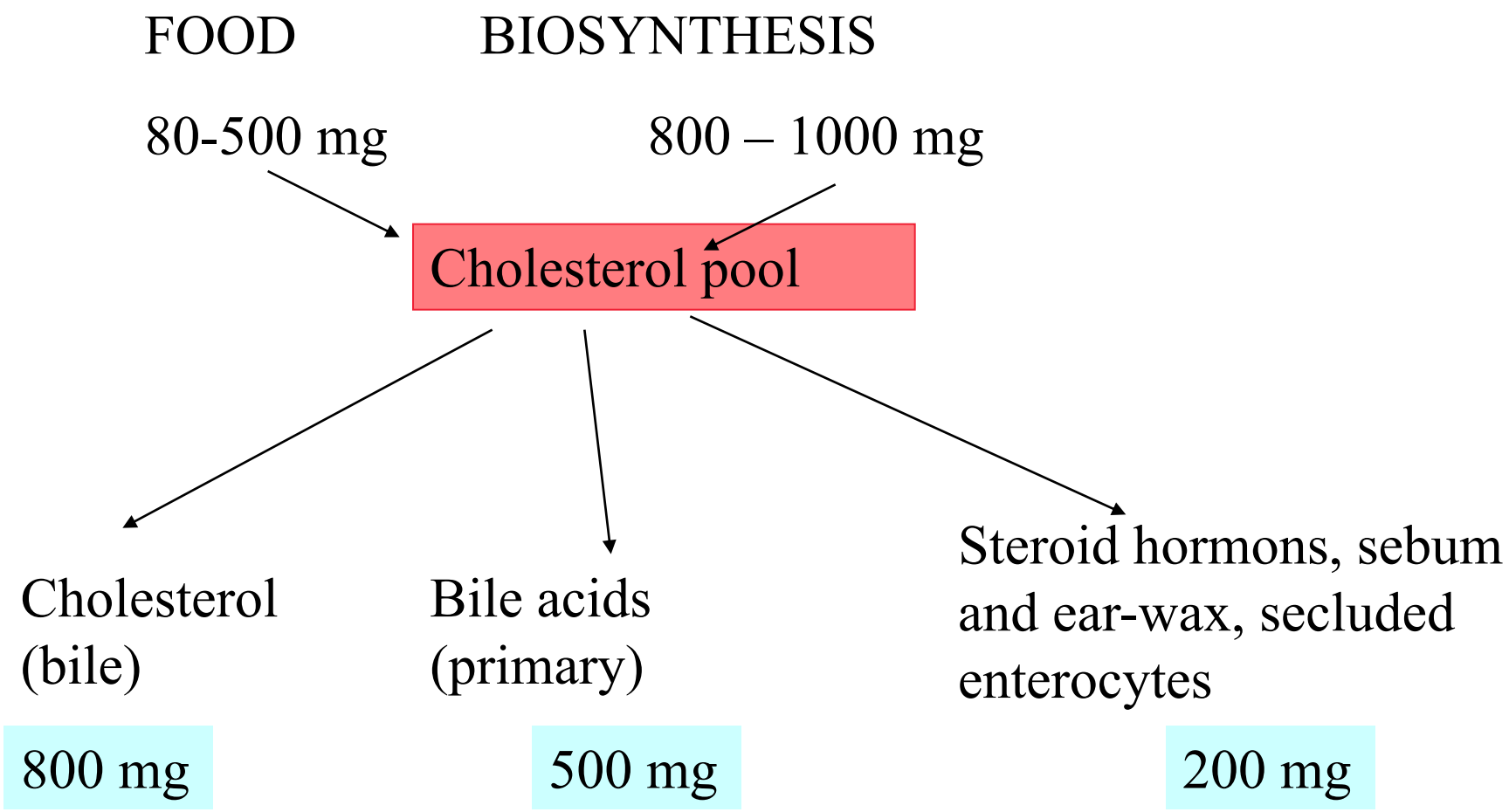
conversion to bile acids and their excretion

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

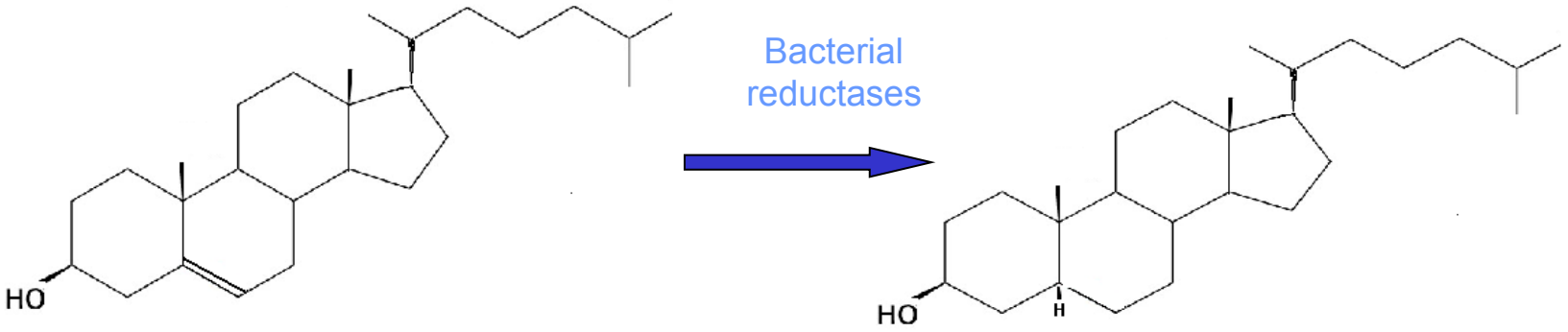
Cholesterol balance per 24 h



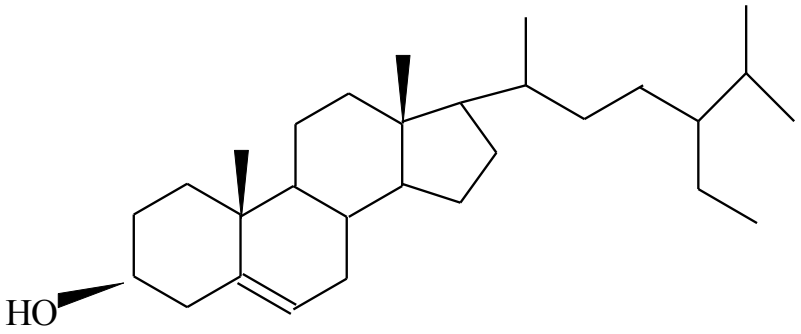
1000-1500 mg/day is excreted

Cholesterol in the gut

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



Phytosterols - sterols of plant origin



β-sitosterol

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

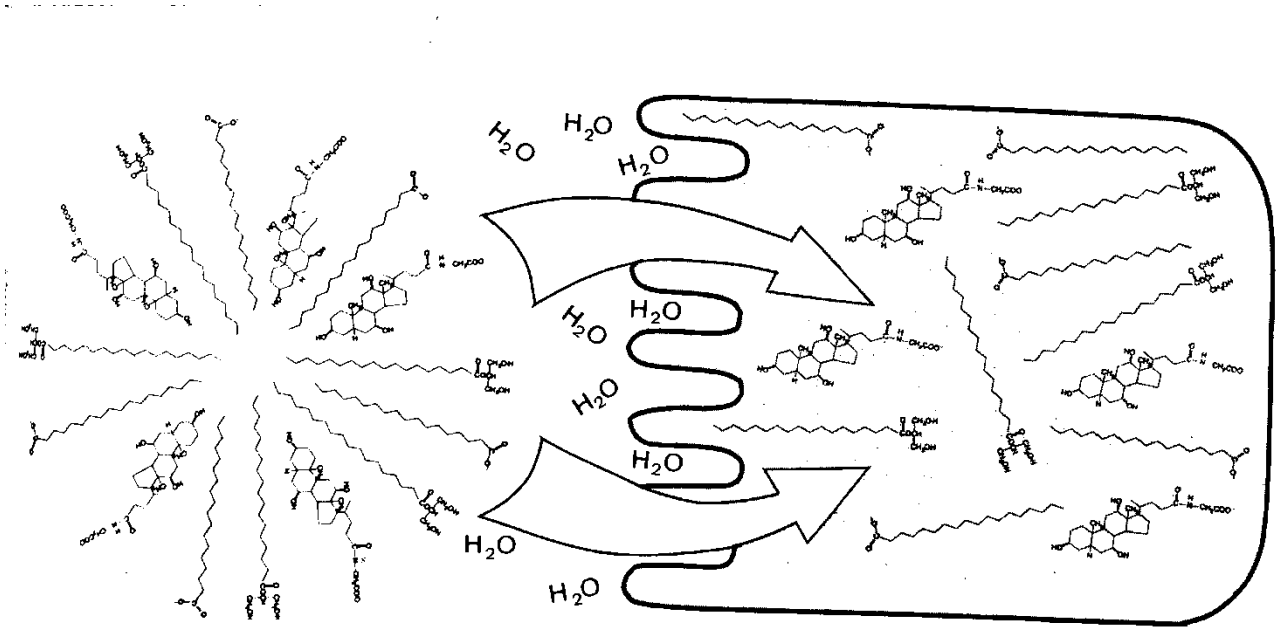
Consumption of phytosterols reduces the resorption of cholesterol.

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

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

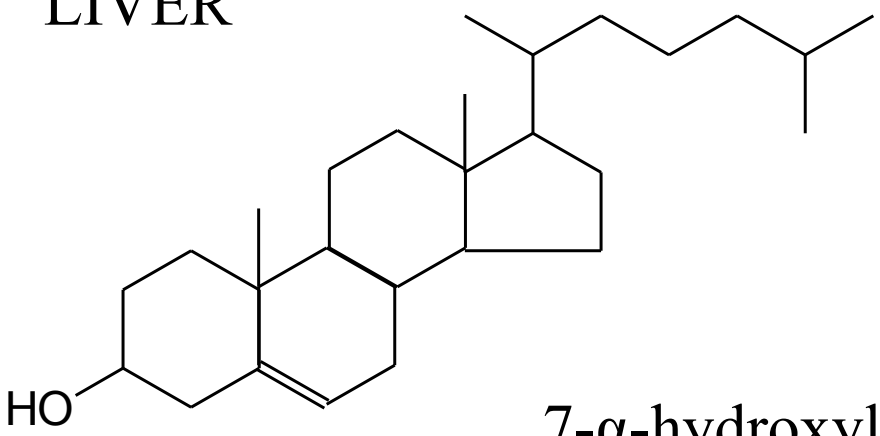
How do phytosterols function?

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

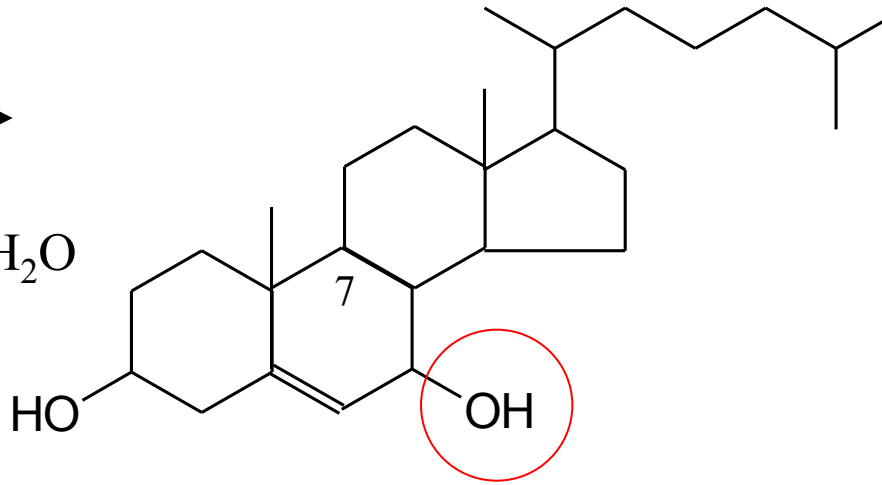


rate-limiting step
in the synthesis

7-α-hydroxylase

cytP450

NADPH, O_2 → $NADP^+$, H_2O



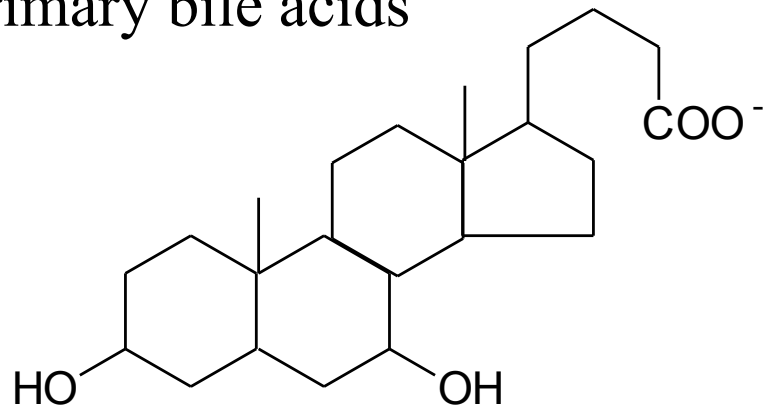
Located in ER (monooxygenase reaction)

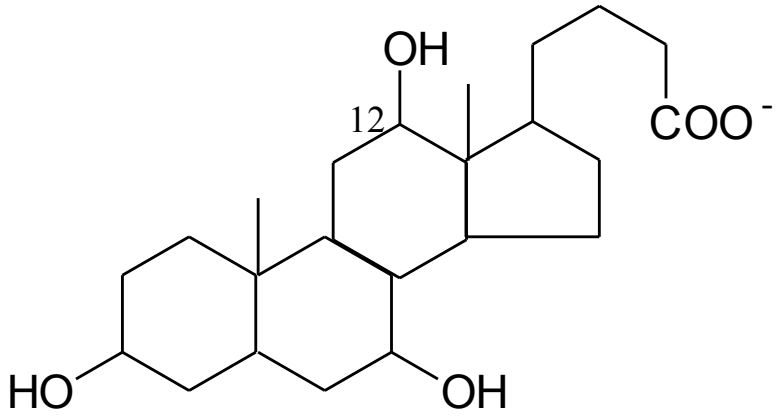
LIVER

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 α-hydroxylgroups 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



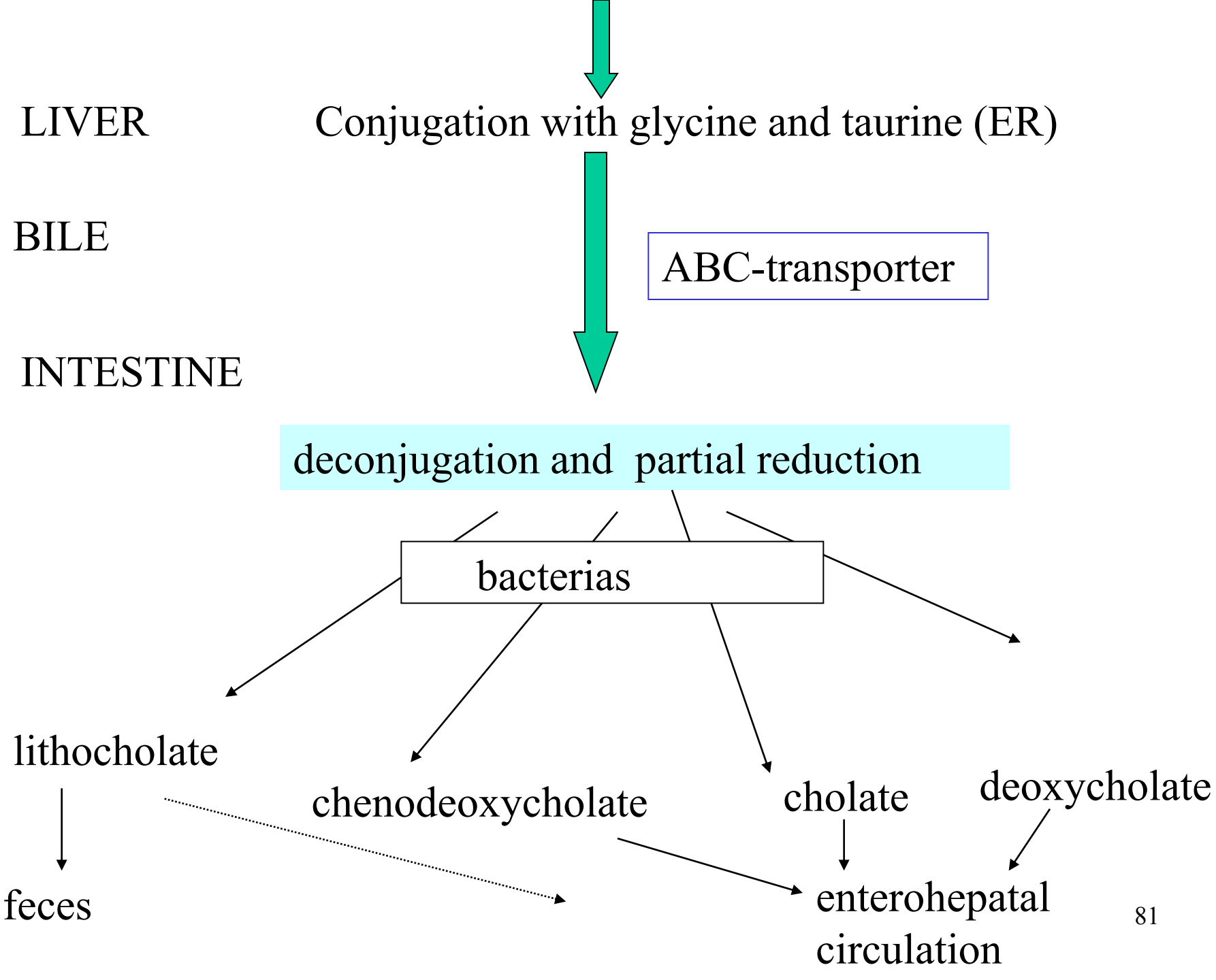


chenodeoxycholate

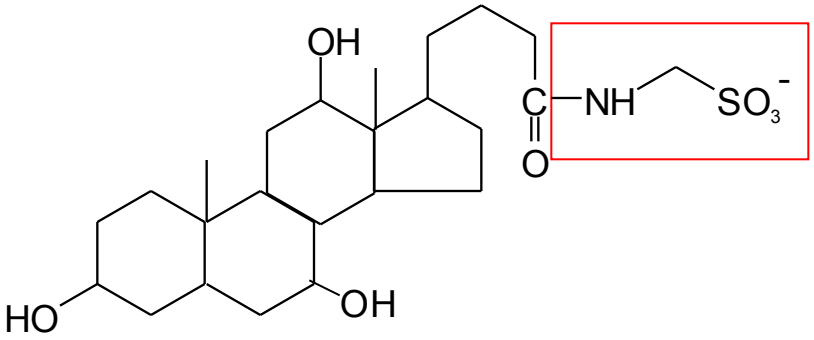
$pK_A \approx 6$

cholate

$pK_A \approx 6$

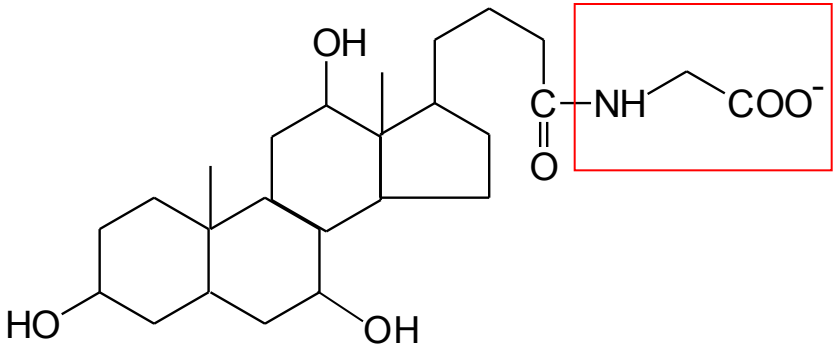

LIVER
Conjugation with glycine and taurine (ER)
BILE
ABC-transporter
INTESTINE
deconjugation and partial reduction
bacterias
lithocholate
chenodeoxycholate
cholate
deoxycholate
feces
enterohepatal circulation

Conjugated bile acids



taurocholic

$pK_A \approx 2$

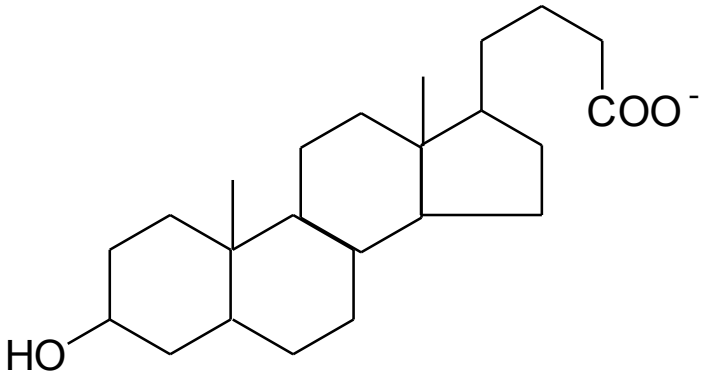


glycocholic

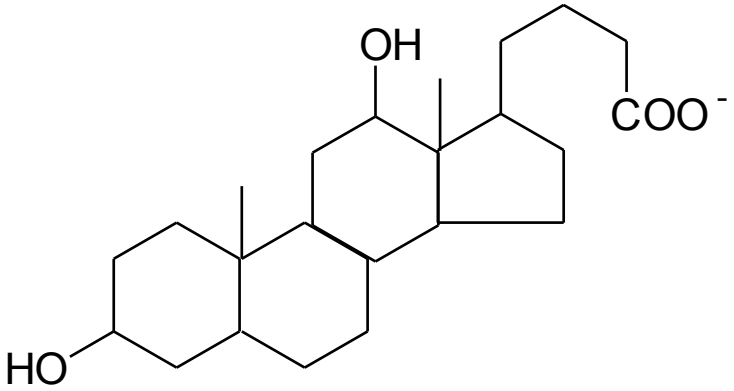
$pK_A \approx 4$

Conjugation increase 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