

## **Lipid metabolism II**

**Phospholipids and glycolipids**

**Eicosanoids**

**Cholesterol and bile acids**

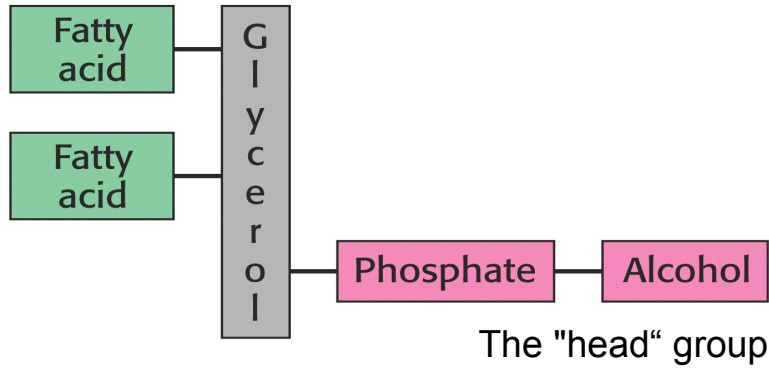
Biochemistry I

Lecture 9

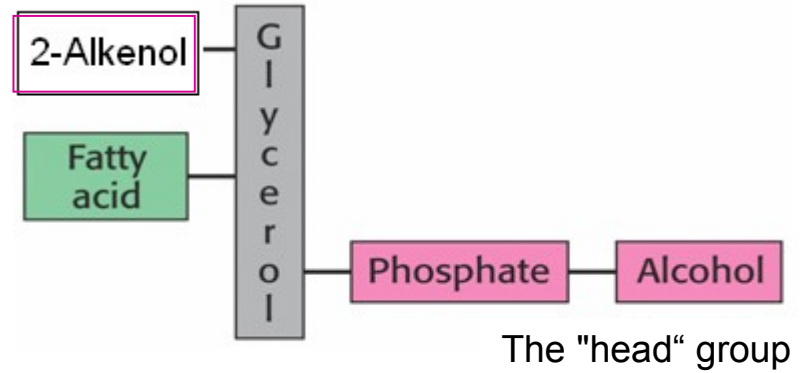
2008 (J.S.)

# Schematic structure of complex lipids

## Glycerophospholipids

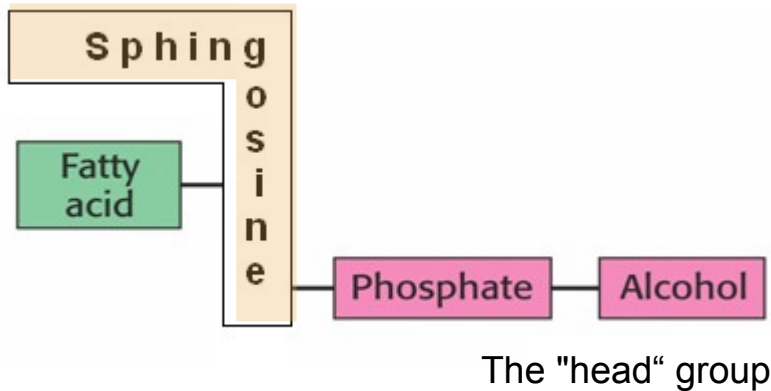


## Plasmalogens

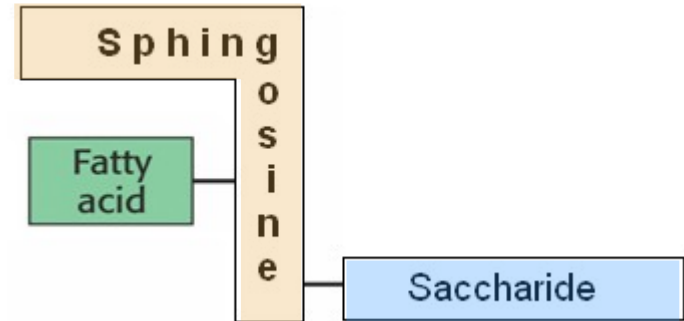


## Sphingolipids:

### Sphingophospholipids



### Glycolipids



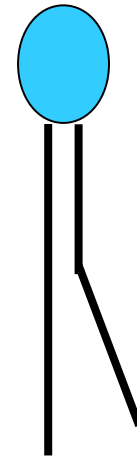
In spite of the difference in the structures of glycerophospholipids and sphingophospholipids, the over-all shape of the both types of phospholipid molecules is very similar:



**Glycerophospholipid**



**Sphingophospholipid**



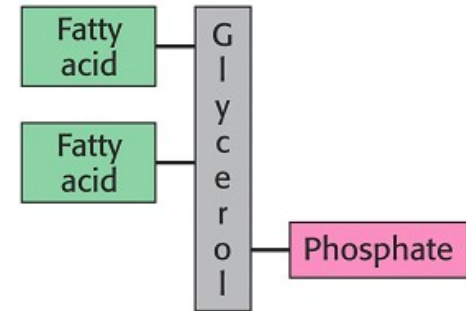
Polar head

Two hydrophobic chains

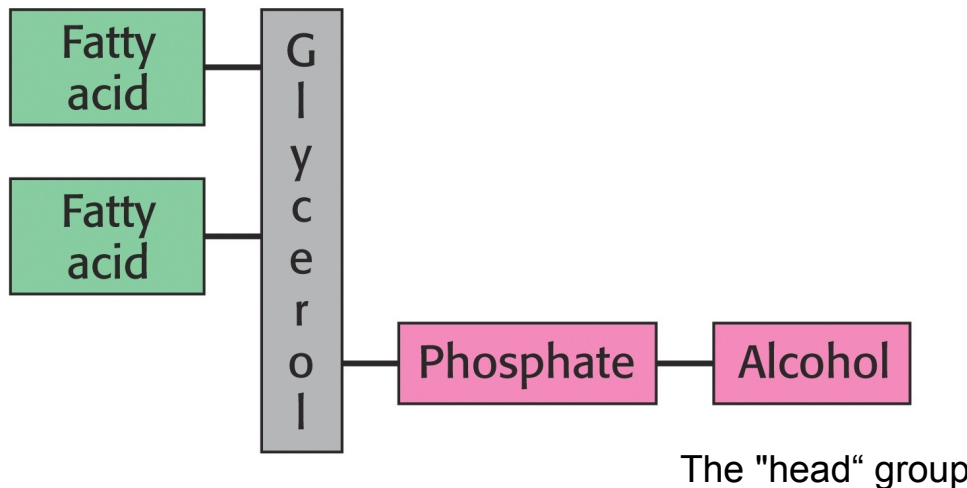
Simplified icon of a phospholipid molecule

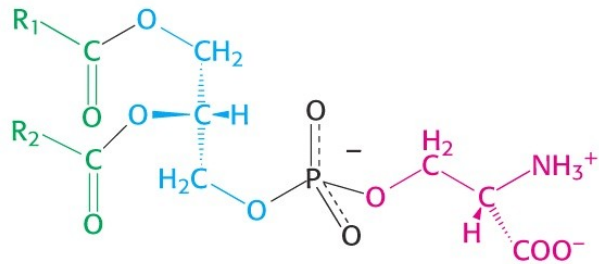
# Glycerophospholipids

The simplest glycerophospholipid is **phosphatidic acid** (phosphatidate, *sn*-1,2-diacylglycerol 3-phosphate). Only very small amounts of phosphatidate are present in membranes. However, the molecule is a key intermediate in the biosynthesis of the other glycerophospholipids.

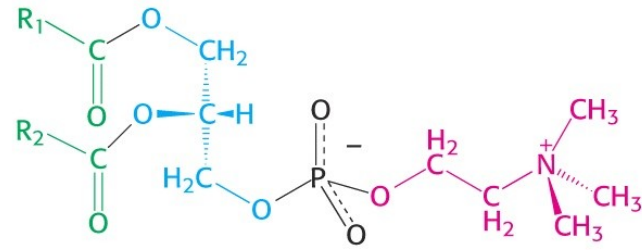


## The major glycerophospholipids

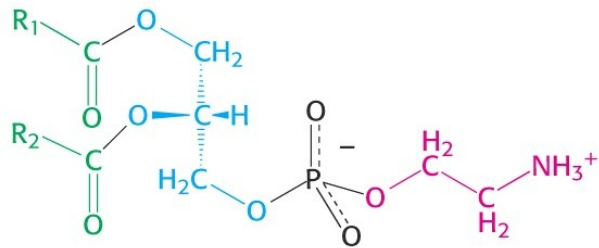




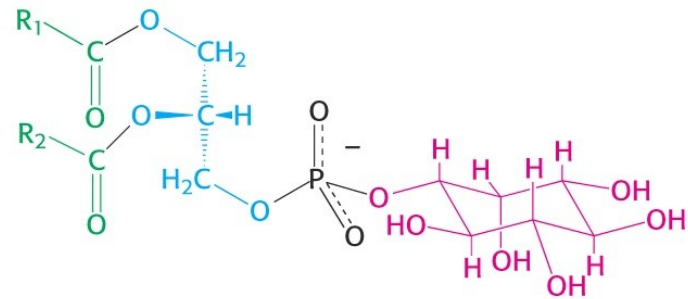
**Phosphatidyl serine**



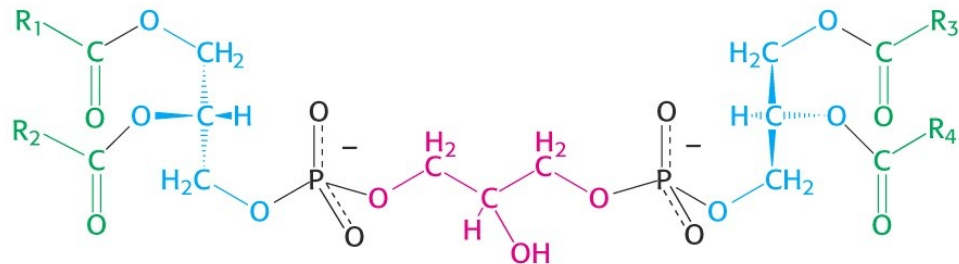
**Phosphatidyl choline**



**Phosphatidyl ethanolamine**



**Phosphatidyl inositol**



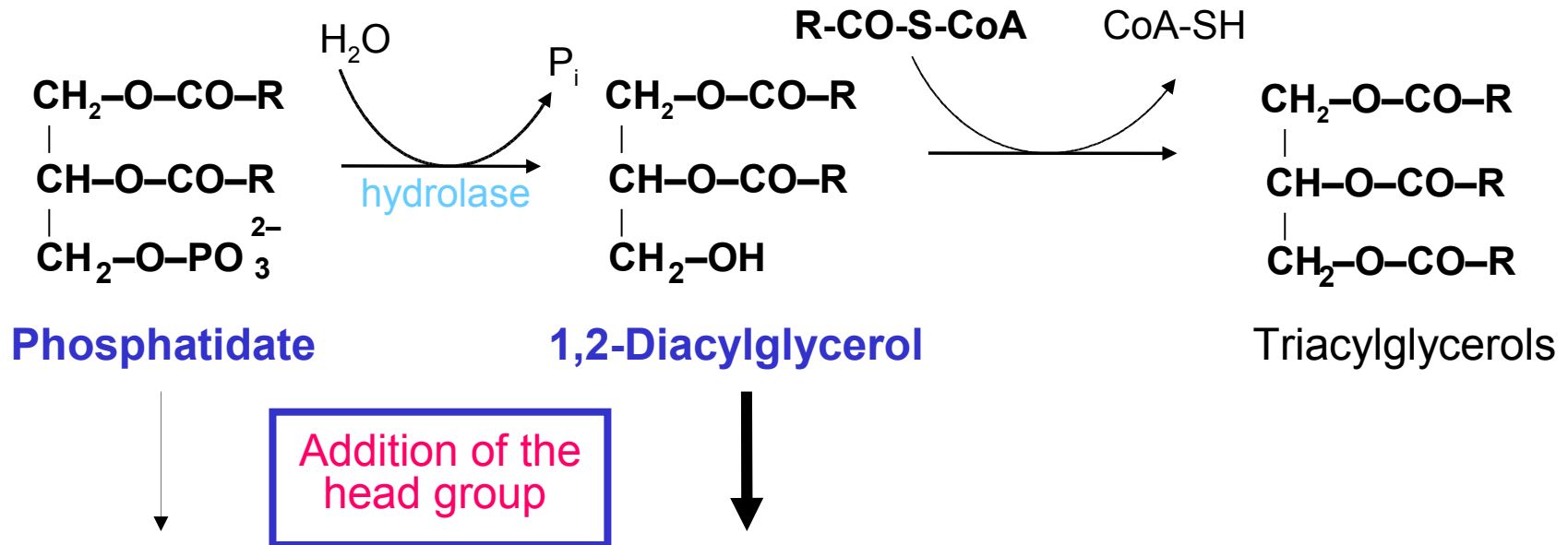
**Diphosphatidyl glycerol (cardiolipin)**

# Biosynthesis of glycerophospholipids

The synthesis is localized on the **membranes of endoplasmic reticulum**. The competent enzymes are integral membrane proteins, the active sites are accessible on the cytoplasmic side of ER.

The new molecules formed in the outer layer of ER membranes are transferred into the inner layer by the action of flippases, transported into other membranes in the form of membrane vesicles, released by means of phospholipid-transfer proteins into the cytoplasm.

The initial steps in the synthesis are similar to those of the triacylglycerol synthesis:



## GLYCEROPHOSPHOLIPIDS

There are two mechanisms of addition of the head group.

In both cases, the reaction is driven by **CTP (cytidine triphosphate)**:

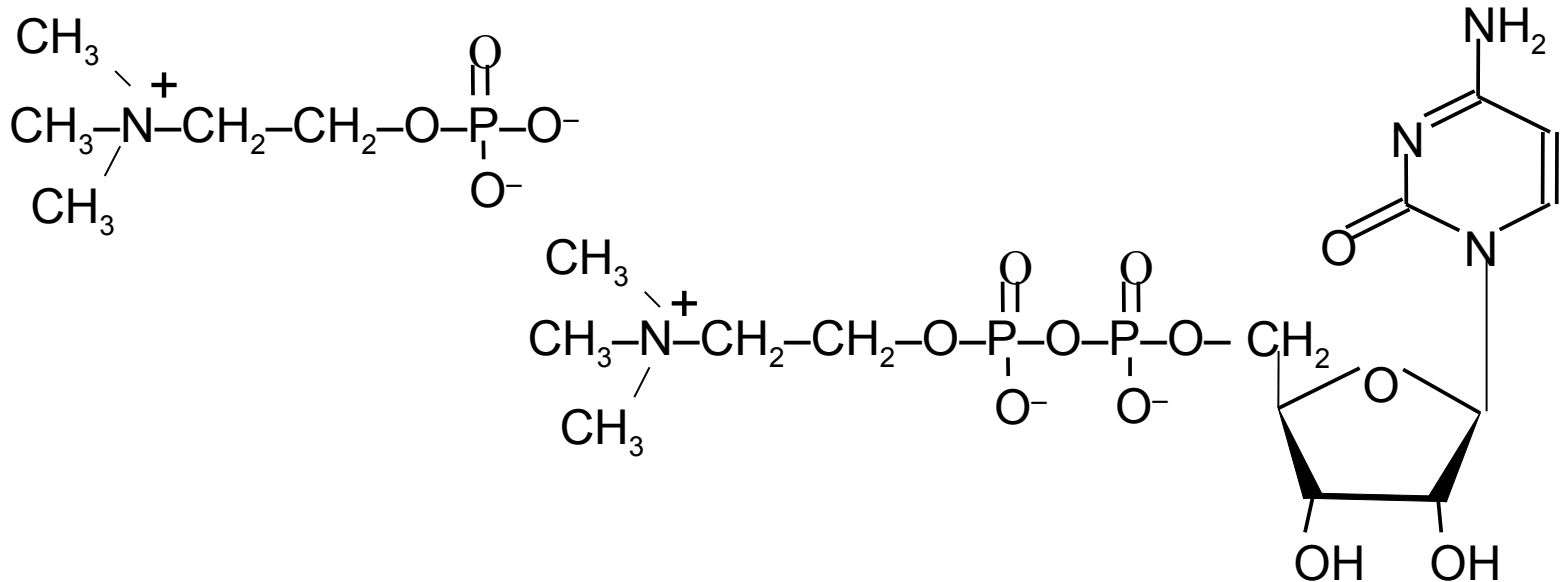
**1 – diacylglycerol** can accept **CDP-activated choline** or **ethanolamine** (synthesis of phosphatidyl choline, phosphatidyl ethanolamine, resp. phosphatidyl serine).

**2 – phosphatidate** is activated to **CDP-diacylglycerol** that can accept the head group (synthesis of phosphatidyl inositol or cardiolipin),

# 1 Synthesis of phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl serine

Diacylglycerol accepts **CDP-activated choline** or **ethanolamine**.

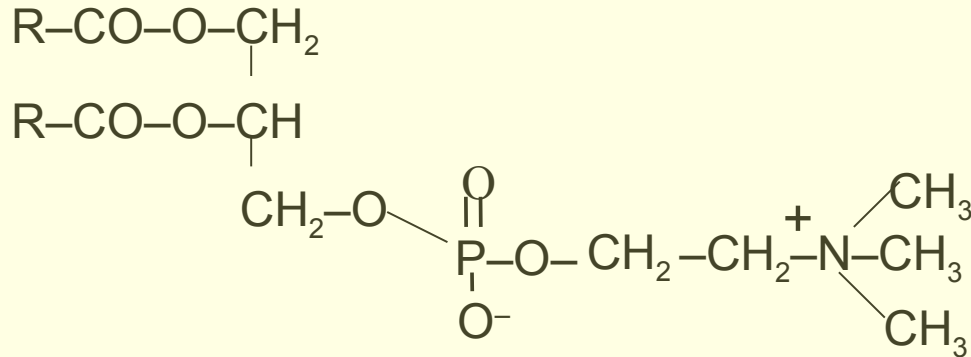
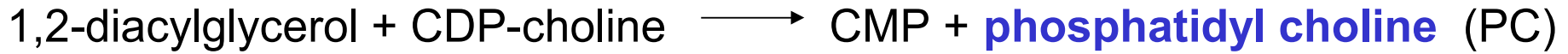
Activation of choline in two steps:



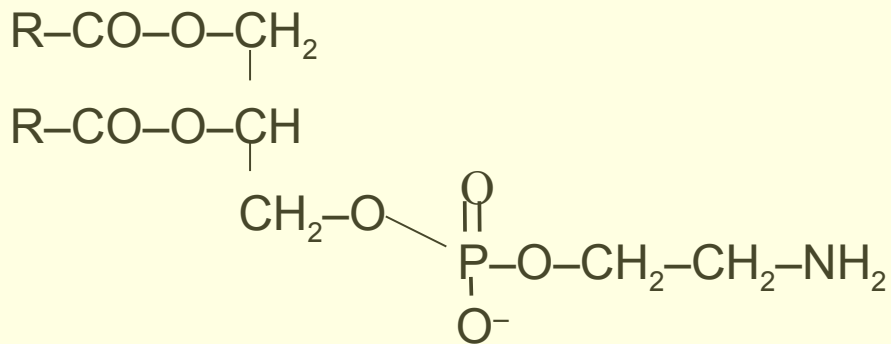
CDP-choline plays a part formally similar to that of UDP-glucose in the synthesis of glycogen.



Cytidine diphosphate (CDP) is used as a carrier, from which **choline phosphate is transferred**, the acceptor being a 1,2-diacylglycerol.

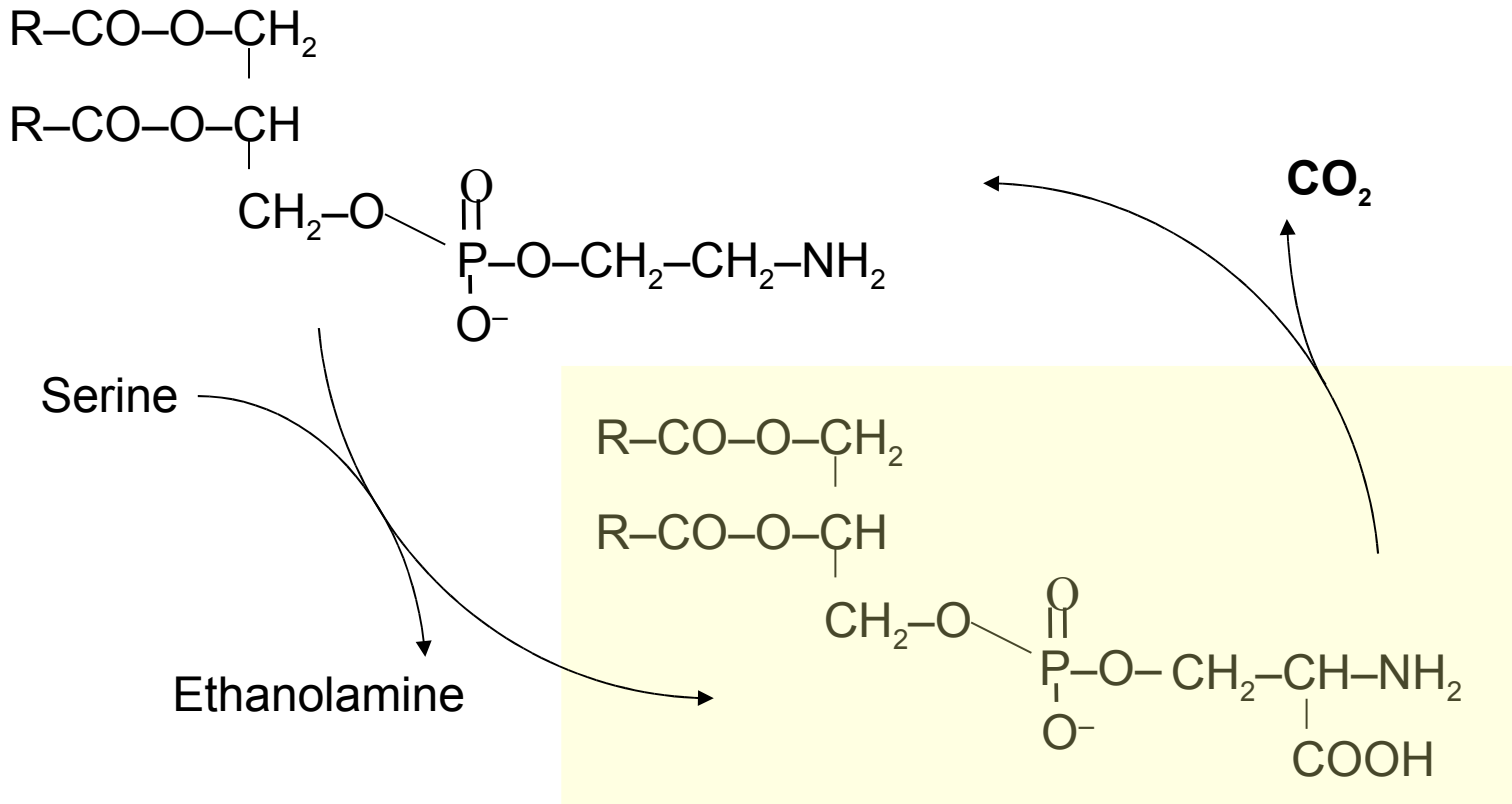


The biosynthesis of **phosphatidyl ethanolamine (PE)** is similar.



**N-Methylation of PE** (in the liver, the donor of methyl group is S-adenosylmethionine) to give PC is not as important in higher animals as incorporation of choline *de novo*.

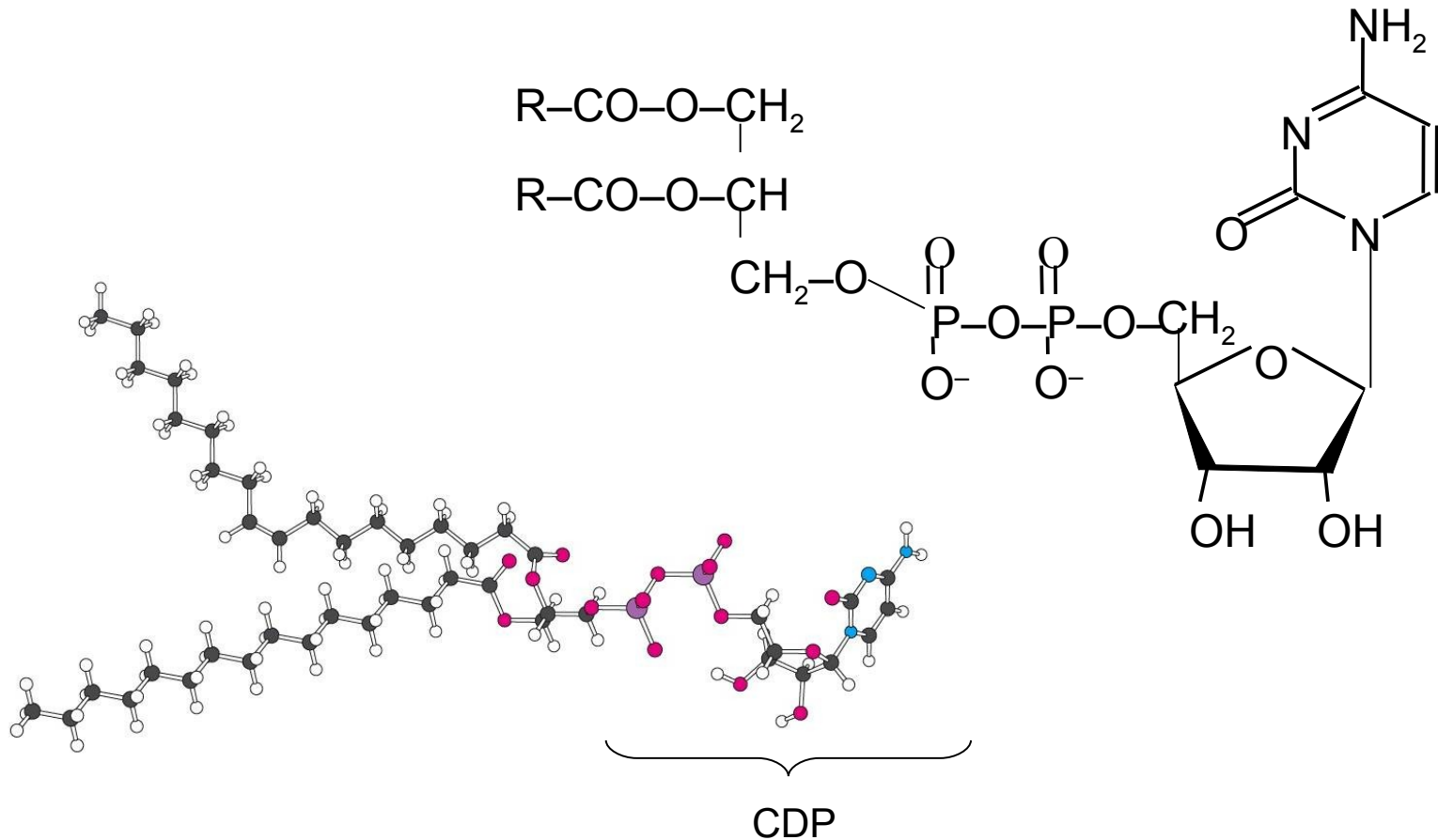
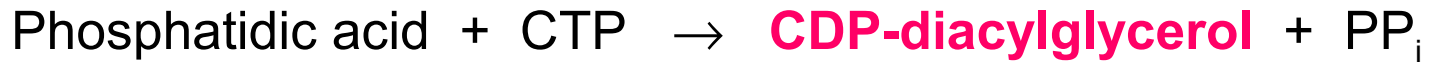
**Phosphatidyl serine** (PS) is not, in animals, formed directly in this way, but as exchange of serine for the ethanolamine of PE:



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

## 2 Synthesis of phosphatidyl inositol and cardiolipin

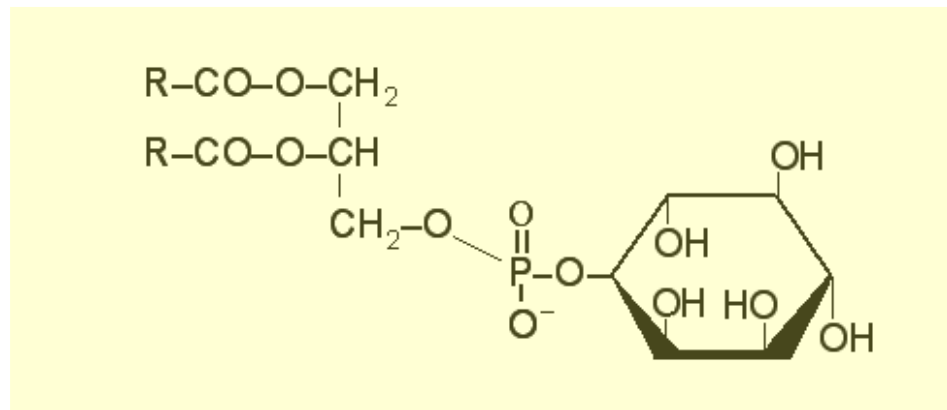
Phosphatidic acid is activated in a reaction with CTP to **CDP-diacylglycerol**:



CDP-Diacylglycerol then reacts with **free inositol** to give phosphatidyl inositol (PI), or with **glycerol phosphate** to form phosphatidyl glycerol (PG), resp.

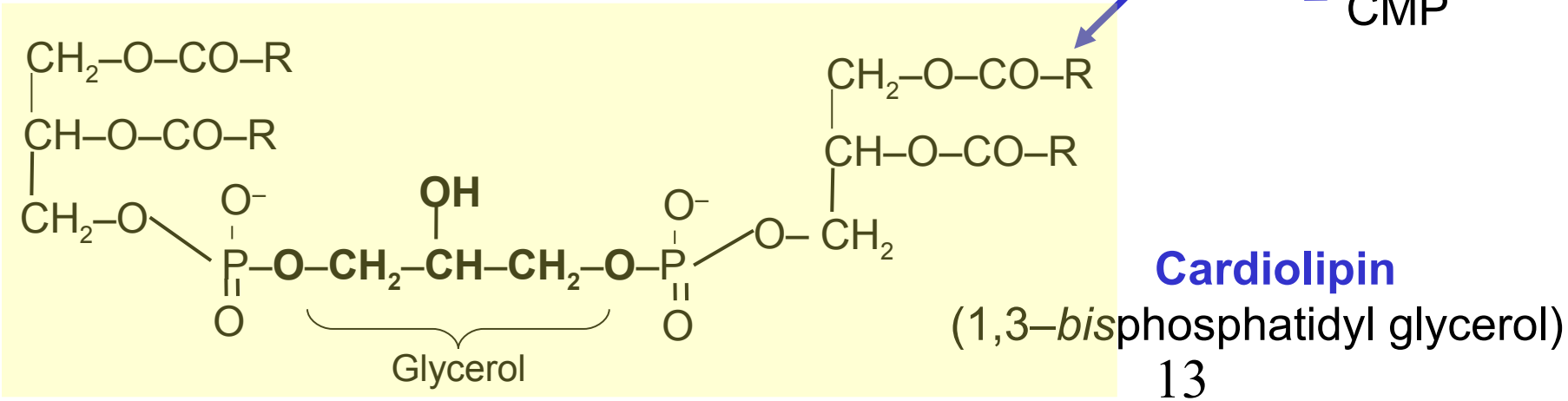
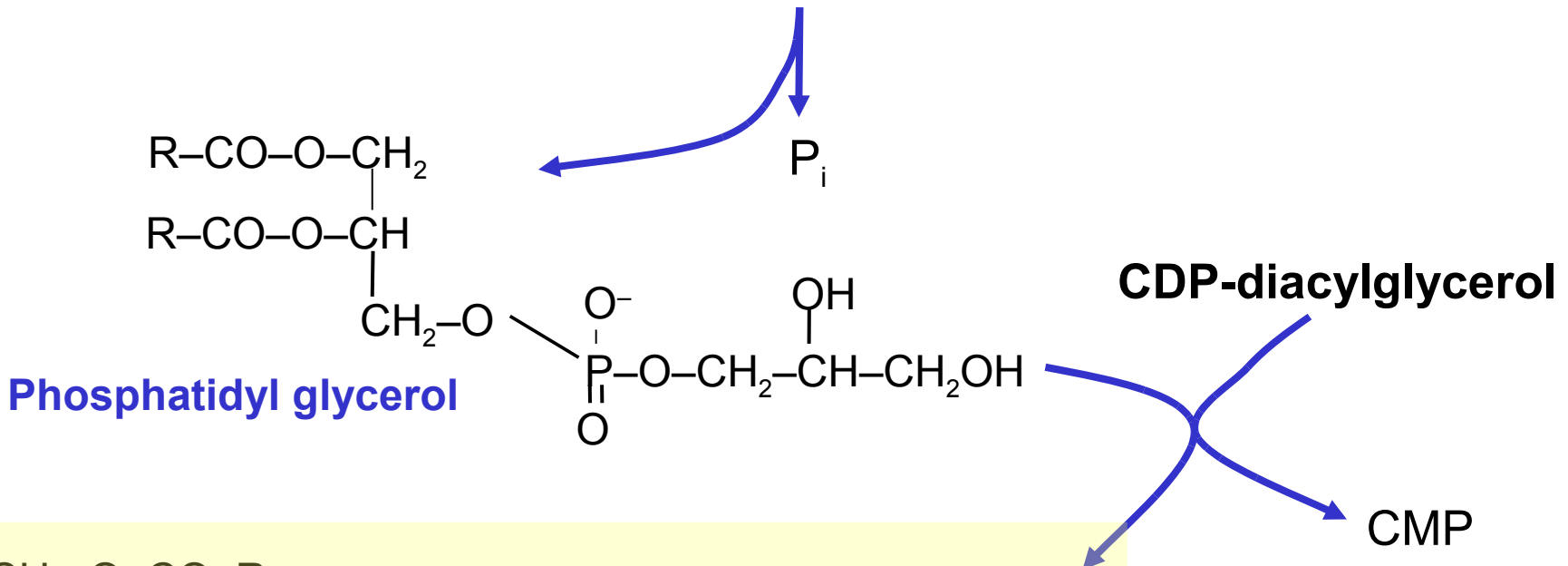
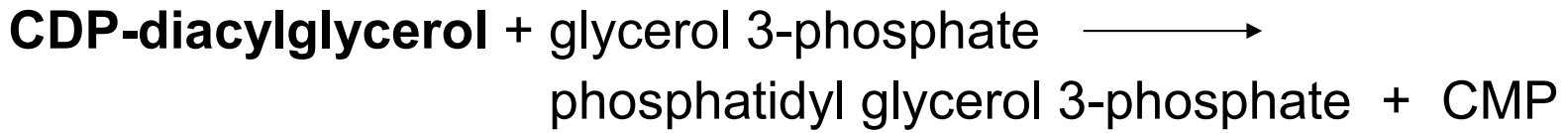
### Phosphatidyl inositol:

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



Further phosphorylations of PI generate phosphatidyl inositol *bis*phosphate (**PIP<sub>2</sub>**) which is an intermediate of the phosphatidyl inositol cycle generating important intracellular messengers IP<sub>3</sub> and diacylglycerol.

# Cardiolipin (constituent of the inner mitochondrial membrane)



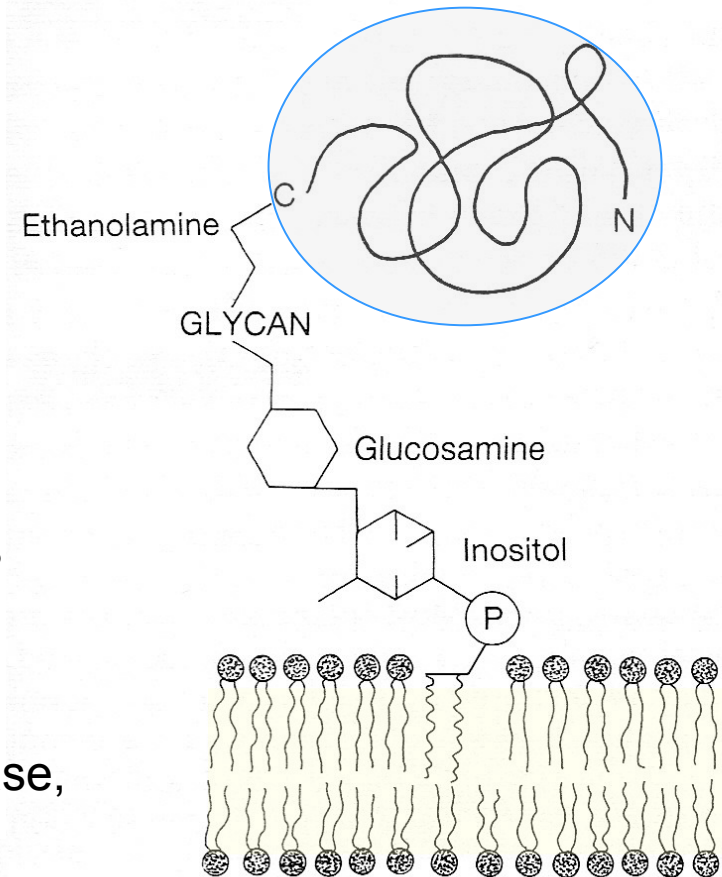
## Glycerophospholipids

are

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

## Anchoring of proteins to membrane

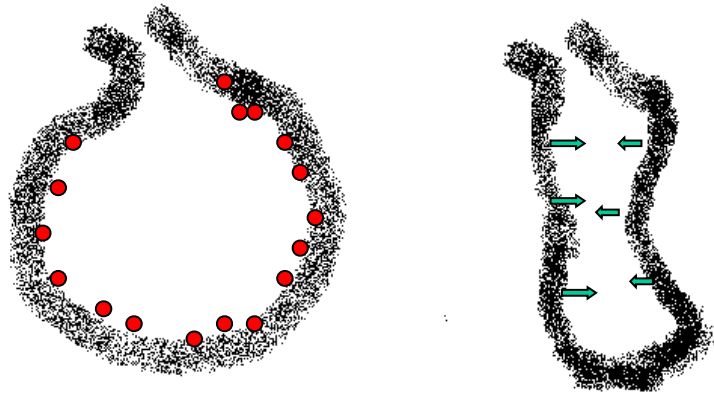
The linkage between the COOH-terminus of a protein and phosphatidylinositol fixed in the membrane lipidic dilayer exist in several ectoenzymes (alkaline phosphatase, acetylcholinesterase, some antigens).



## Lung surfactant

The major component of lung surfactant 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.



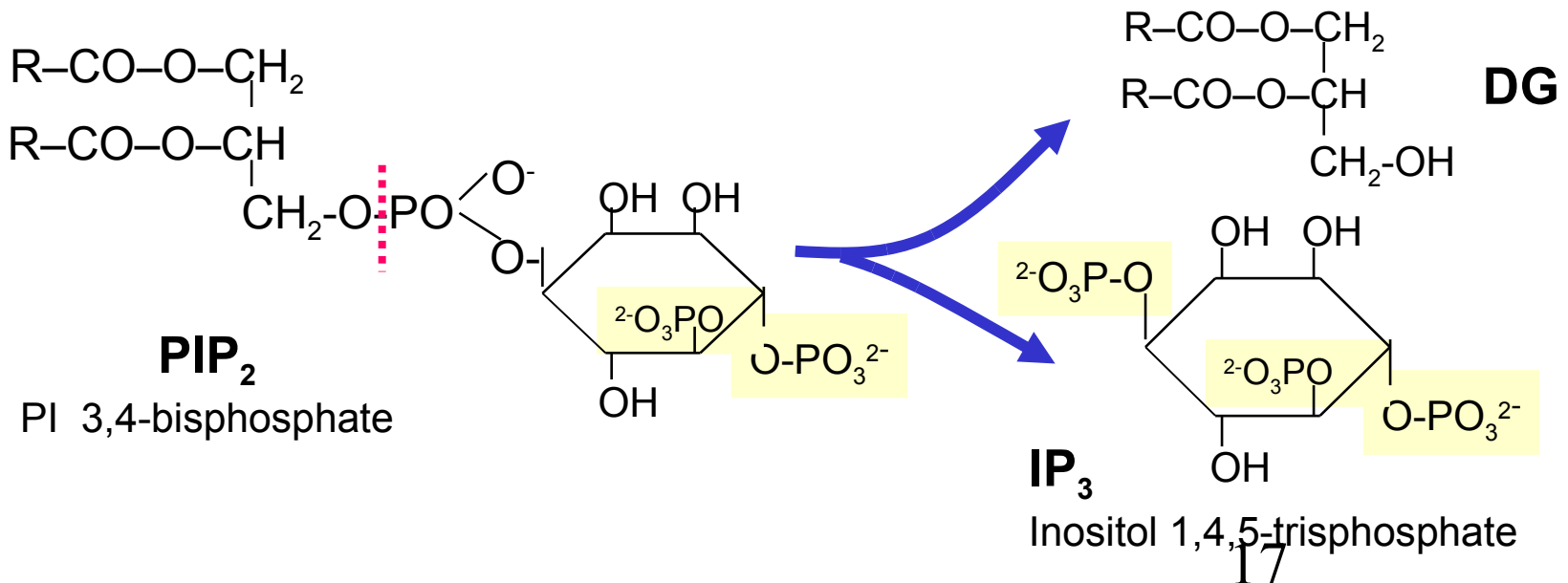
## Phosphatidyl inositol phosphates

(PIP, PIP<sub>2</sub>, PIP<sub>3</sub>) are minor components of plasma membranes, and their turnover is stimulated by certain hormones.

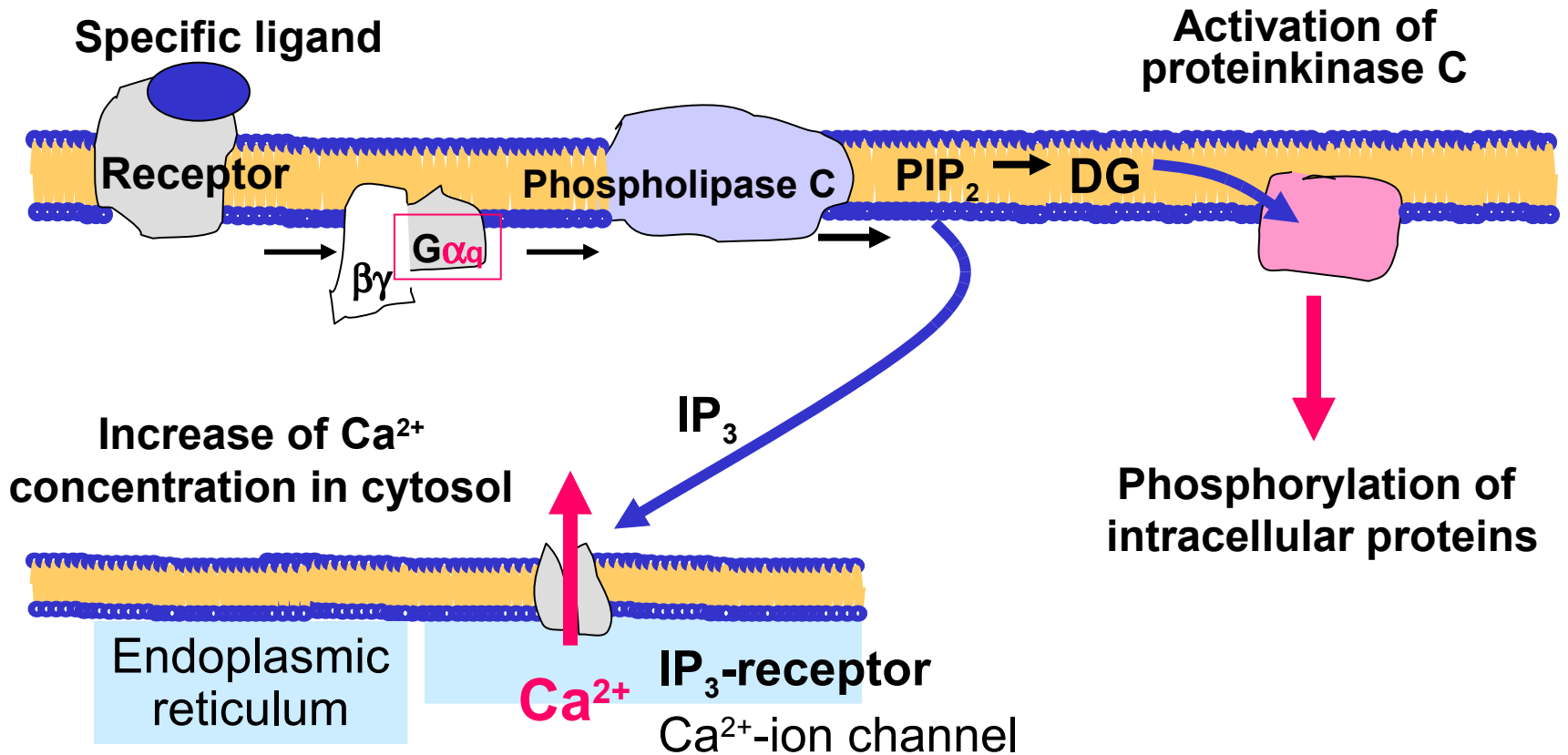
A specific phospholipase C, under hormonal control, hydrolyses phosphatidyl 4,5-bisphosphate (PIP<sub>2</sub>) to **diacylglycerol** and **inositol 1,4,5-trisphosphate** (IP<sub>3</sub>), both of which have **second messenger functions**.

### Second messengers

are intracellular compounds the concentration of which raises as a consequence of binding of the hormone or the neurotransmitter to the membrane receptor. The hormone-receptor complex controls the synthesis (or release) of the second messenger and this control is mediated by a third type of protein, called G-protein.



# Phosphatidyl inositol cascade

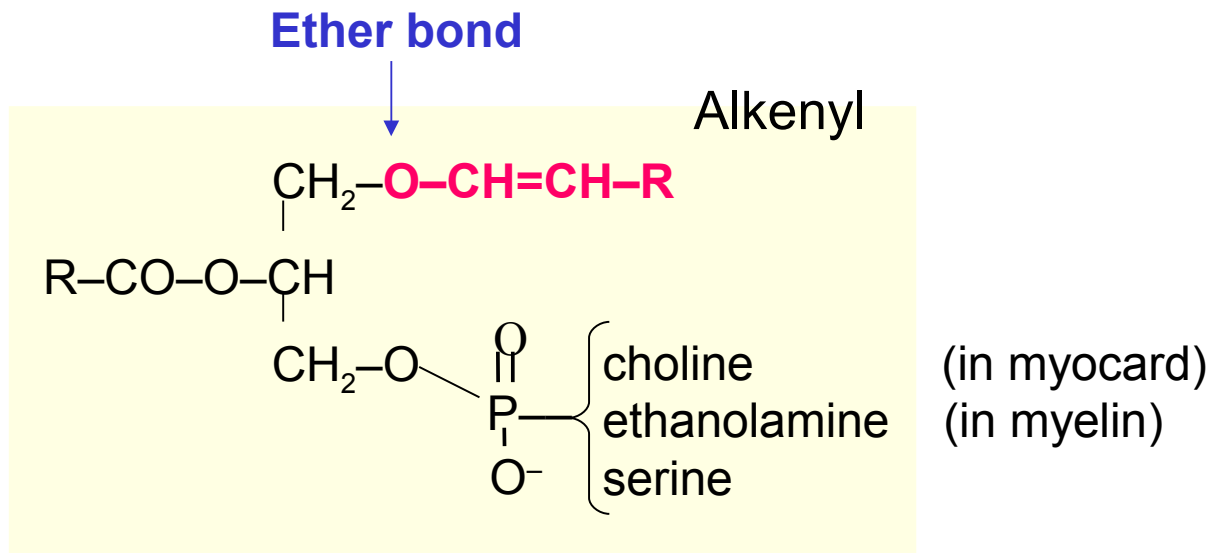


# Plasmalogens

are modified glycerophospholipids – called alkoxylipids or ether glycerophospholipids.

Plasmalogens represent about 20 % of glycerophospholipids.

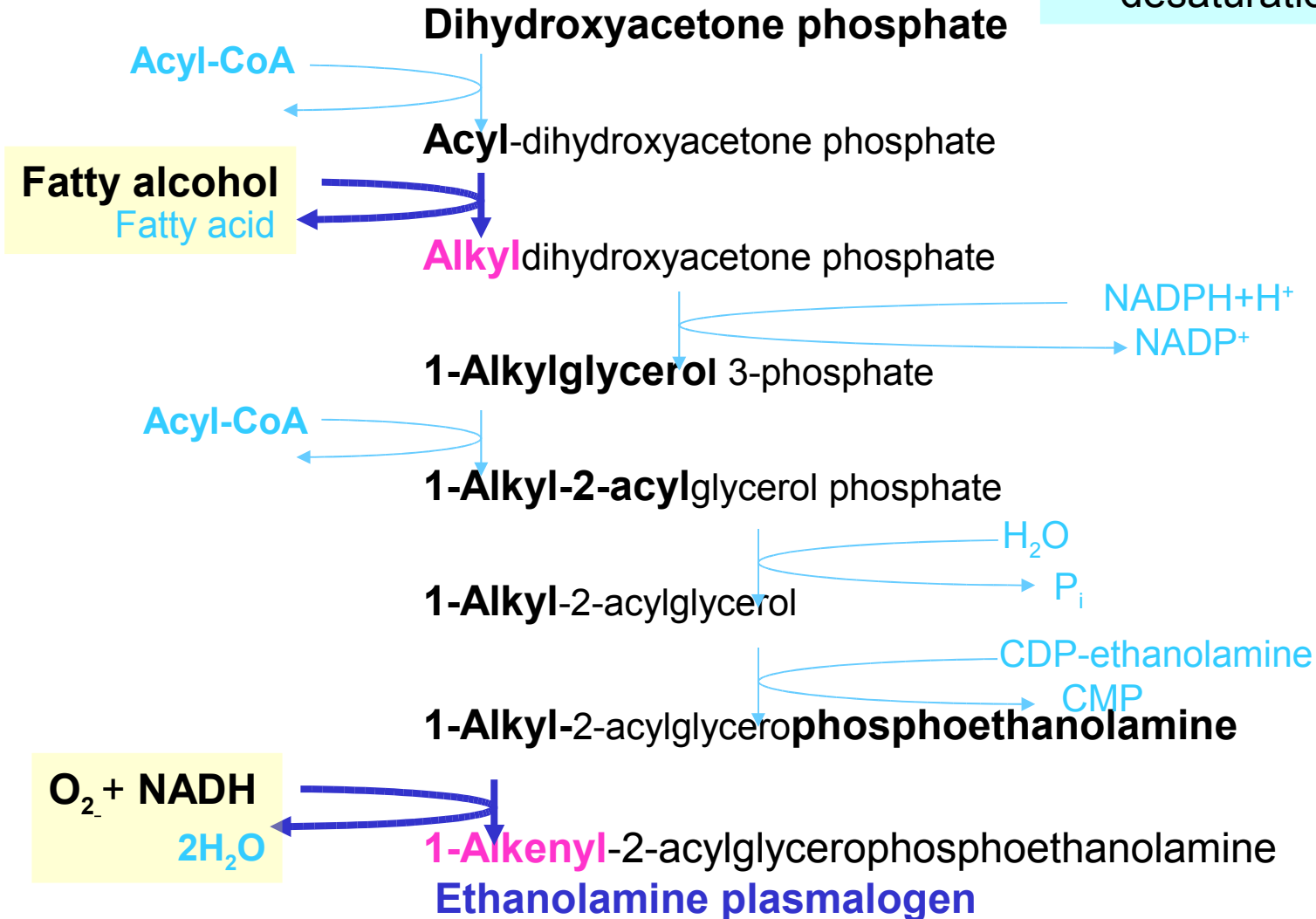
Choline plasmalogen is found in myocard, in the liver (~1 %), and ethanolamine plasmalogen in myelin (~ 23 %).



# Synthesis of plasmalogens

(ether glycerophospholipids, alkoxylipids)

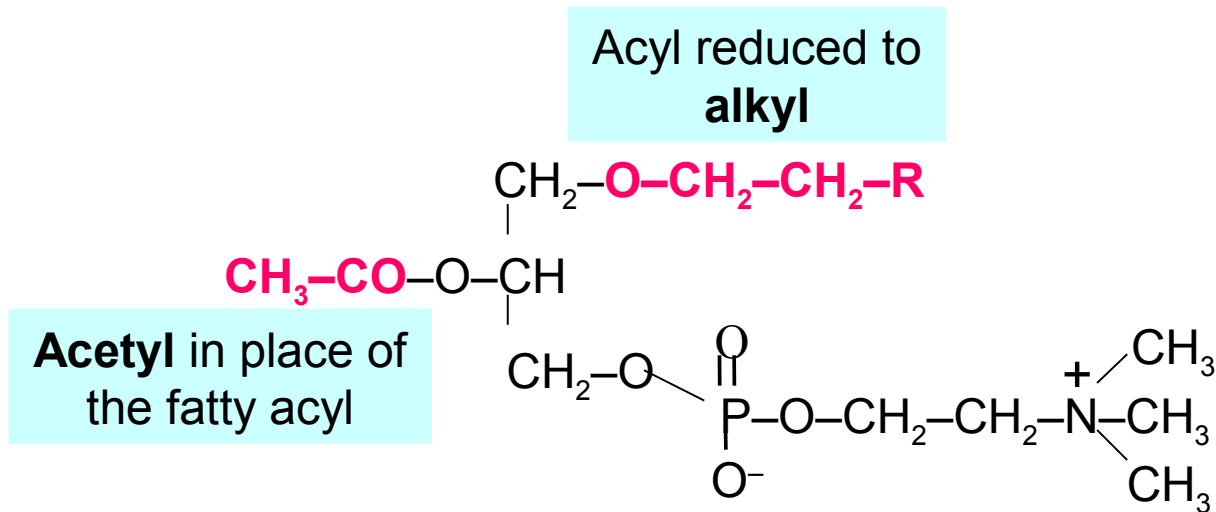
Exchange of the acyl for an alcohol and the desaturation of it



## PAF (platelet activating factor)

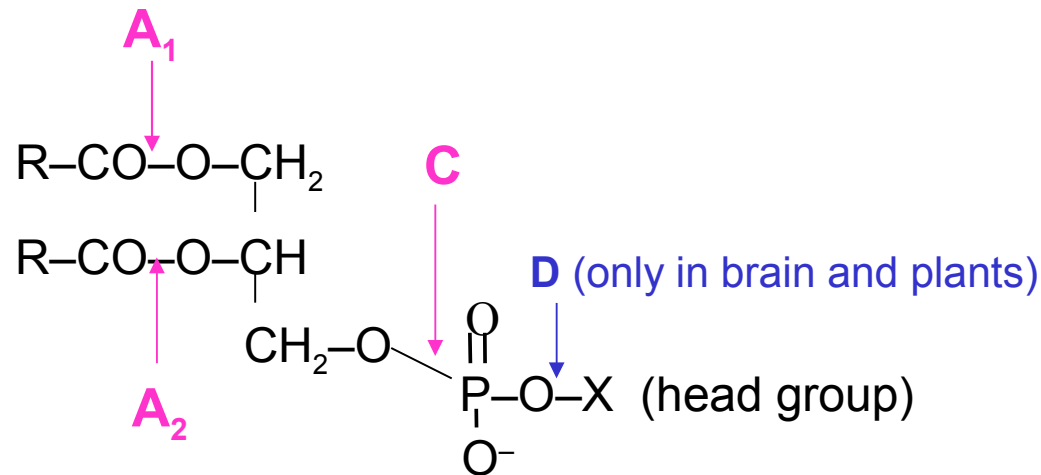
is an unusual alkoxy lipid in which the alkenyl group of plasmalogens was reduced to saturated **alkyl** and the fatty acyl at position 2 was exchanged for **acetyl**.

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.



# 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:



Phospholipase A<sub>1</sub> (PL A<sub>1</sub>) exhibits preference for phosphatidyl ethanolamines.

Phospholipase A<sub>2</sub> obviously prefers phosphatidyl cholines and is of special importance because it liberates arachidonic acid as a precursor of eicosanoids.

Either PL A<sub>1</sub> or A<sub>2</sub> set free only **one acyl residue** and leaves a **lysophospholipid** which is not further attacked by either enzyme.

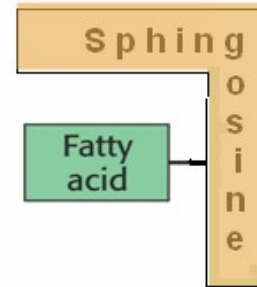
The remaining acyl group is removed by the action of **lysophospholipase-transacylase** (formerly called phospholipase B).

The enzyme removes the **remaining acyl group** from the lysophospholipid, and transfers it either to water (hydrolysis), or to a second lysophospholipid (transacylation).

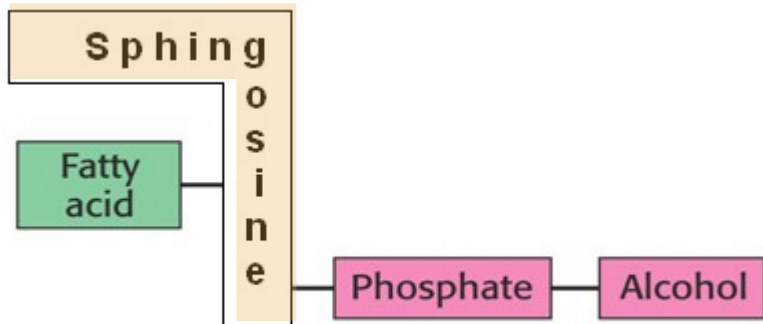
Phospholipase C is stimulated by some hormonal signals and some neurotransmitters. It hydrolyses PIP<sub>2</sub> to IP<sub>3</sub> and DG – the crucial step in phosphatidyl inositol cascade.

# Sphingolipids – schematic structure

**Ceramide**  
*N*-Acylsphingosine

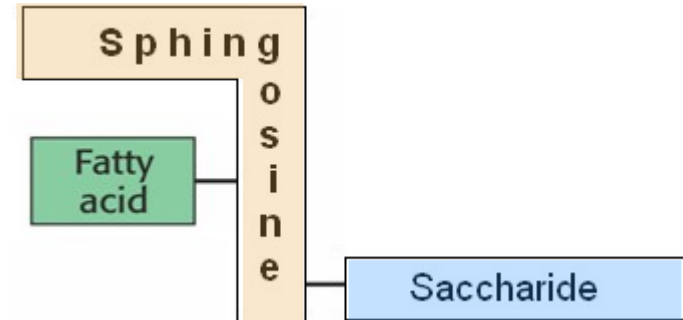


**A sphingophospholipid**



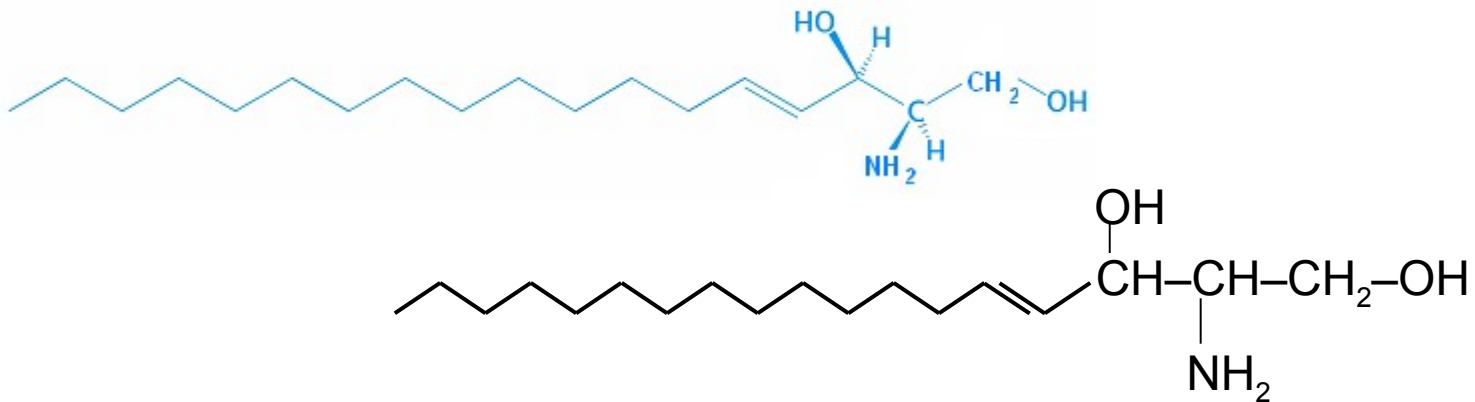
The "head" group

**A glycolipid**

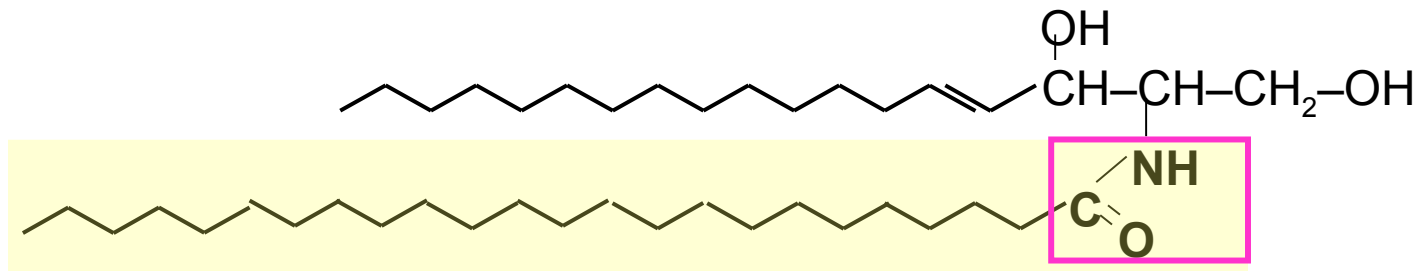




**Sphingosine** contains 18 carbon atoms, *trans*-double bond in position 4, amino group at position 2, and two hydroxyls at position 1 and 3. Its alternative name is 4-sphingenine (syst. **2-aminooctadec-4-ene-1,3-diol**).



**Ceramides** are ***N*-acylated sphingosines**. The acyl residue is attached to the amino group of sphingosine by an **amide link**:

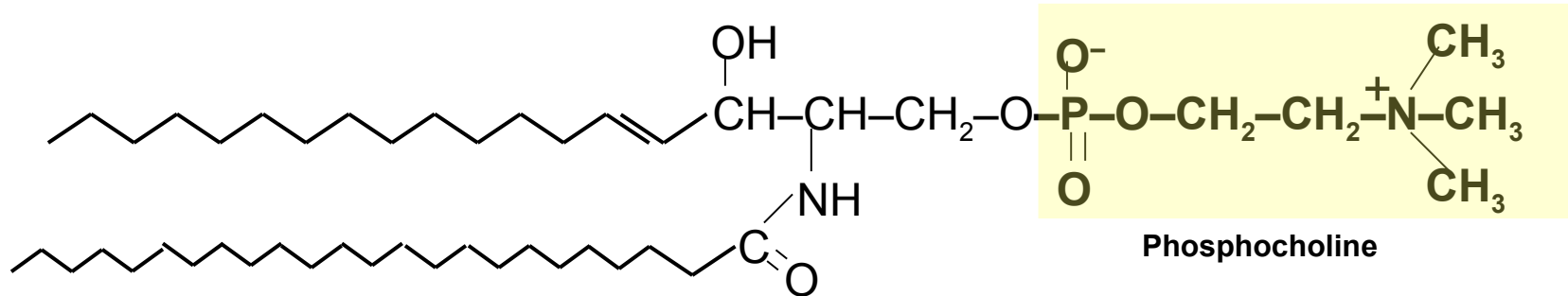


The acyl residue has often 24 carbon atoms (lignoceric acid and its derivatives).

**Sphingolipids** Ceramide is the lipidic part of all types of sphingolipids.

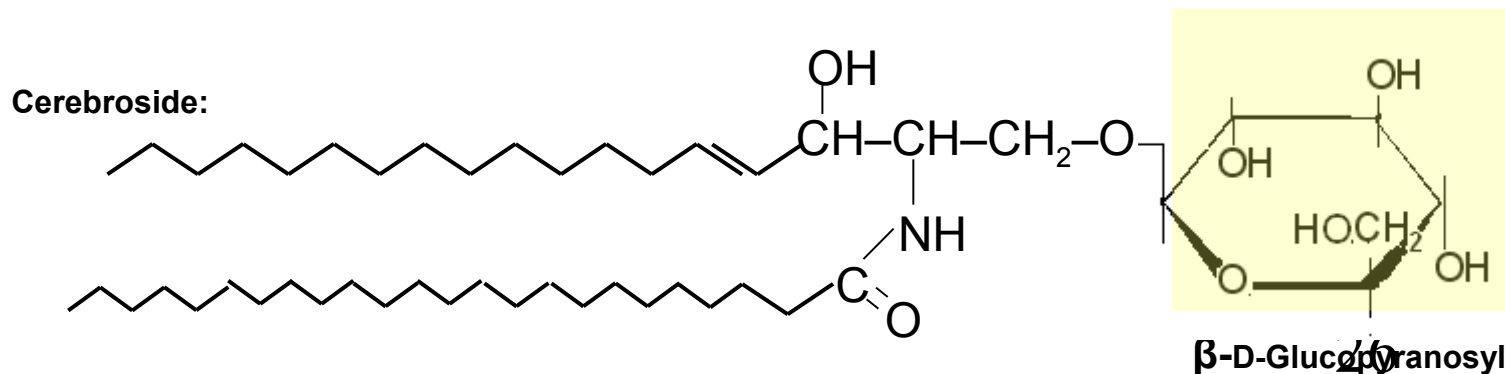
## **Sphingophospholipids**

are esters of ceramide-1-phosphate and ethanolamine or (mostly) choline. Ceramidephosphocholines are called **sphingomyelins**.



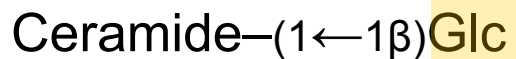
## **Glycolipids**

are ceramides to which a saccharidic component is attached by glycosidic bond: monoglycosylceramides – **cerebrosides**, oligoglycosylceramides, acidic sulphoglycosylceramides, and sialoglycosylceramides – **gangliosides**.

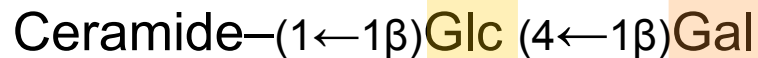


## Saccharidic components of glycolipids - examples:

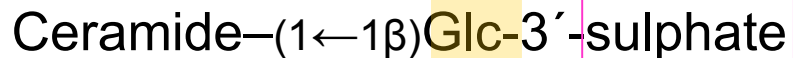
**Cerebroside**



**Oligoglycosylceramide**

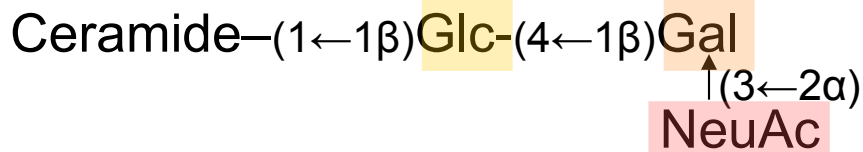


**Sulphoglycosphingolipid**

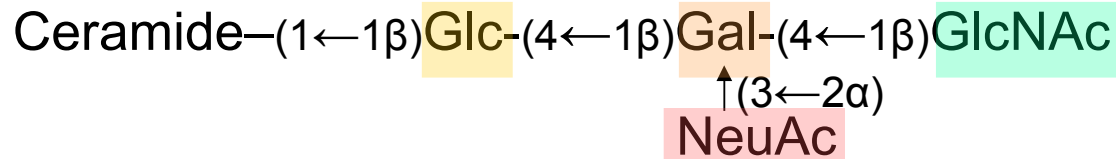


## Gangliosides

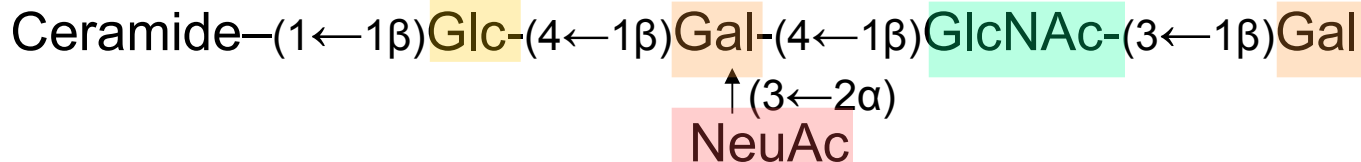
$G_{M3}$  (monosialo ganglioside type III)



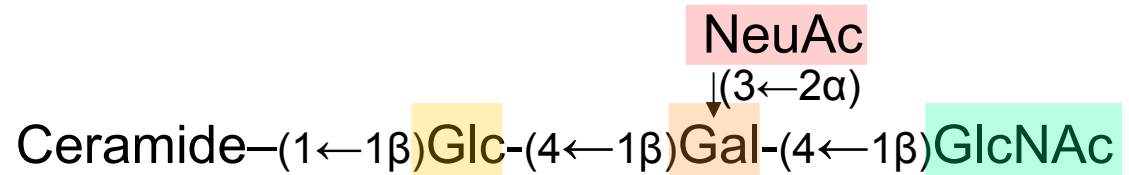
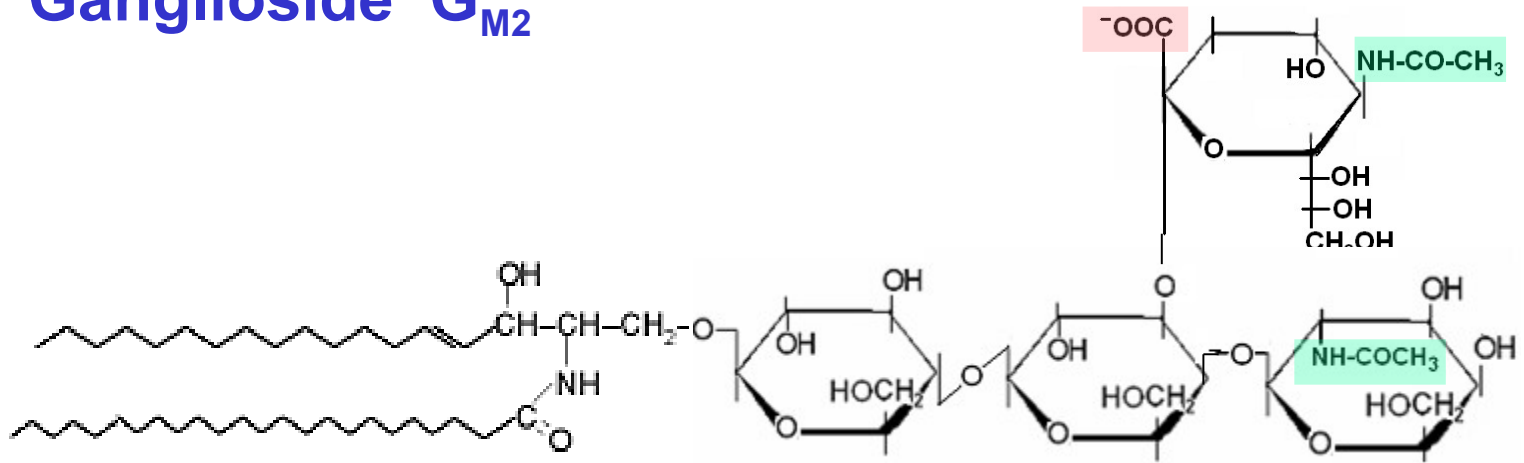
$G_{M2}$



$G_{M1}$

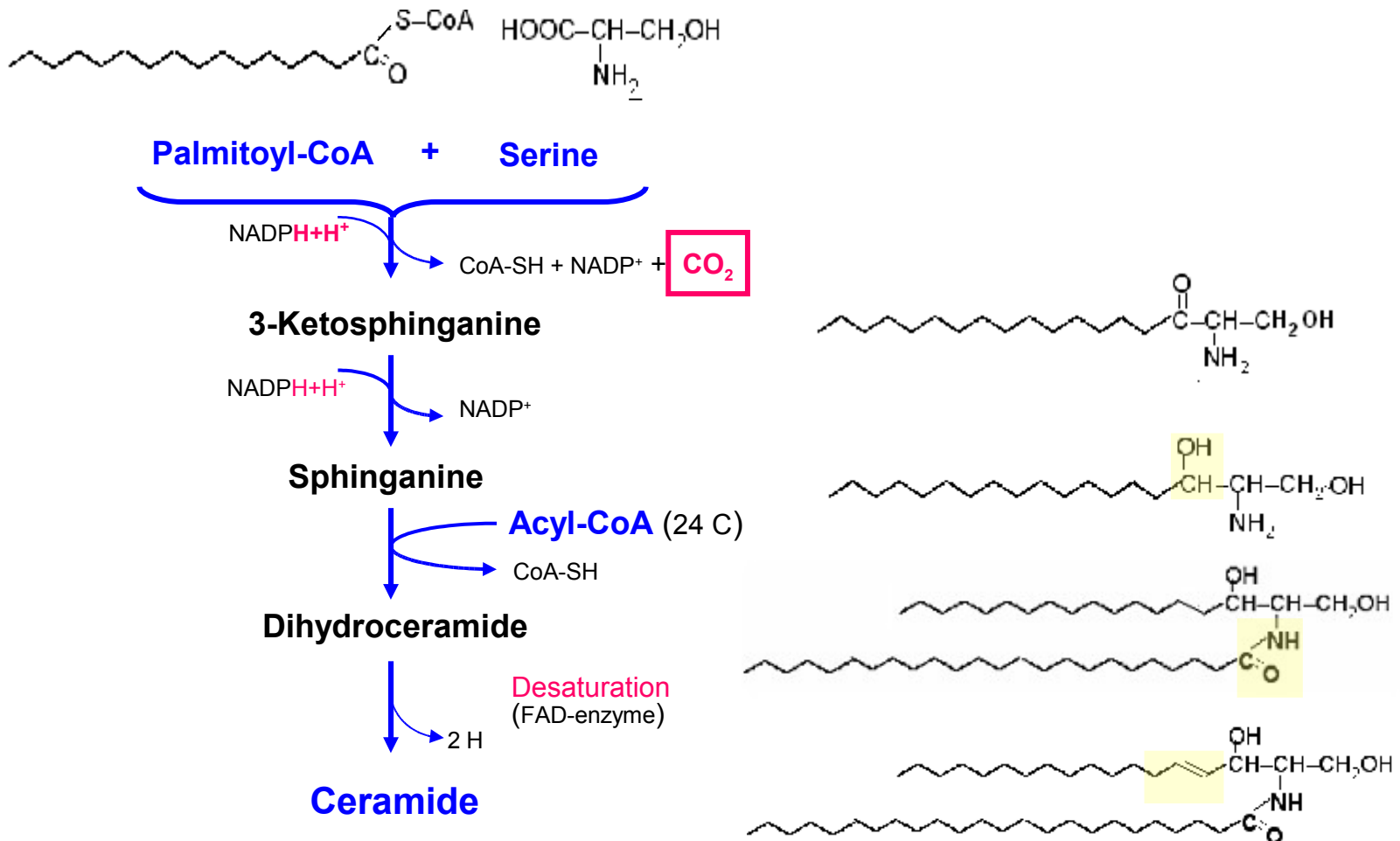


# Ganglioside G<sub>M2</sub>



# Biosynthesis of sphinganine and *N*-acylsphingosine (ceramide)

The carbon chain of sphingosine is formed by condensations between acyl-CoA – usually **palmitoyl-CoA** – and **serine**:

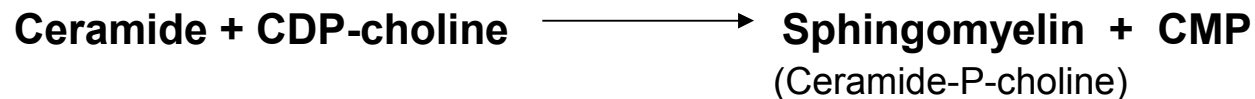


# Biosynthesis of sphingomyelin and glycolipids

All sphingolipids are formed by attachment of an **activated group** to the free 1-hydroxyl of a ceramide.

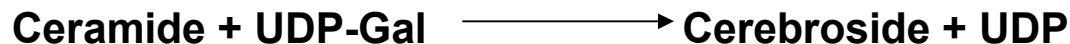
## Synthesis of sphingomyelin

CDP acts as a carrier of phosphoryl choline:



## Synthesis of glycolipids

A glycosyl is supplied by the transfer from UDP-monosaccharide:



Attachment of further glycosyls proceeds in a similar way.

Sialyl group (NeuAc in gangliosides) is transferred from CMP-NeuAc.



Sulphosphingolipids are formed by transfer of sulphate from 3'-phosphoadenosine-5'-phosphosulphate (abbr. PAPS).

# Degradation of sphingolipids in lysosomes

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

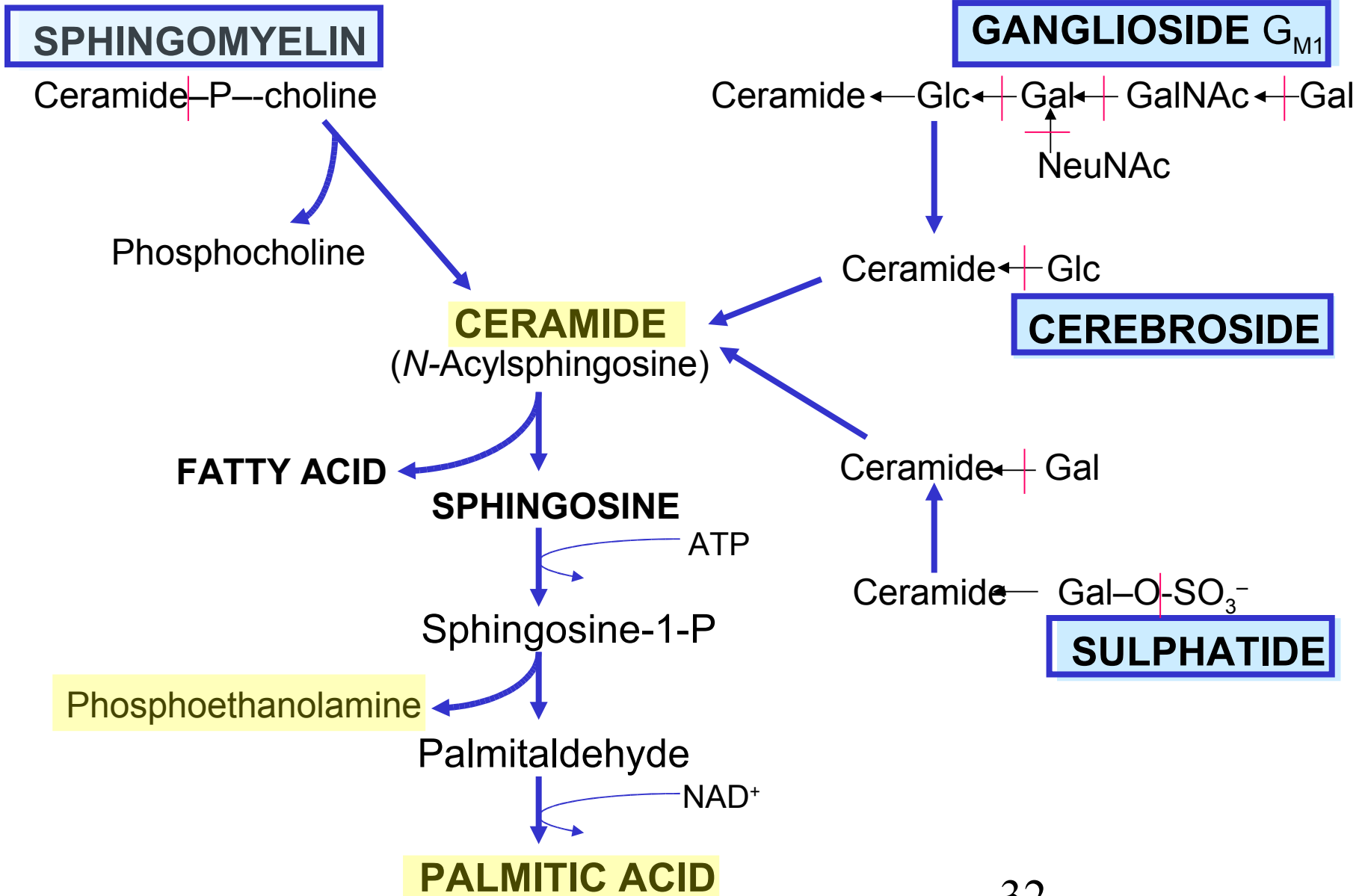
**Sphingomyelins** lose phosphocholine to give **ceramide**.

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

**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





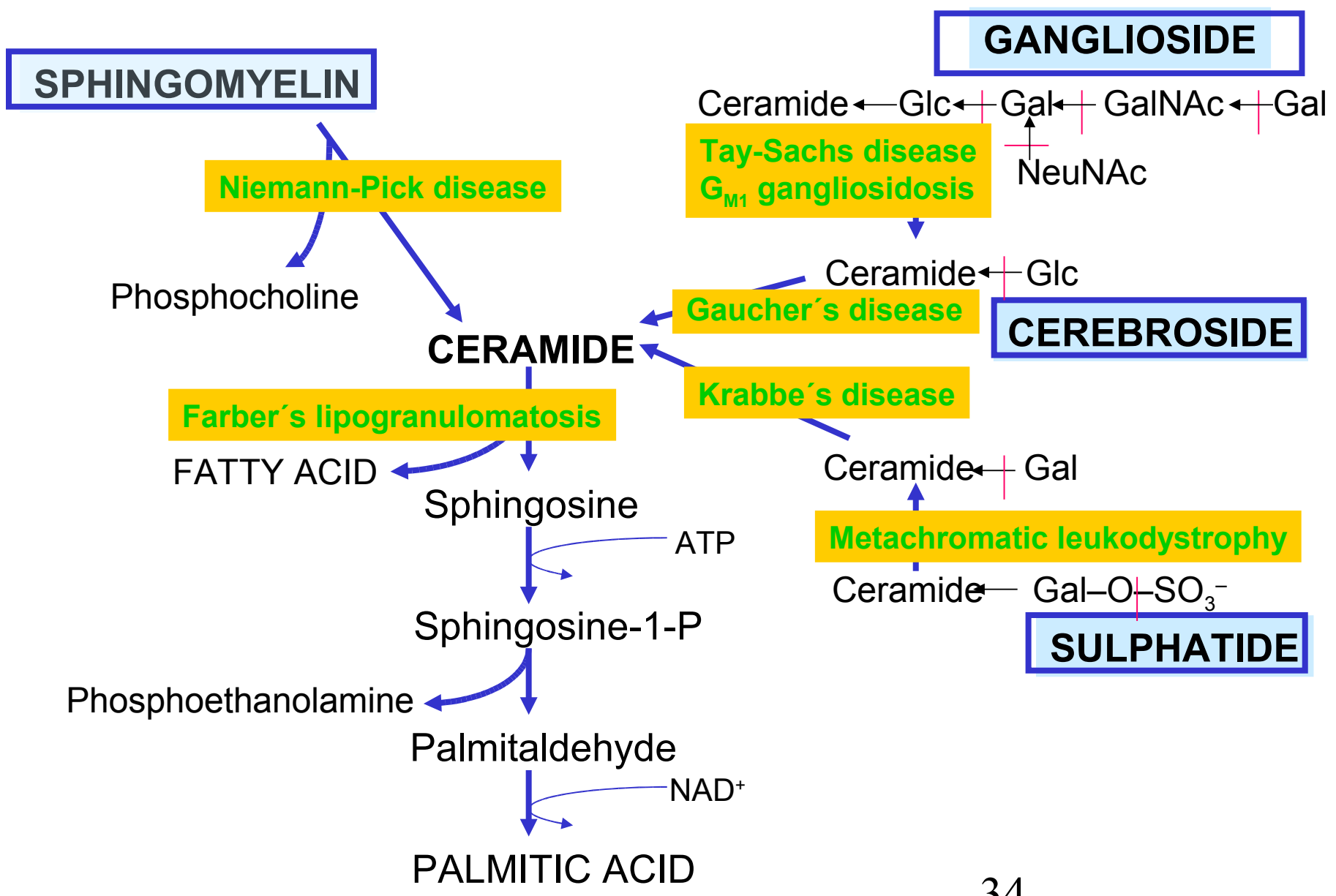
In general, the turnover of sphingolipids is very slow, particularly in brain.

## **Sphingolipidosis**

Inherited defects in production of the enzymes that catabolize sphingolipids 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.

# Spingolipidoses – genetic defects (deficiency of lysosomal enzymes)



# Eicosanoids

# Eicosanoids

are a family of polyunsaturated C<sub>20</sub> fatty acid derivatives,

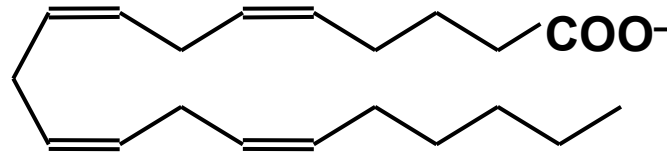
(Greek *eikosi* – “twenty”),

which act as local hormones and have a wide range of biological functions.

The major **precursors** are essential polyunsaturated fatty acids

– **arachidonic acid** (eicosatetraenoic, abbr. ETE)

20:4 (5,8,11,14) from the *n*-6 series,



– **eicosapentaenoic acid** (abbr. EPE)

20:5 (5,8,11,14,17) from the *n*-3 series,

and, in part, non-essential

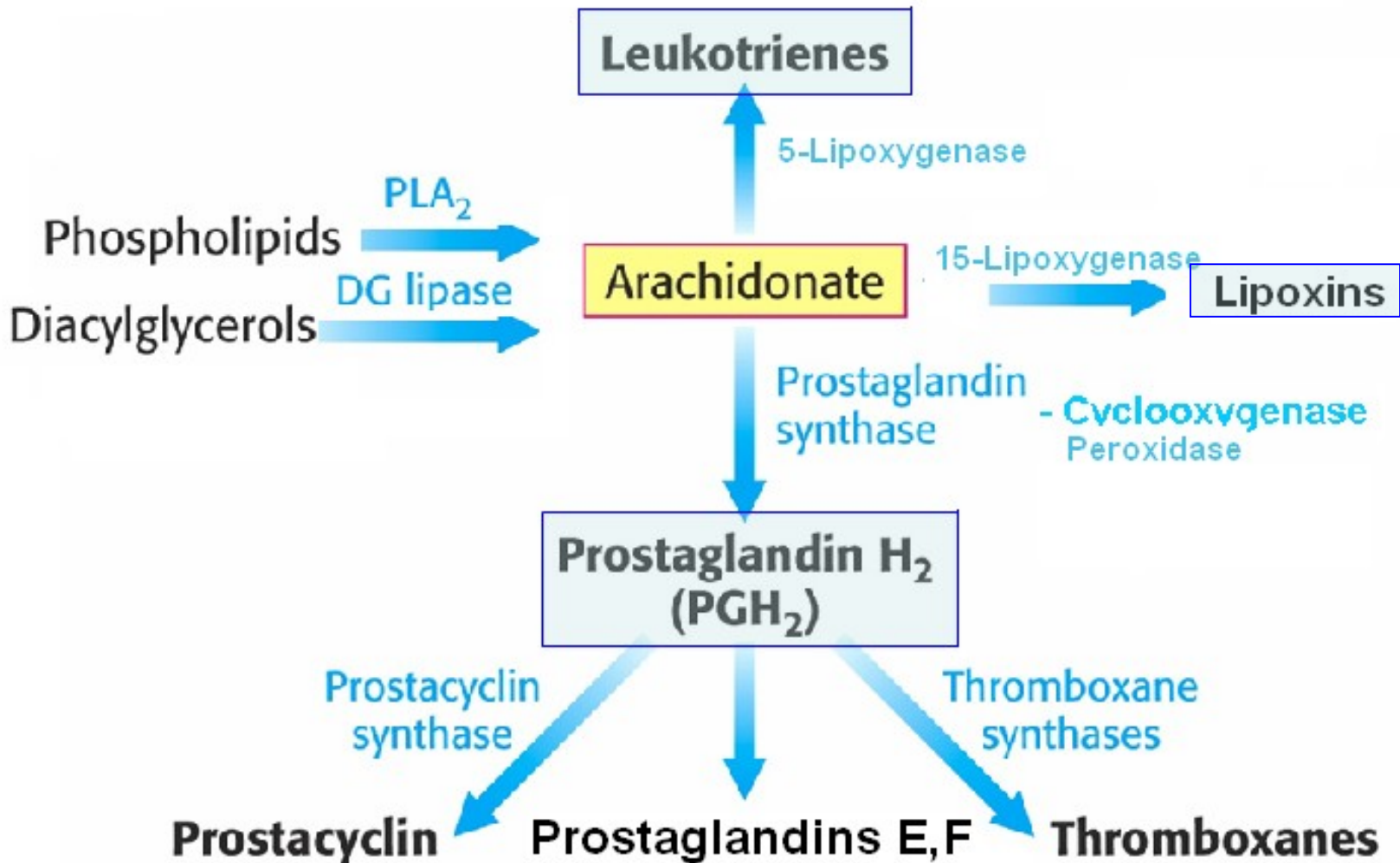
– **eicosatrienoic acid** 20:3 (5,8,11) from the *n*-9 series.

Although the intracellular concentration of free precursors is very low, they can be released **from C-2 of membrane phospholipids** by the action of **phospholipase A<sub>2</sub>** and also by the degradation of diacylglycerol generated in the PI cycle.

The activity of phospholipase A<sub>2</sub> is a process closely regulated by extracellular mediators (adrenaline, thrombin, angiotensin II, bradykinin). On the other hand, corticosteroids through induction of lipocortin inhibit the activity of phospholipase A<sub>2</sub>.

**Cyclooxygenase pathway** leads to the synthesis of **prostaglandin H**, an endoperoxide, the precursor of cyclic **prostaglandins**, **prostacyclins**, and **thromboxanes**.

**Lipoxygenase pathway** converts precursor acids to acyclic **hydroperoxyacids** (HETEs), from which either **leukotrienes** (action of 5-lipoxygenase) or **lipoxins** (action of 15- and 12-lipoxygenase) are formed.



# Cyclooxygenase pathway

## Synthesis of cyclic eicosanoids - prostanoids

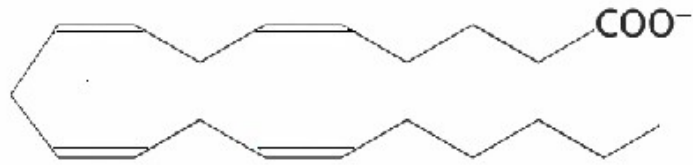
**Cyclooxygenase** (**COX**, prostaglandin endoperoxide synthase) is a membrane-bound enzyme, which has cyclooxygenase and peroxidase activities. It exists in two forms:

**COX-1** is a constitutive enzyme, expressed in almost all tissue;

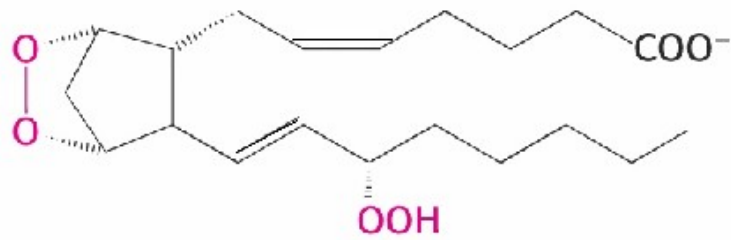
**COX-2** is inducible – its synthesis is induced by cytokines in  
inflamed tissue.

COX catalyses the conversion of **arachidonate** to **PGH<sub>2</sub>** – the common precursor of all the prostanoids of the 2-series (diene prostanoids): after formation of the ring, from four double bonds of arachidonate there will remain only two double bonds in the side chains.

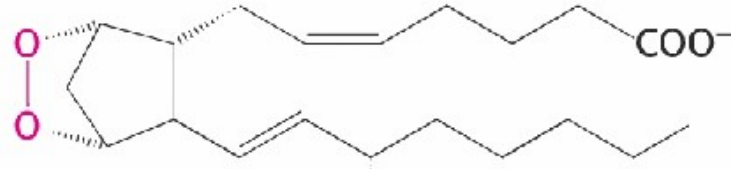
Similarly, COX catalyses conversion of **eicosapentaenoate** to **PGH<sub>3</sub>**, the precursor of the prostanoids of the 3-series (triene prostanoids), and conversion of **eicosatrienoate** to **PGH<sub>1</sub>**



**Arachidonate**



**Prostaglandin G<sub>2</sub>**

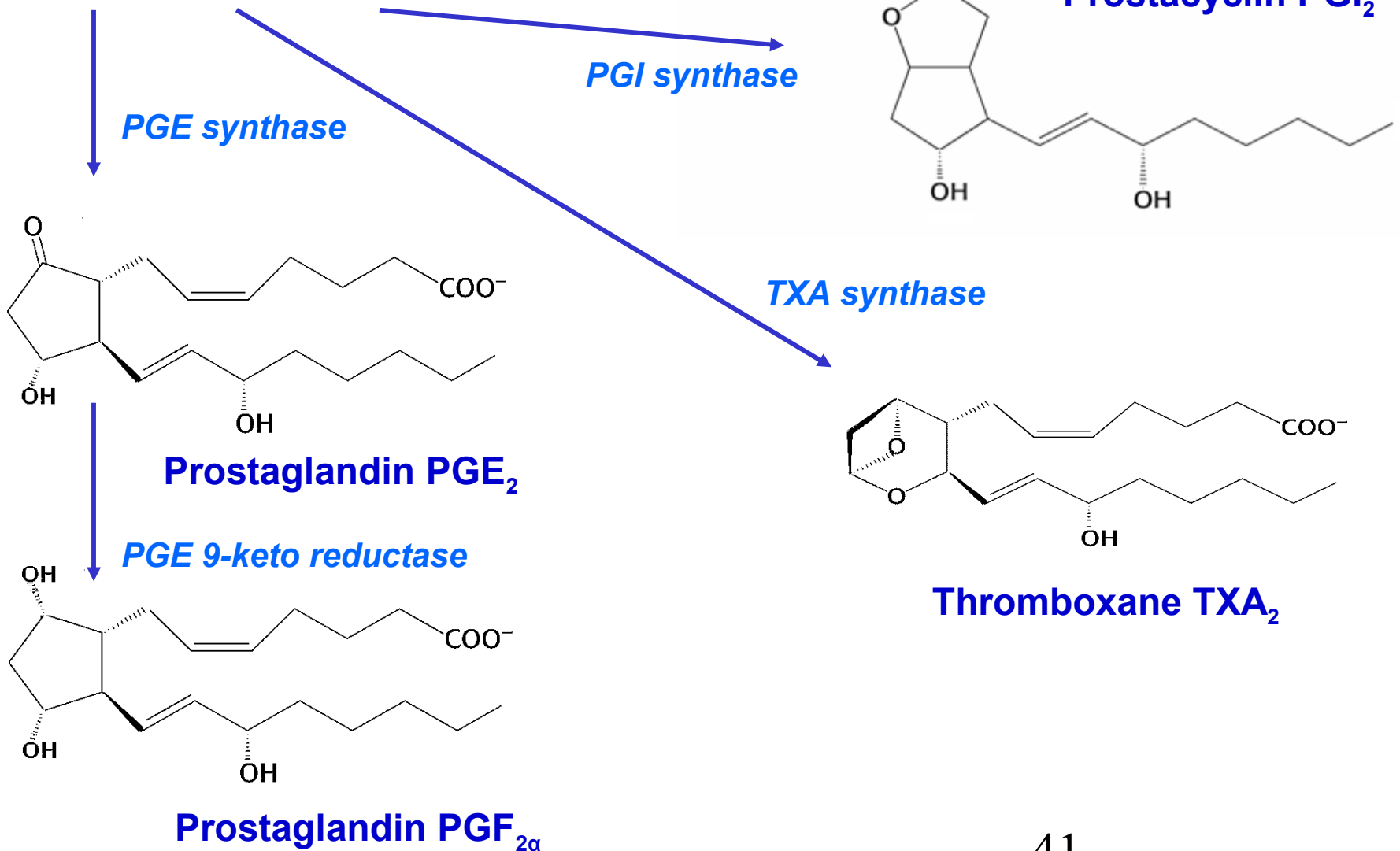


**Prostaglandin H<sub>2</sub>**

Precursor of all prostanoids  
of the 2-series



# Prostaglandin H<sub>2</sub>



# Inhibition of cyclooxygenase blocks prostanoid production

Prostanoids mediate, at least partly, the inflammatory response.

## **Advisable effects of suppressed prostanoid production:**

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.

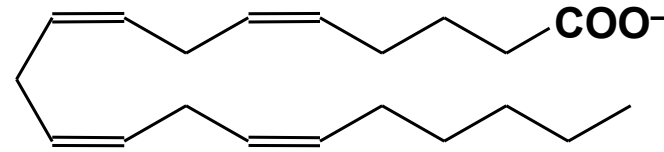
**Inhibitors** of cyclooxygenase act as **nonsteroidal anti-inflammatory drugs** (NSAIDs, analgetics-antipyretics):

- **acetylsalicylic acid** (aspirin) – inhibits both COX-1 and COX-2 irreversibly by acetylation the enzyme at its active site.,
- **acetaminophen** and **ibuprofen** – reversible COX inhibitors.

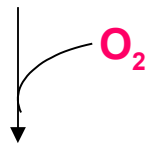
Drugs are being developed which will act as selective inhibitors of COX-2 (named coxibs, e.g. celecoxib, rofecoxib) without the adverse gastrointestinal and anti-platelet side effects of non-specific inhibitors of COX.

# Lipoxygenase pathway

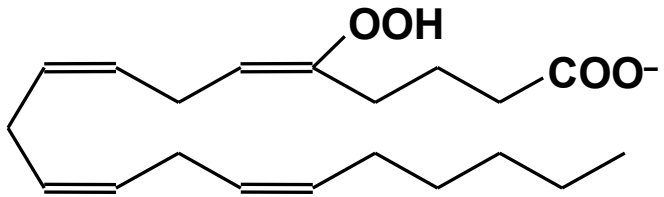
## Synthesis of leukotrienes



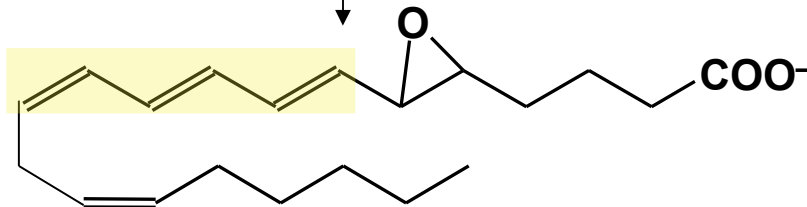
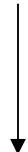
Arachidonate



*5-Lipoxygenase*



5-HydroperoxyETE

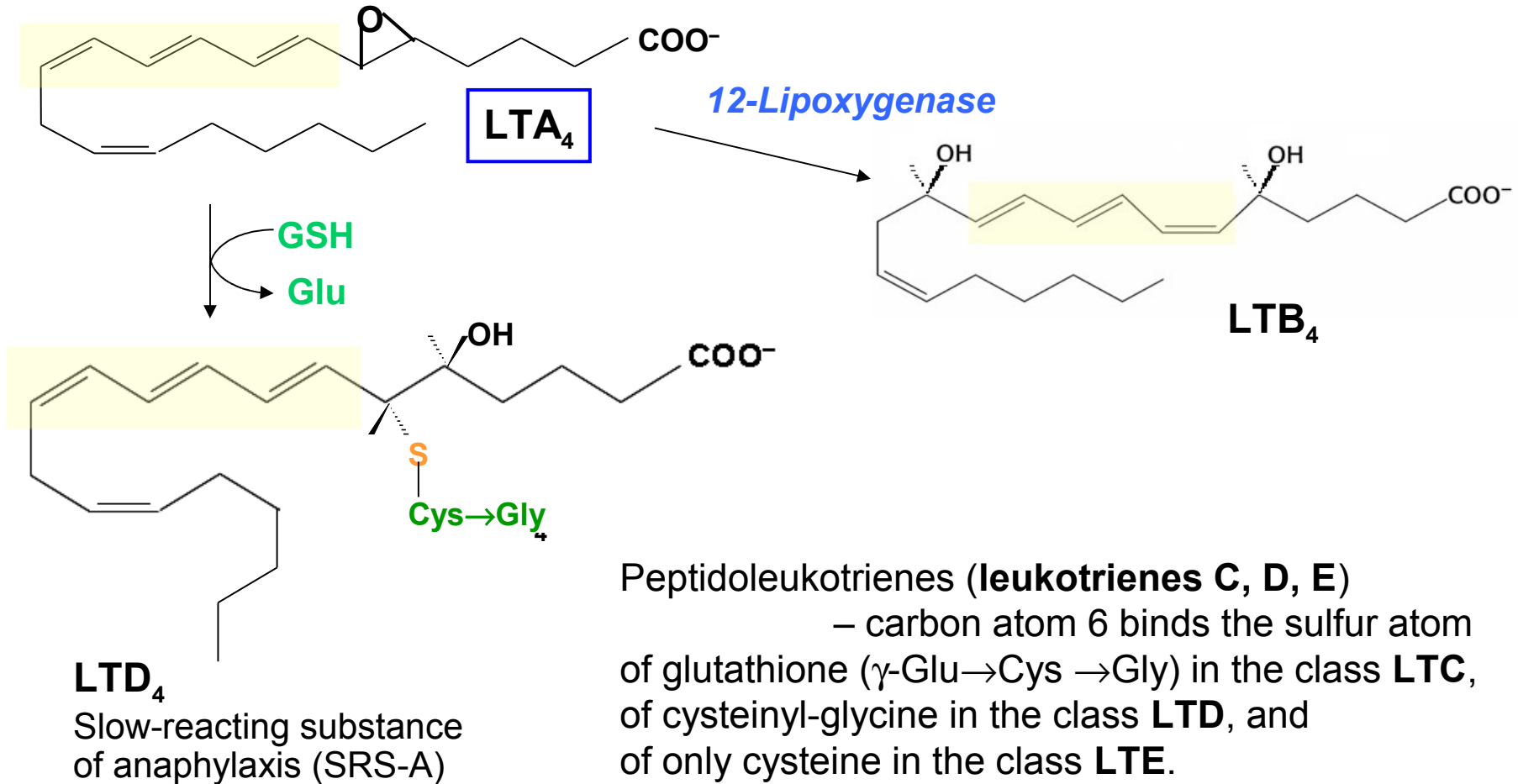


Leukotriene LTA<sub>4</sub>

Precursor of all leukotrienes  
of the 4-series

Leukotrienes are produced primarily in leukocytes and mast cells and all of them have three conjugated double bonds (trienes), the position of which may be different and the configuration either *trans* or *cis*.

The classes of LTs are designated by letters A – E), the subscript denotes the total number of double bonds.



Leukotrienes are the most effective eicosanoids, e.g. their vasodilating effect is about 5 000 times more intensive than that of the same amount of histamine.

**Eicosanoids are produced in various types of tissue.**

The site of their synthesis depends on expression of genes for the enzymes which take part in the synthetic pathways.

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.

**Catabolism of eicosanoids** is rapid.

The biological half-life of prostanoids  $t_{1/2}$  was found to be in the range from seconds to few minutes.

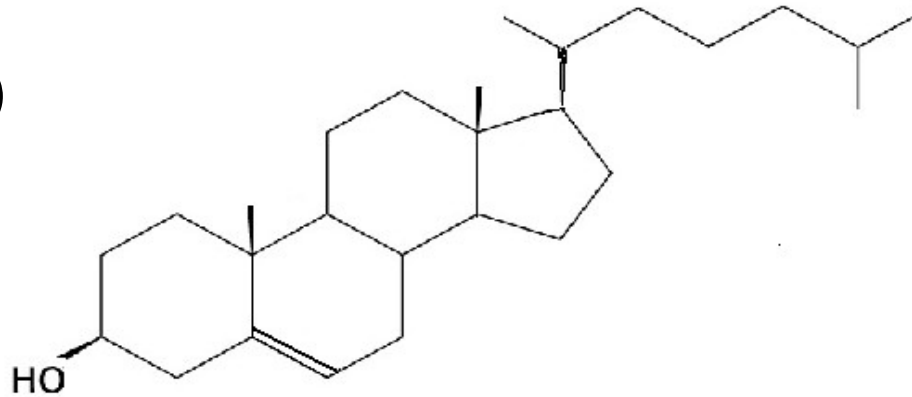
## Eicosanoids

Examples	Structural group	Synthesized in	The most remarkable effect:
<b>PGE<sub>2</sub></b>	prostaglandin E	nearly all cell types	inflammatory reaction, vasodilation, inhibition of HCl secretion
<b>PGF<sub>2α</sub></b>	prostaglandin F	nearly all cell types	vasoconstriction increase of body temp.
<b>PGI<sub>2</sub></b>	prostacyclin	endothelial cells, smooth muscle cells of blood vessels	vasodilation, inhibition of platelet aggregation
<b>TXA<sub>2</sub></b>	thromboxane	blood platelets	platelet aggregation, vasoconstriction
<b>LTD<sub>4</sub></b>	leukotriene	leukocytes, mast cells	bronchoconstriction, vasoconstriction
<b>LXA<sub>4</sub></b>	lipoxin	various cell types	bronchoconstriction, vasodilation

# **Cholesterol and bile acids**

# Cholesterol

(Cholest-5-ene-3 $\beta$ -ol)



**Constituent of all animal membranes** which modulates the fluidity of cell membranes. It also occurs in trace amounts in plants.

**Necessary precursor** of the synthesis of bile acids, steroid hormones and calciols (vitamin D).

Although much cholesterol is obtained from the diet, the animal body can synthesize all the cholesterol it requires.

Cholesterol is synthesized in all nucleated cells.

**Biosynthesis:** approx. 800-1000 mg per day.

**Dietary intake:** approx. 500 mg per day

(egg yolk, animal fat and meat, fat dairy products).



# Biosynthesis of cholesterol

Cholesterol is synthesized **from acetyl coenzyme A**, all 27 carbon atoms of cholesterol are derived from acetyl-CoA.

The synthesis is localized **in the cytosol** and **on the membranes of endoplasmic reticulum** (some enzymes catalysing the synthesis are integral membrane proteins of ER).

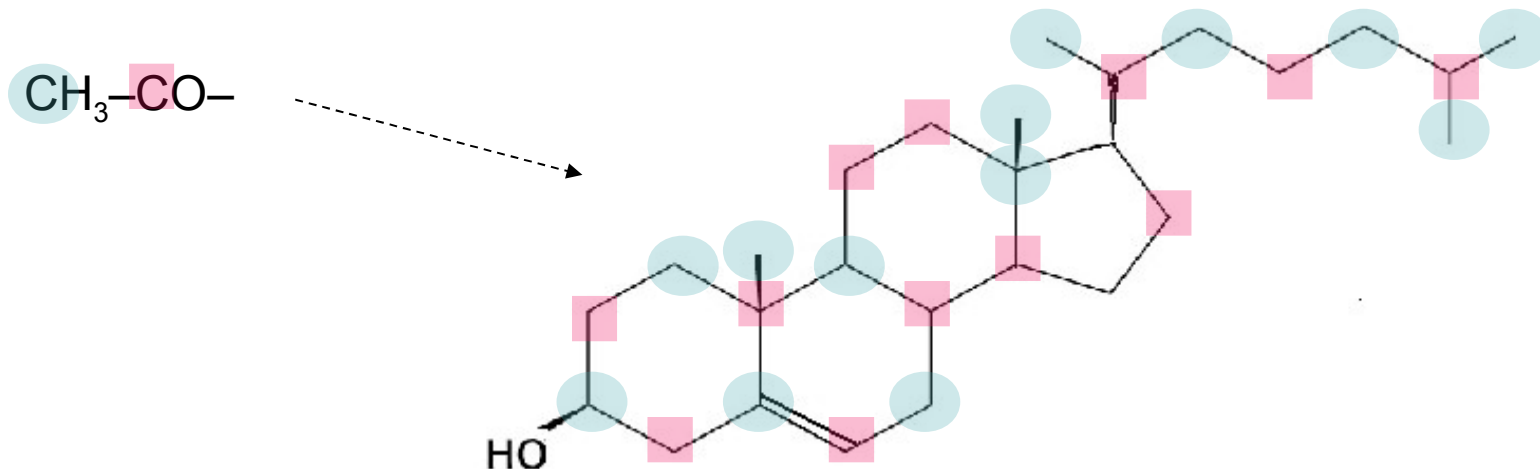
About 1/3 of cholesterol is formed in the liver, substantial amounts are also formed in the gut and skin. High rates of the synthesis are observed in the adrenal cortex and gonades.

The synthesis is a **four-stage proces**:

- 1 The synthesis of **mevalonate** from acetyl-CoA.
- 2 The conversion of two mevalonates to two **activated isoprene units** that are the key building blocks of cholesterol.
- 3 The condensation of six molecules of activated isoprenes to form **squalene**.
- 4 The **cyclization** of squalene in an astonishing reaction and the **conversion** of the four-ring steroid nucleus into cholesterol.

15 **Acetyl-CoA** ----- (tens of reactions) ----- **cholesterol**

The result of isotope-labeling experiment show the source of carbon atoms. Cholesterol was synthesized from acetate labeled in its methyl (blue) or carboxylate (red) atom:

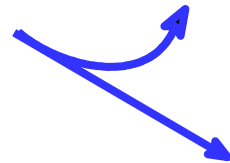


# 1 The synthesis of mevalonate from acetyl-CoA.

Cytosol, ER membrane

2  $\text{CH}_3\text{CO-CoA}$   
Acetyl-CoA

CoA



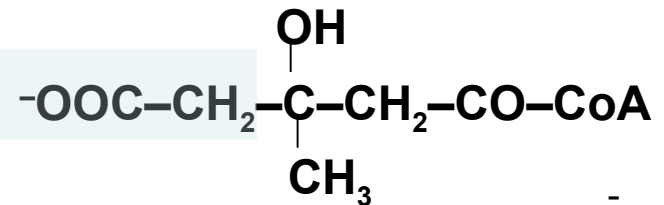
$\text{CH}_3\text{-CO-CH}_2\text{-CO-CoA}$   
Acetoacetyl-CoA

$\text{CH}_3\text{CO-CoA}$

CoA



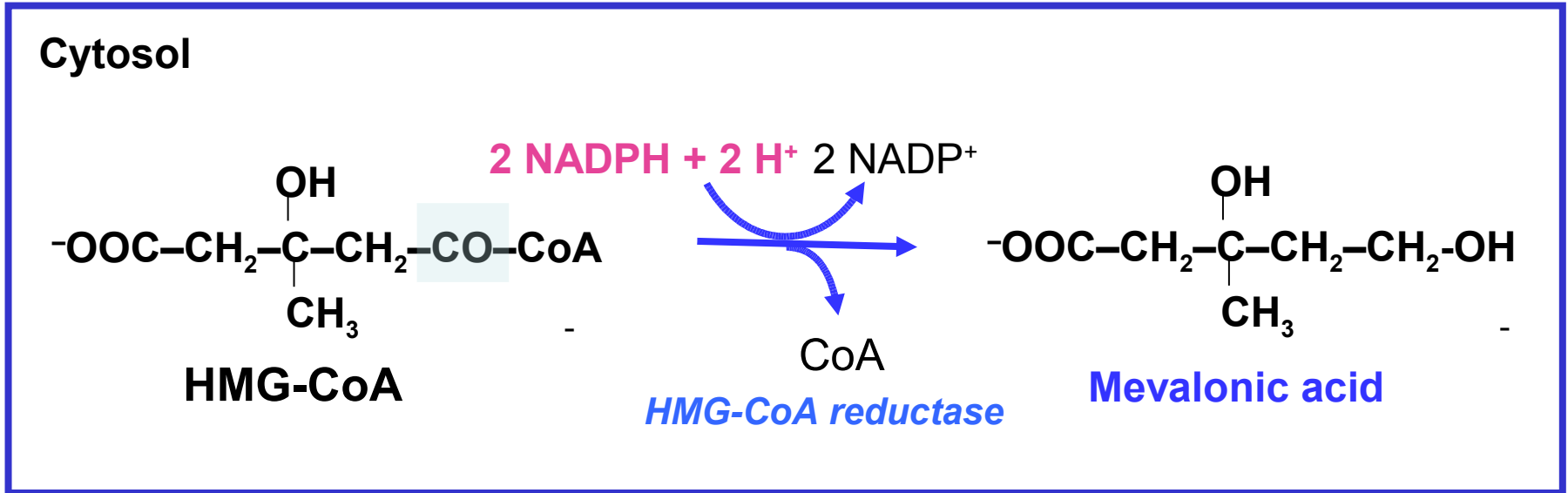
HMG-CoA synthase



**3-Hydroxy-3-methylglutaryl-CoA**  
( HMG-CoA )

Compare with the first steps  
of ketogenesis in the matrix  
of mitochondria!

3-Hydroxy-3-methylglutaryl-CoA is then **reduced** in the 4-electron reaction to mevalonate (3,5-dihydroxy-3-methylvalerate)::



This reduction of HMG-CoA to mevalonate catalysed by HMG-CoA reductase is the **rate-limiting step** in the pathway of cholesterol synthesis. Both the amount of the enzyme and its activity is strictly controlled

The fate of HMG-CoA synthesized in the **mitochondrial matrix** is different – HMG-CoA is split into free acetoacetate and coenzyme A (ketogenesis).

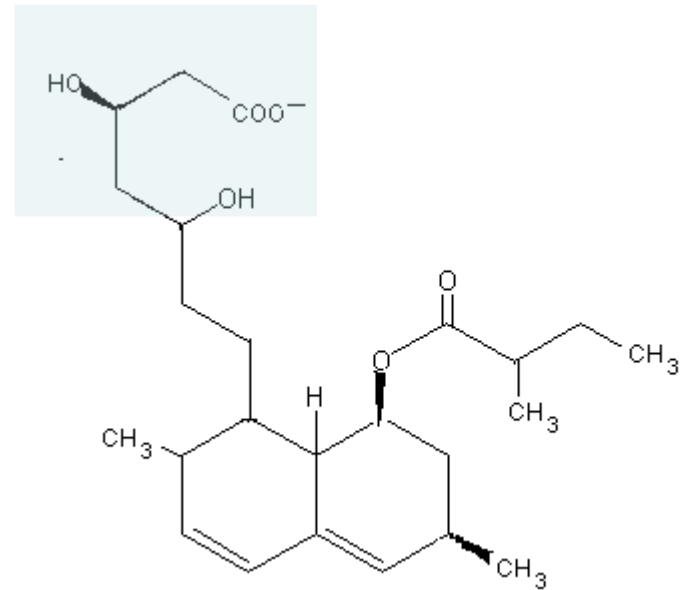
# Control of cholesterol biosynthesis by regulating the activity of HMG-CoA reductase:

## Inhibition

- by cytosolic free cholesterol  
(feed-back control; Brown and Goldstein)
- by reversible phosphorylation of the enzyme
- by drugs called statins.

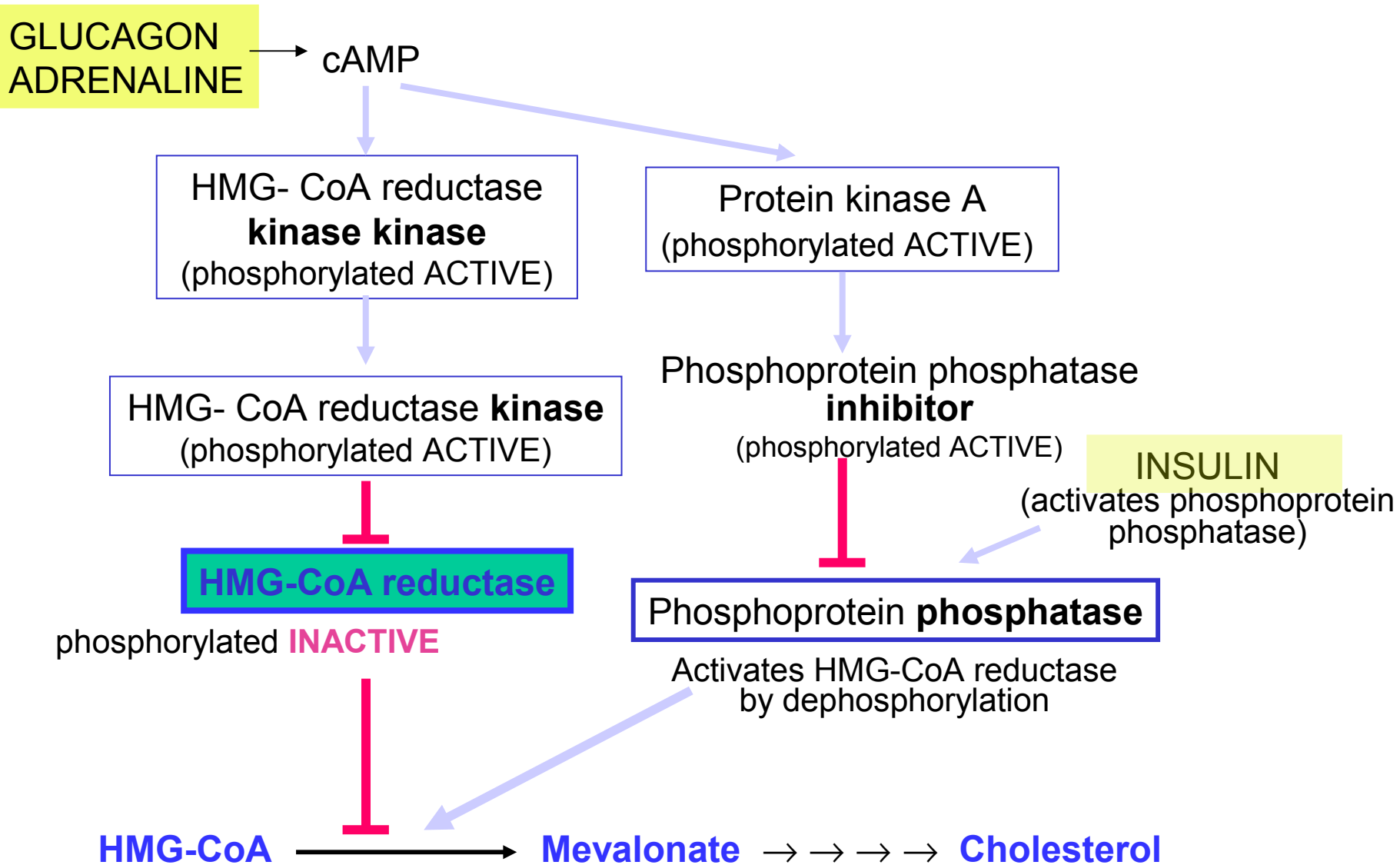
**Statins** are competitive inhibitors of HMG-CoA reductase, either fungal products (e.g. lovastatin), or quite synthetic compounds (3<sup>rd</sup> generation of statins, e.g. cerivastatin).

The highlighted part of the lovastatin molecule resembles the HMG-moiety.

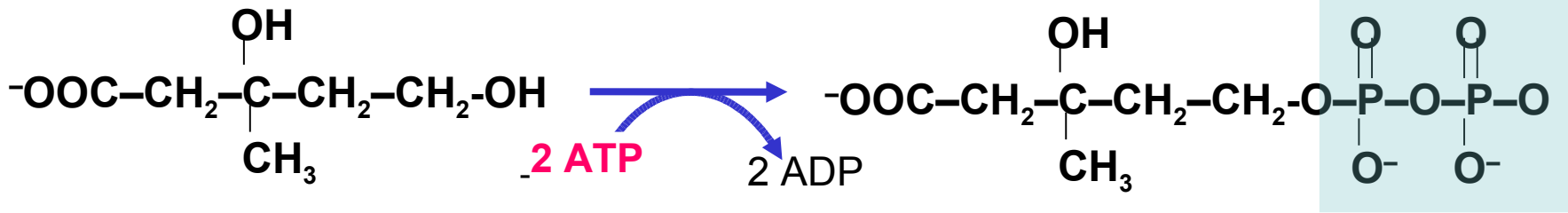


**Lovastatin**

# Hormonal control of the HMG-CoA reductase activity through reversible phosphorylation:

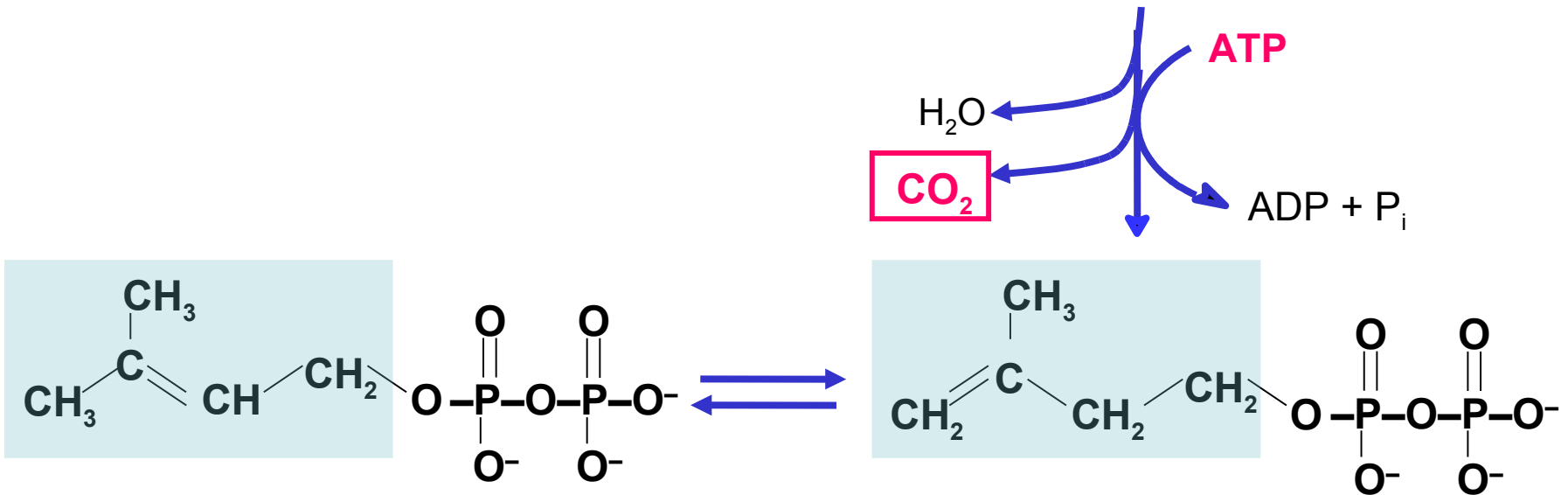


## 2 The conversion of mevalonate to activated isoprene units



Mevalonate

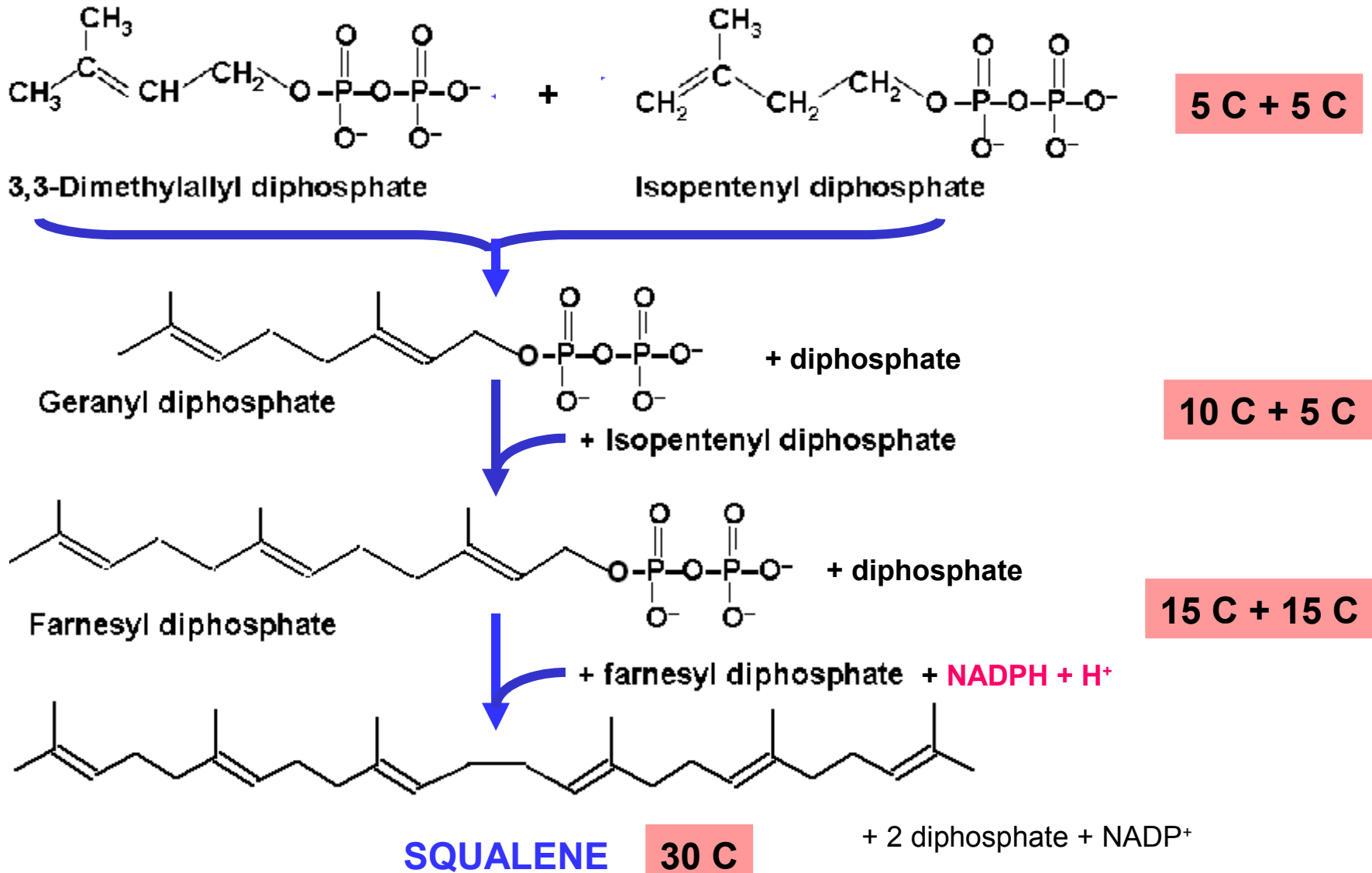
Mevalonate 5-diphosphate



3,3-Dimethylallyl diphosphate

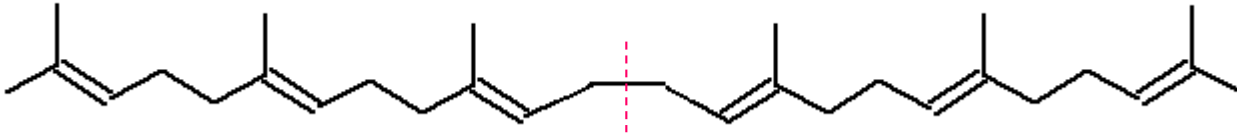
Isopentenyl diphosphate

### 3 The condensation of molecules of activated isoprenes to form squalene (30 C):



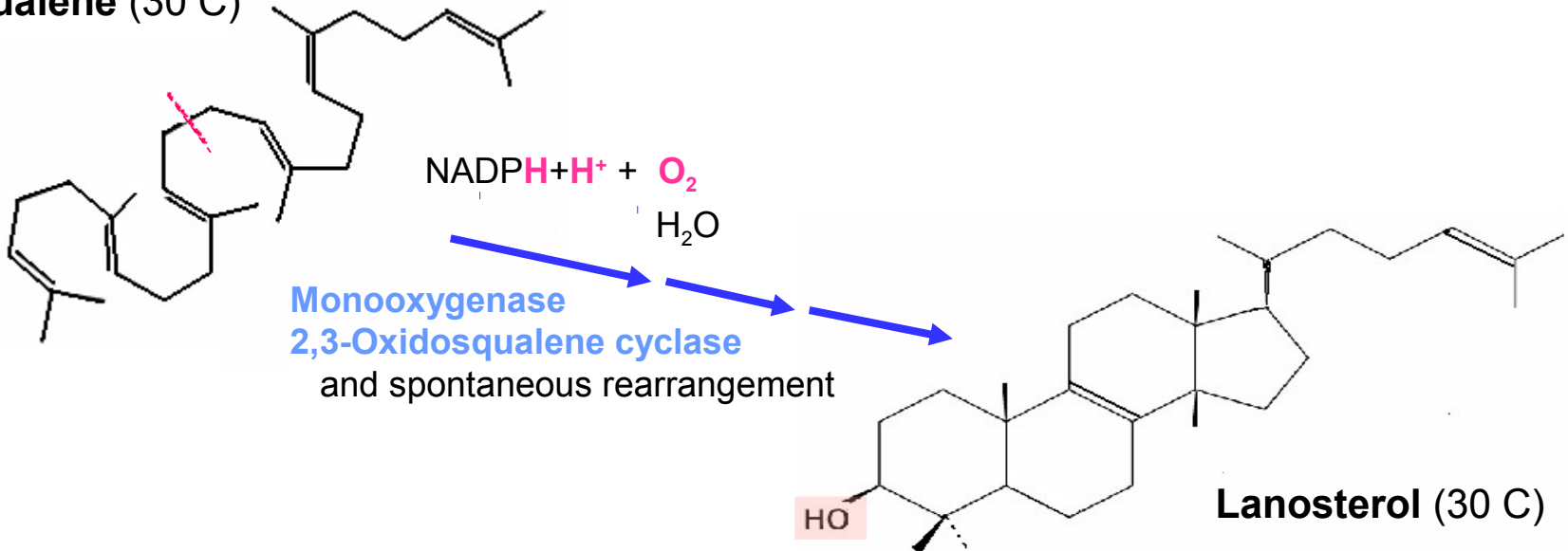


## 4 The cyclization of squalene and the conversion of the steroid nucleus into cholesterol.



Due to free rotation round single covalent bonds, the „stretched“ form of squalene may take also the conformation that suggests the interactions causing the subsequent closure of the four-ring steroid nucleus:

**Squalene (30 C)**



Lanosterol is merely an **intermediate** in man, but occurs free in wool fat.

## The final conversion of lanosterol to cholesterol

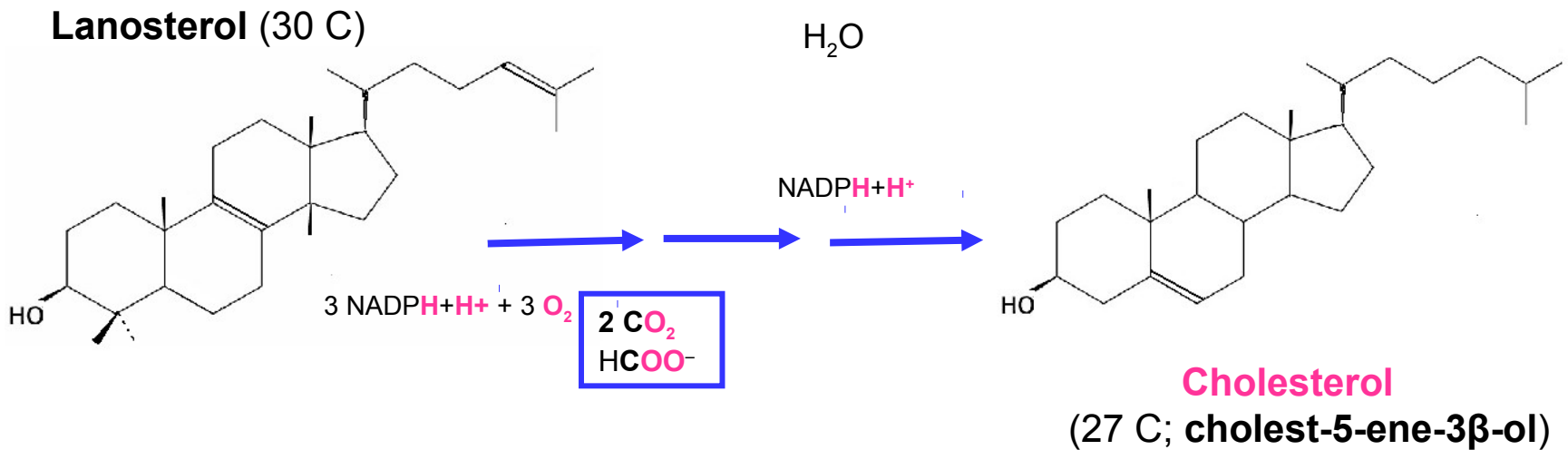
involves more than 5 steps:

-oxidative removal of three  $-\text{CH}_3$  groups

(catalysed by a monooxygenase) as  $2 \text{CO}_2$  and  $\text{HCOO}^-$ ,

- rearrangement of double bonds,

- reduction (saturation) of one of the two double bonds.



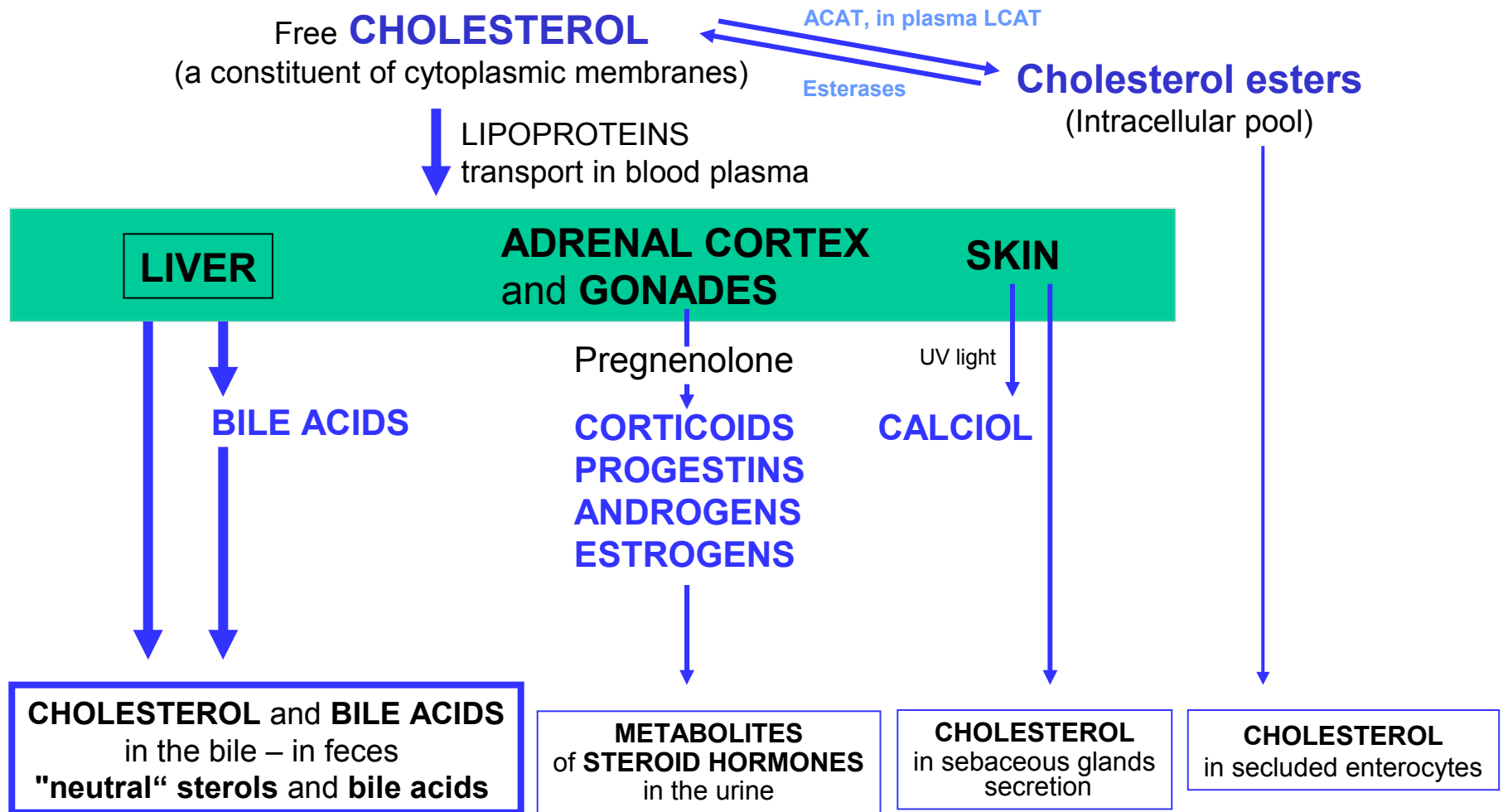
**Squalene (30 C)  $\rightarrow \rightarrow \rightarrow$  lanosterol (30 C)  $\rightarrow \rightarrow \rightarrow$  cholesterol (27 C)**

Almost all the reactions in cholesterol synthesis take place **on the endoplasmic reticulum**. The products become successively less water-soluble, a carrier protein (SCP, **steroid carrier protein**) is required to transport the intermediates from one enzyme site to another.

# Cholesterol utilization and elimination from the body

In higher animals, the steroid nucleus of cholesterol is **neither decomposed** to simple products **nor oxidized** to  $\text{CO}_2$  a  $\text{H}_2\text{O}$ .

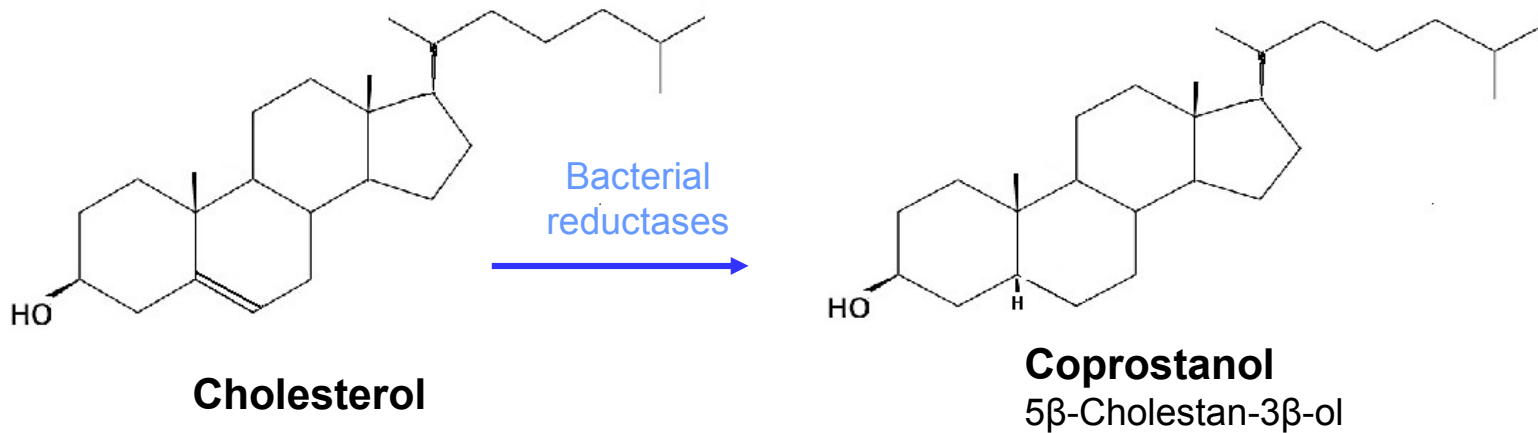
**The liver** is the organ which excretes most of the cholesterol, either directly or as bile acids.



# Cholesterol in the gut

In the small intestine, dietary cholesterol as well as cholesterol secreted in the bile is not absorbed completely (only about 40-50 %).

Most of the cholesterol that escapes absorption and enters the large intestine undergoes **reduction to coprostanol**. The reaction is catalysed by the enzymes of intestinal microflora.

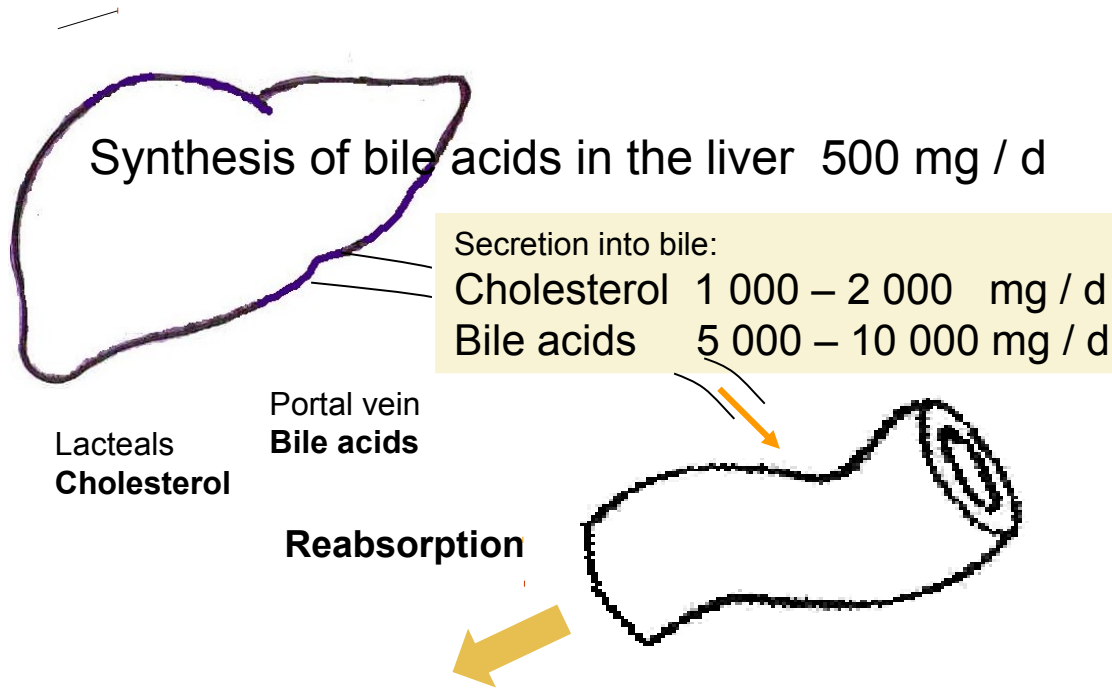


# The balance of cholesterol intake and elimination

## BODY POOL

Cholesterol 150 g  
Bile acids 3 – 5 g

**DIETARY INTAKE of cholesterol 80 – 500 mg per day**  
**BIOSYNTHESIS 800 – 1000 mg per day**



Synthesis of bile acids in the liver 500 mg / d

Secretion into bile:

Cholesterol 1 000 – 2 000 mg / d

Bile acids 5 000 – 10 000 mg / d

Lacteals  
Cholesterol

Portal vein  
Bile acids

Reabsorption

**ELIMINATION**  
**1000-1500 mg / d :**

**Feces** Cholesterol and other neutral sterols 800 mg / d  
Bile acids 500 mg / d

**Other** Steroid hormones, sebaceous secretion,  
cholesterol of intestinal cells 200 mg / d

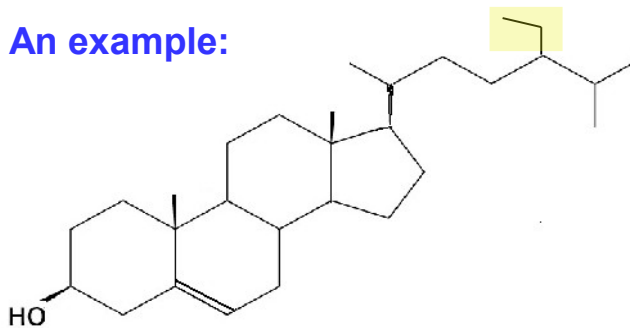
## Phytosterols - sterols of plant origin

are structurally related to cholesterol; only the side chain on C-17 is changed.

Phytosterols are **not resorbed in the gut**.

On the contrary, consumption of phytosterols reduces the resorption of cholesterol.

An example:



### **β-Sitosterol**

– predominant in the sterol fraction of plant oils

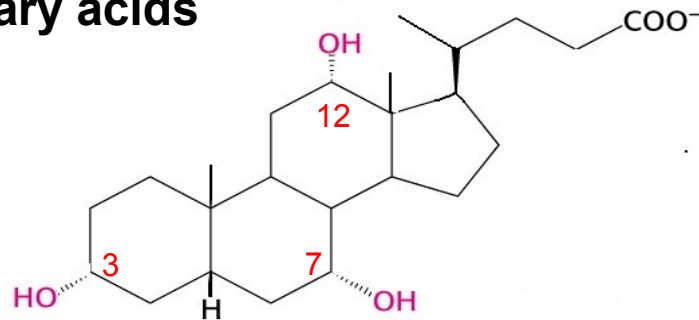
Plant oils (corn, rapeseed, soya, sunflower, walnut) contain up to 0.9 % phytosterols.

Average intake of phytosterols in Czech republic - about 240 mg per day,  
in Finland (some foods are enriched with phytosterols) - 350 mg per day.

# Bile acids

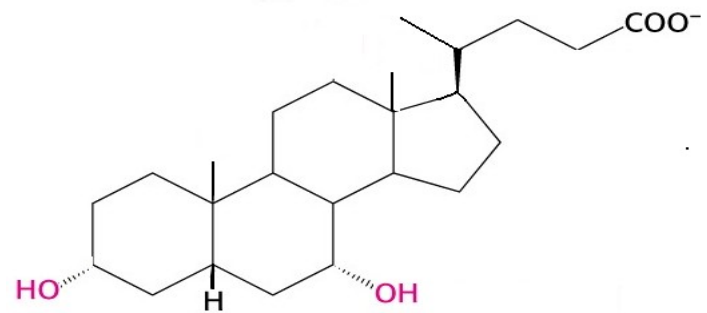
## Structure of the major bile acids

### Primary acids



#### CHOLATE

3<sup>α</sup>,7<sup>α</sup>,12<sup>α</sup>-trihydroxy-5<sup>β</sup>-cholan-24-oate

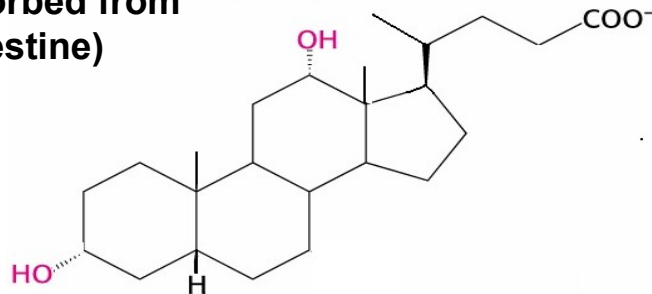


#### CHENODEOXYCHOLATE

3<sup>α</sup>,7<sup>α</sup>-dihydroxy-

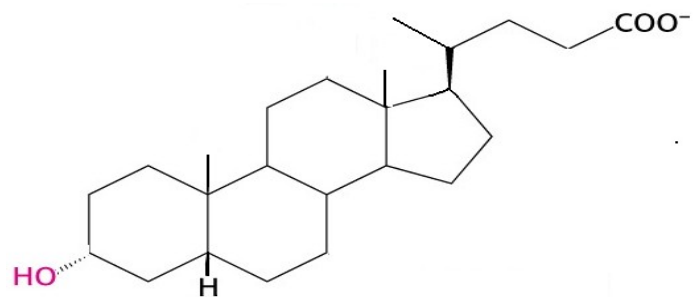
### Secondary acids

(reabsorbed from  
the intestine)



#### DEOXYCHOLATE

3<sup>α</sup>,12<sup>α</sup>-dihydroxy-

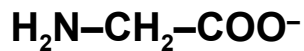


#### LITHOCHOLATE

3<sup>α</sup>-hydroxy-

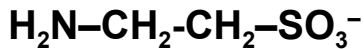
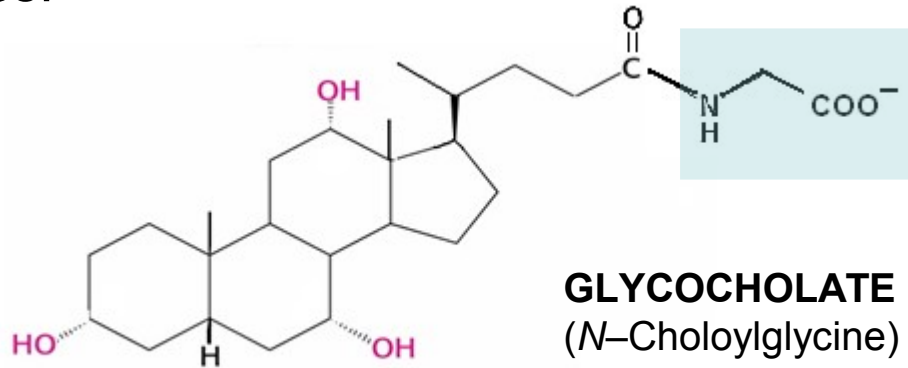
The primary bile acids, cholate and chenodeoxycholate, are **conjugated** within endoplasmic reticulum of the liver cells **with glycine or taurine**.

Those amides called **conjugated bile acids** (or bile „salts“, resp.) are then secreted into bile ductules:



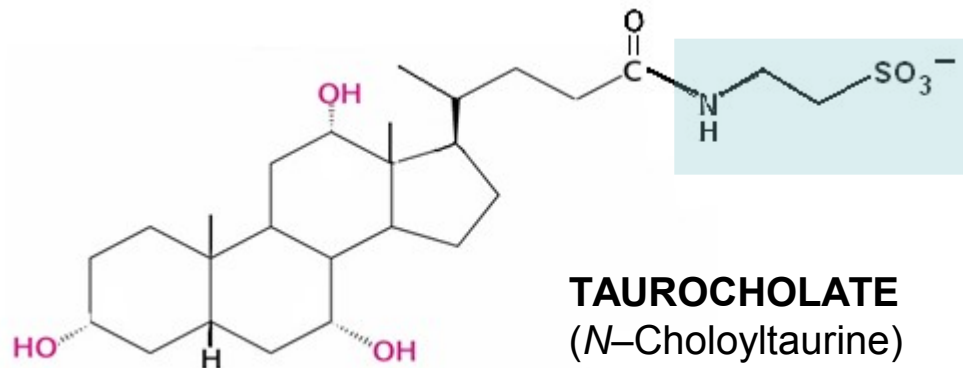
**Glycine**

(Aminoacetic acid)



**Taurine**

(2-Aminoethanesulphonic acid)



The structure of conjugated chenodeoxycholate is analogous to glyco- and taurocholate. Conjugated acids are more acidic ( $\text{pK}_a$  2-4) than the unconjugated acids ( $\text{pK}_a$  6), therefore they are more efficient emulgators than the unconjugated ones.

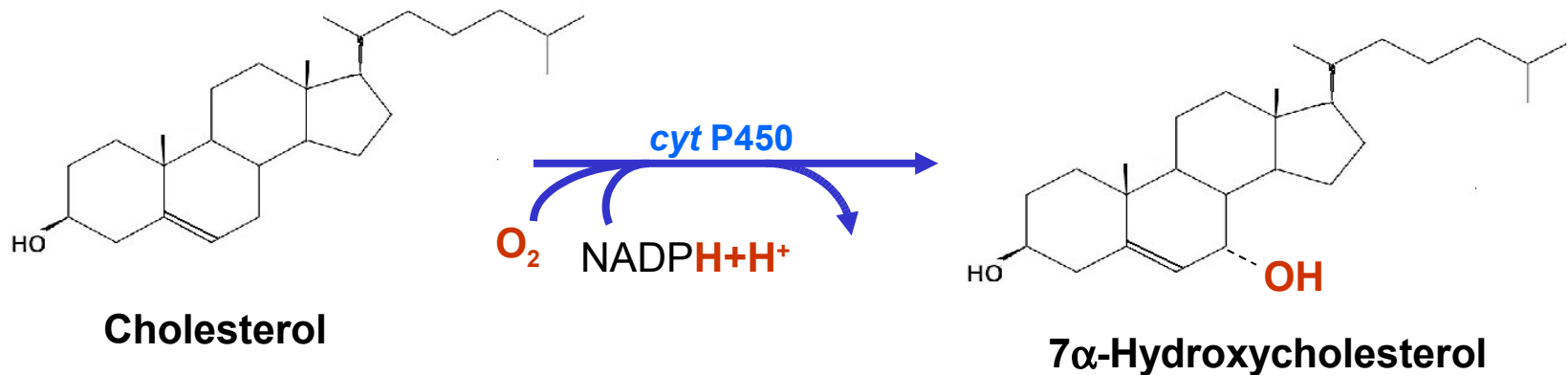


# Biosynthesis of the bile acids

occurs only in the liver cells:

The first and rate-limiting step of the conversion to bile acids is the **hydroxylation of cholesterol at C-7** catalysed by **7 $\alpha$ -hydroxylase**.

The enzyme is a monooxygenase of the cytochrome P450 class, bound in the membrane of endoplasmic reticulum and its activity is supported by the presence of L-ascorbate.



The second **hydroxylation at C-12** in the synthesis of cholate is connected with rearranging of the ring A and B.

(In the synthesis of chenodeoxycholate (not shown) the second hydroxylation is omitted.)

**7 $\alpha$ -Hydroxycholesterol**

ON THE MEMBRANE OF ER

$O_2 + NADPH + H^+$   
Cyt P 450

Dehydrogenation to 3-oxo-  
Isomerization of the double bond  
Hydrogenation of 3-oxo and of double bond at C-4

**5 $\beta$ -Cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -triol**

↓

Coenzyme A, ATP  
Propionyl-CoA, ADP

**Cholate**

**MITOCHONDRION**  
26-Hydroxylation  
Oxidation to C-26 carboxyl  
Activation to acyl-CoA  
Propionyl-CoA released

Coenzyme A, ATP  
ADP

**Choloyl-CoA**

Glycine  
(or taurine)  
Co-A

CONJUGATION  
WITHIN ENDOPLASMIC RETICULUM

**GLYCOCHOLATE**  
(or taurocholate)



Secretion into bile ductules

Cholate and chenodeoxycholate are called **primary bile acids**. They are the direct products of cholesterol degradation in the liver and are secreted in the bile.

In the intestine they may be modified by bacterial action – they are dehydroxylated to give the **secondary bile acids, deoxycholate and lithocholate**.

Bile acids are efficiently reabsorbed and returned to the liver via portal vein and secreted again – bile acid undergo the **enterohepatic circulation**.