Lipid metabolism II

Phospholipids and glycolipids Eicosanoids. Cholesterol

Biochemistry I
Lecture 9

2009 (J.S., J.D.)

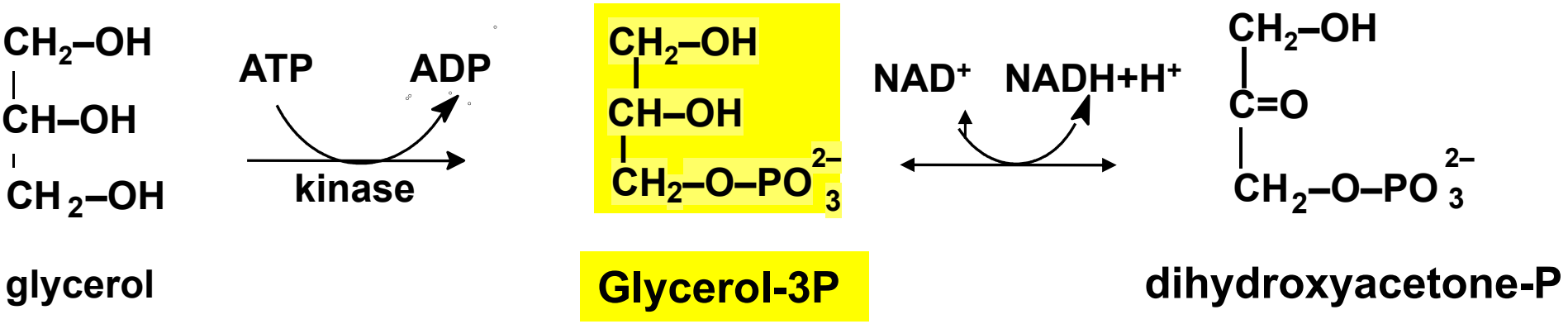
Synthesis of triacylglycerols

is provided by esterification of **glycerol 3-phosphate** (or dihydroxy-acetone phosphate) by activated fatty acids - **acylcoenzymes A**.

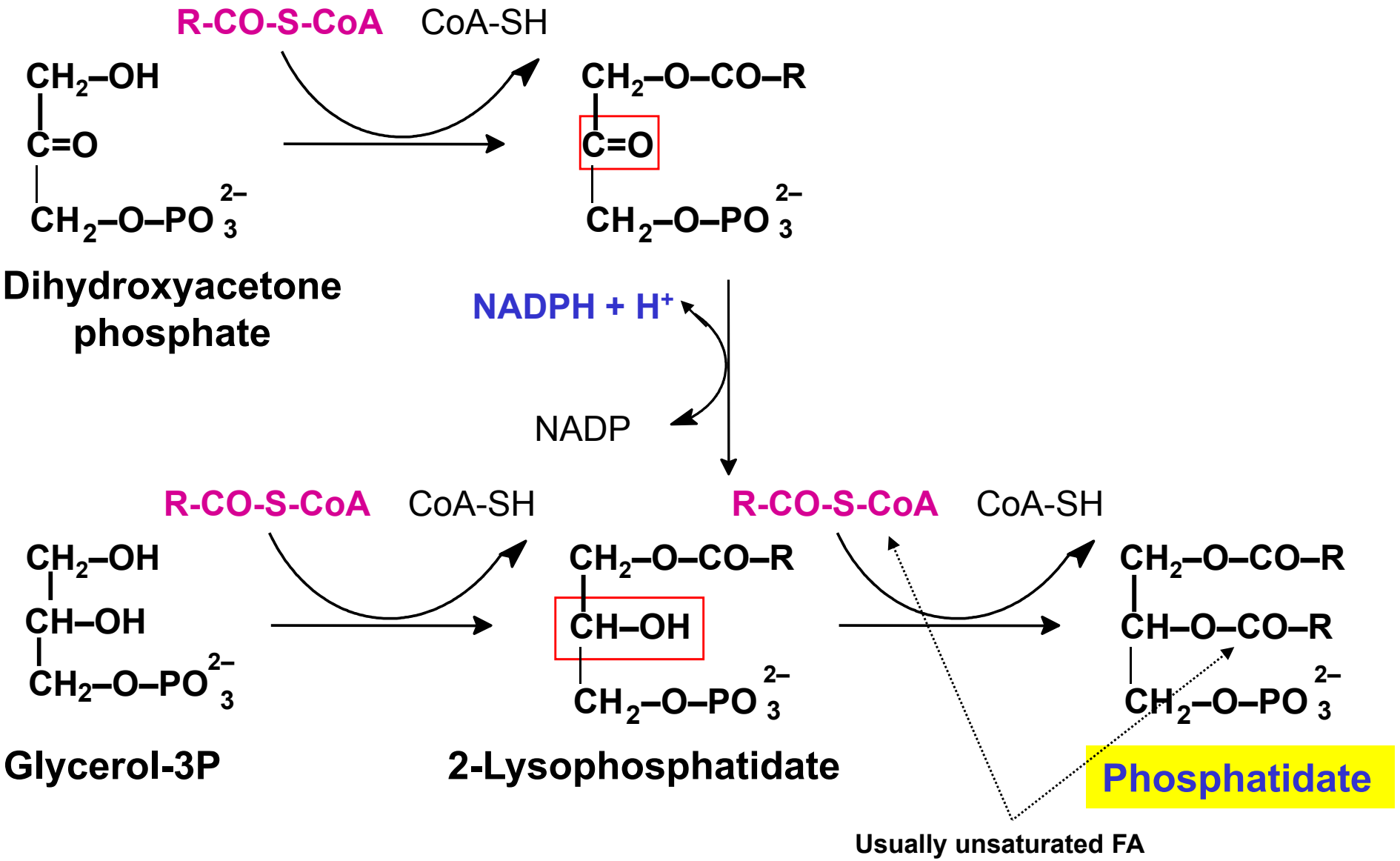
There are **two sources** of glycerol phosphate:

In **liver and small intestine** (but **not in adipose tissue**) is glycerol phosphorylated by **glycerol kinase.**

In **most other tissues** glycerol phosphate originates by reduction of dihydroxyacetone phosphate, an intermediate of glycolysis, by the action of **glycerol phosphate dehydrogenase**

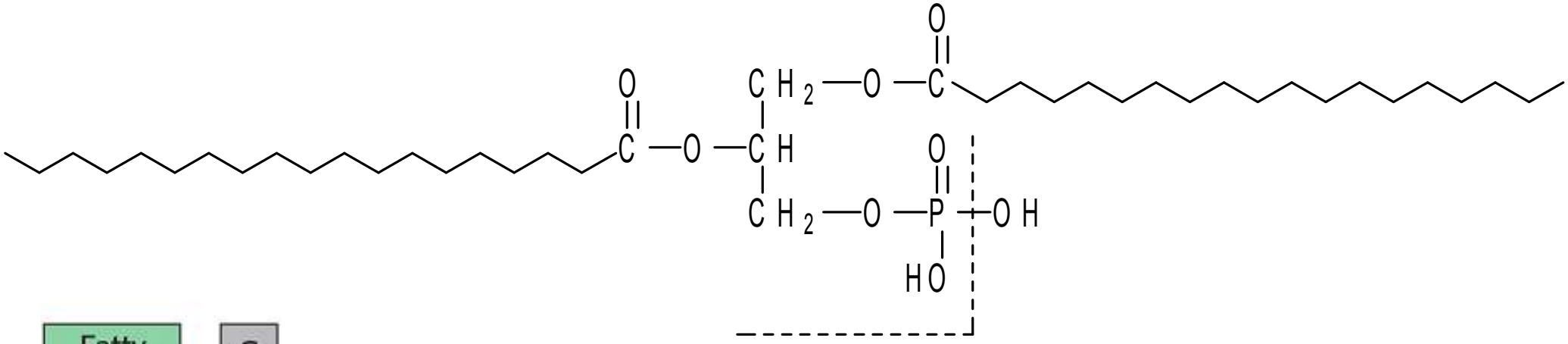


Phosphatidate is an intermediate in the synthesis of triacylglycerols and glycerophospholipids in the endoplasmic reticulum:

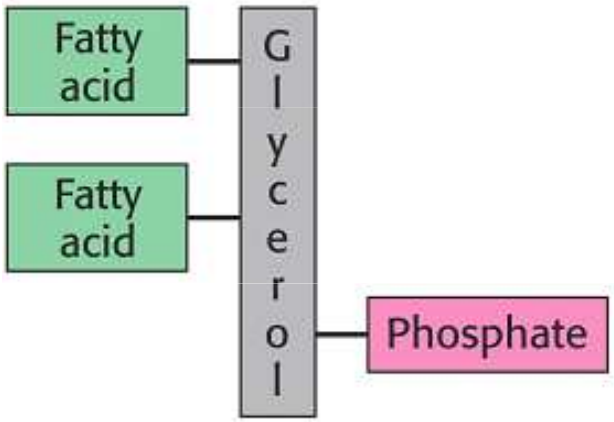


Phosphatidic acid

1,2-diacylglycerol-3-phosphoric acid



phosphatidyl



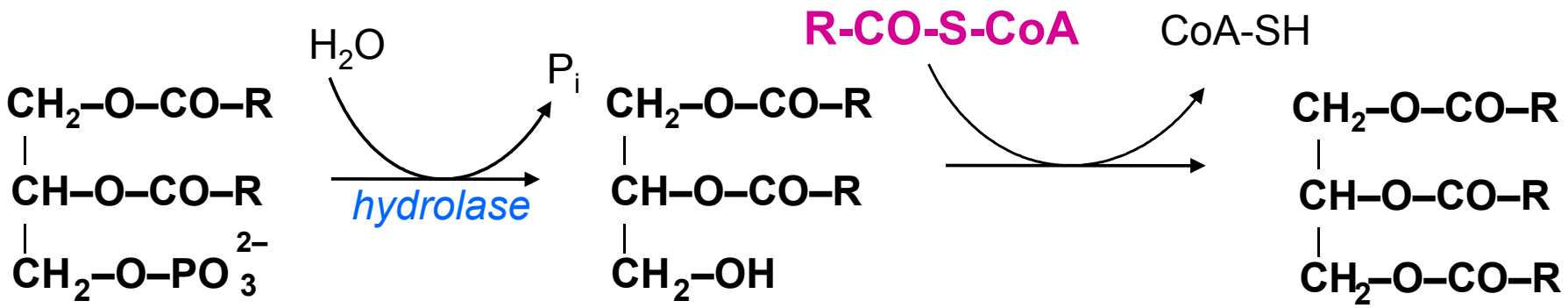
Distinguish

- **Phosphatidate** = anion of phosphatidic acid

  (after dissociation of two H^+)

- **Phosphatidyl** = acyl of phosphatidic acid

  (after removing one –OH group)



Phosphatidate **1,2-Diacylglycerol** **TRIACYLGLYCEROLS**

Small intestine → **Chylomicrons**
Liver cells → **VLDL**
Adipocytes → **Reserve fat**

Glycerophospholipids

Phosphatidylserine
Phosphatidylinositol
Cardiolipin
Phosphatidylcholine
Phosphatidylethanolamine

Fat reserve in adult body

Feature	Males	Females
Total body water	60 – 67 %	50 - 55 %
Total body fat	10 – 20 %	20 – 30 %
Main fat distribution	abdomen (apple)	buttocks (pear)

Compare

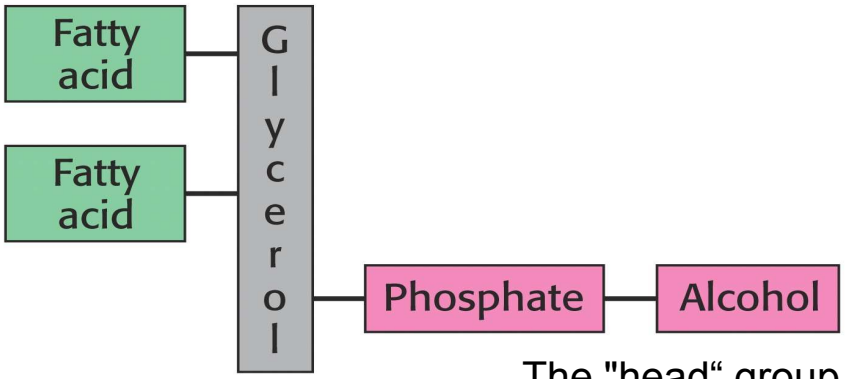
$$\text{alcohol in blood (‰)} = \frac{m_{alcohol}(\text{g})}{m_{body}(\text{kg}) \times f}$$

0.67 (males)
0.55 (females)

Schematic structure of complex lipids

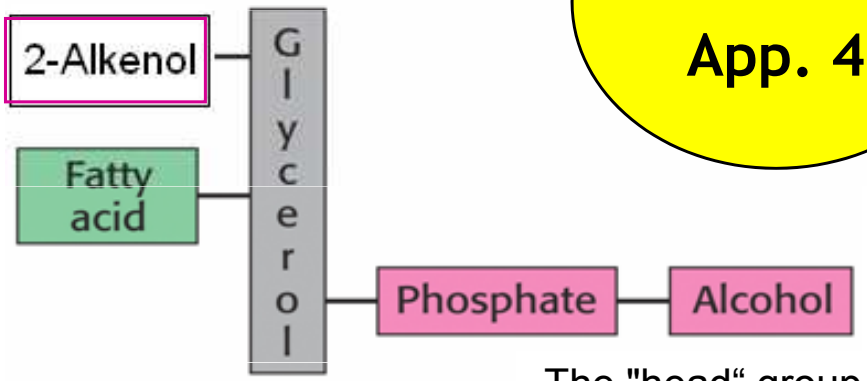
see MCH II App. 4

Glycerophospholipids



The "head" group

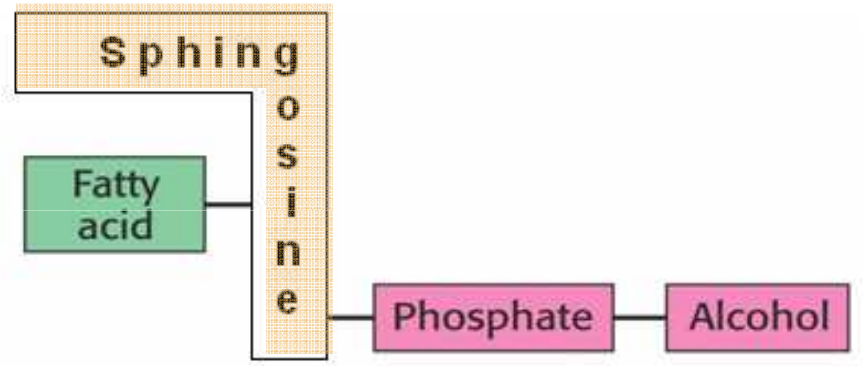
Plasmalogens



The "head" group

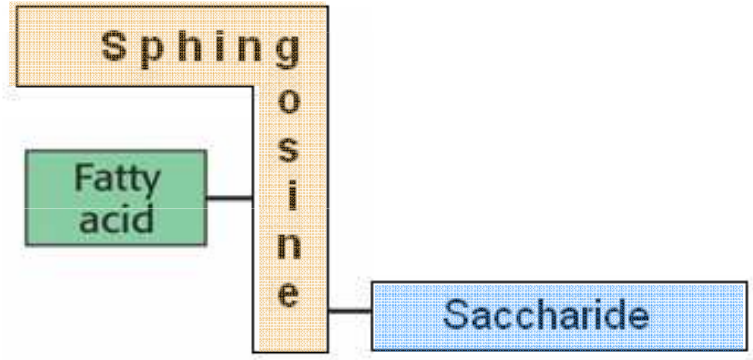
Sphingolipids:

Sphingophospholipids

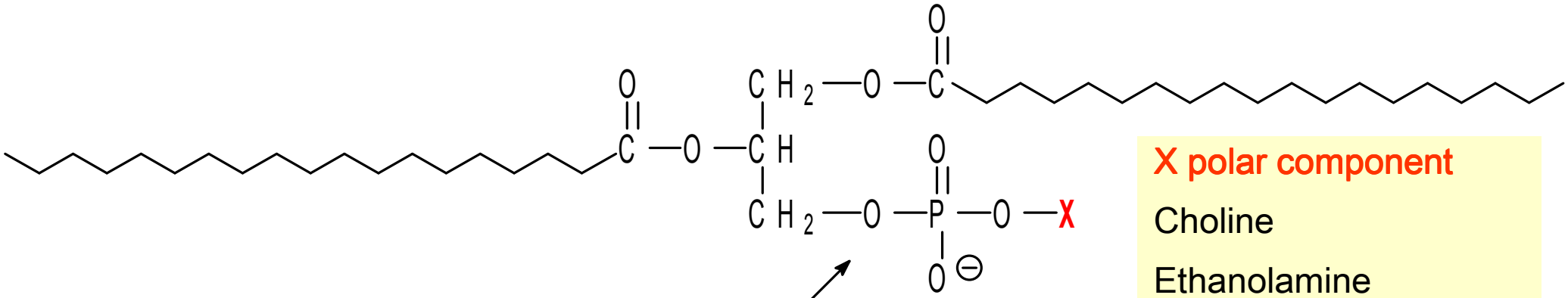


The "head" group

Glycolipids



Glycerophospholipids



diester of phosphoric acid

Disociated – negative charge

X polar component

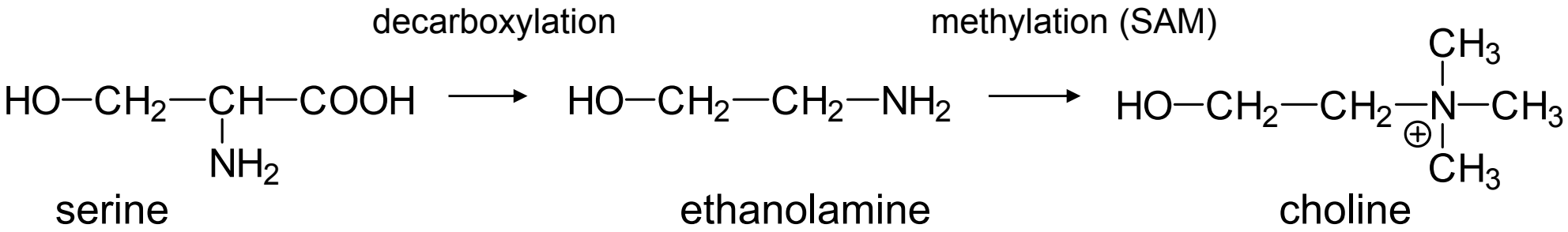
Choline

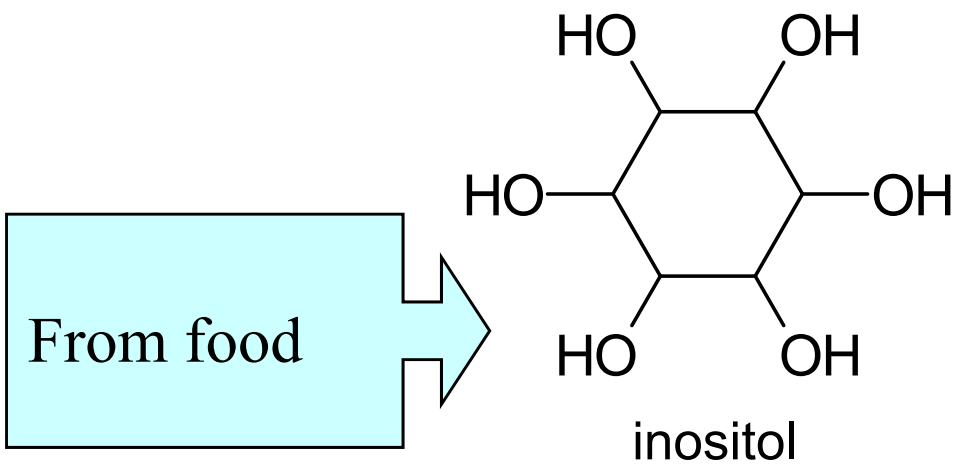
Ethanolamine

Serine

Inositol

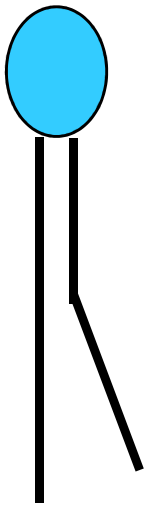
Polar components – Metabolic origin





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

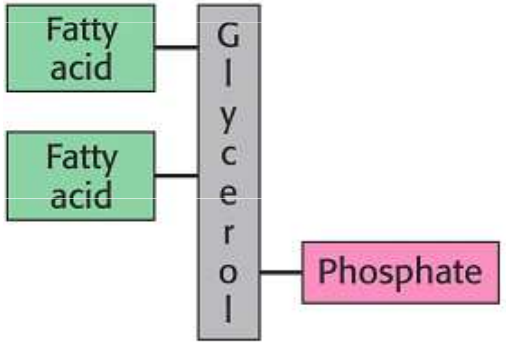
Glycerophospholipid **Sphingophospholipid**



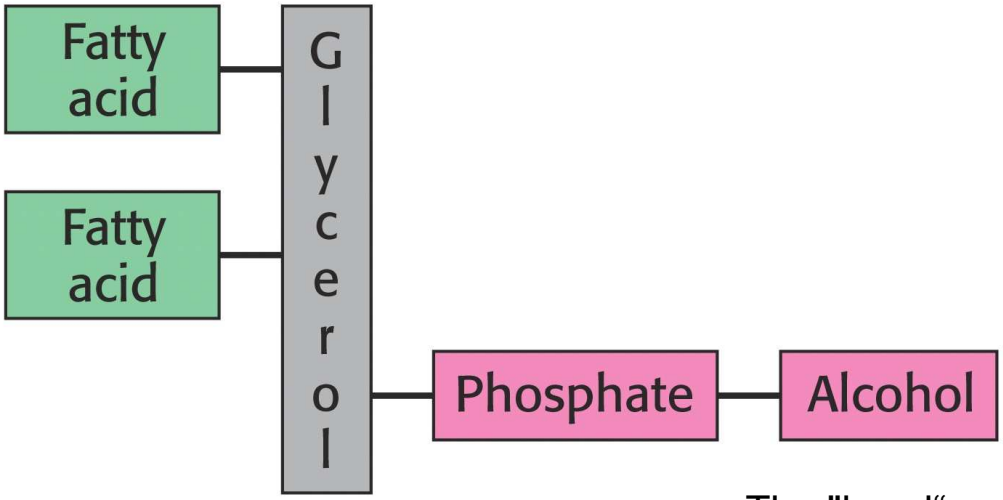
Pictogram of
a phospholipid molecule

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



The "head“ group

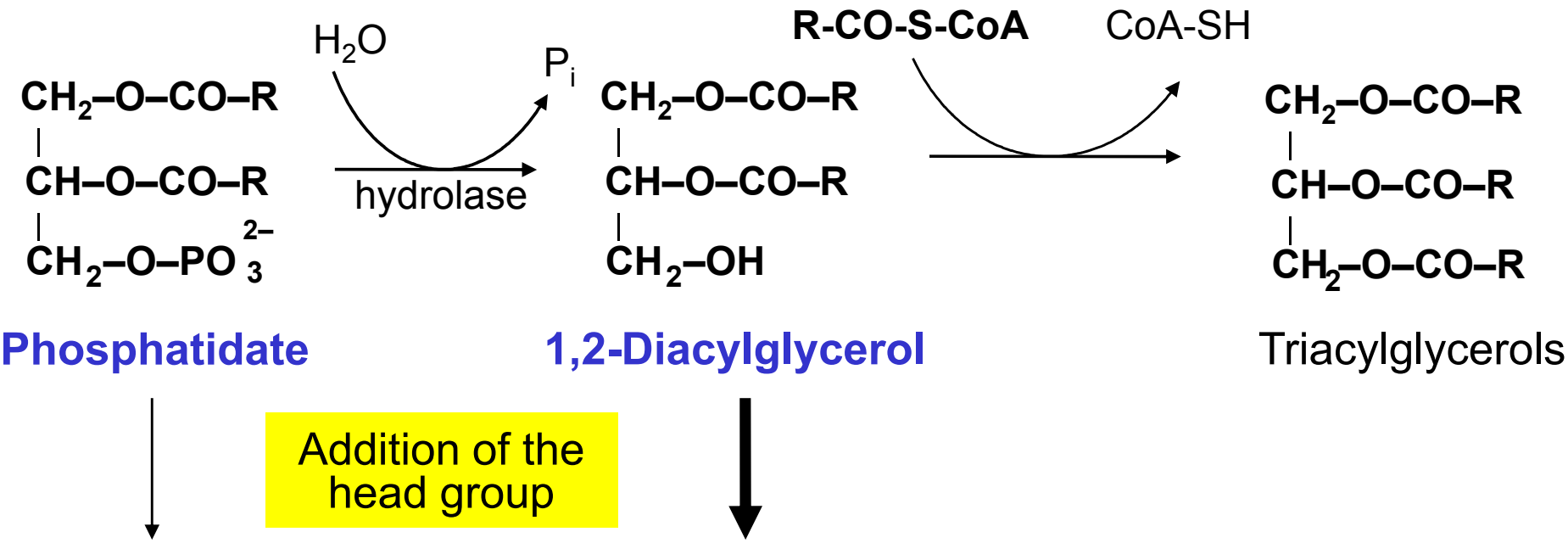
Biosynthesis of glycerophospholipids

The synthesis is localized on the **membranes of endoplasmic reticulum**. The competent enzymes are integral membrane proteins, the active sites are accesible 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 flipases,
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:



There are two mechanisms of additing 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, phosphatidyl serine).

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

Two ways of activation

diacylglycerol

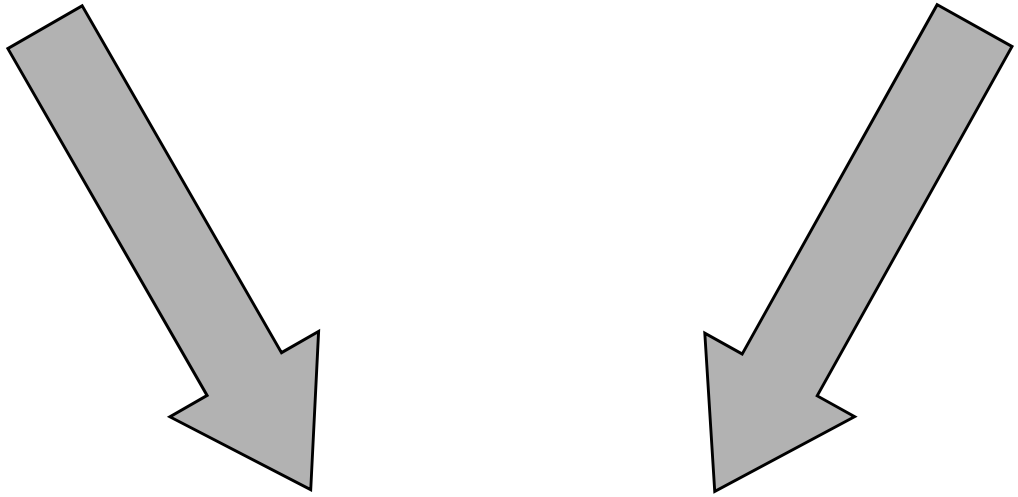
+

activated head group

activated phosphatidate

+

head group



glycerophospholipid

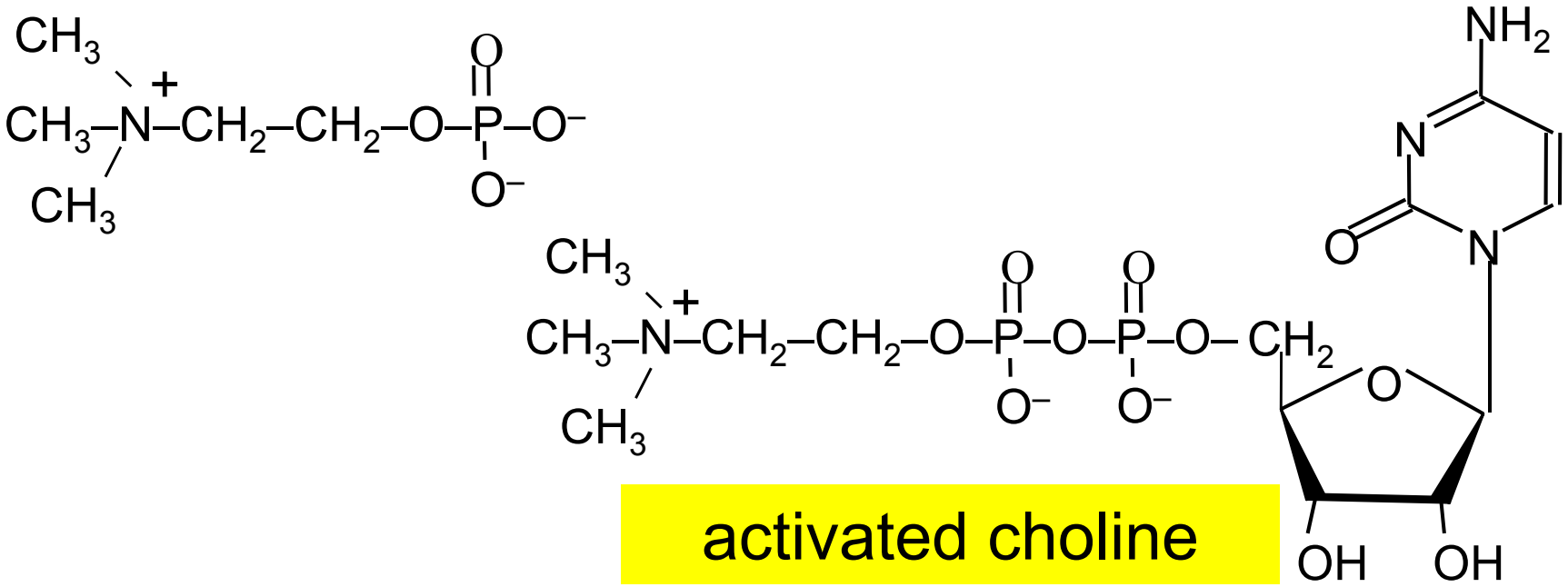
1 Synthesis of phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl serine

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

Activation of choline in two steps:

Choline + ATP → **choline phosphate** + ADP

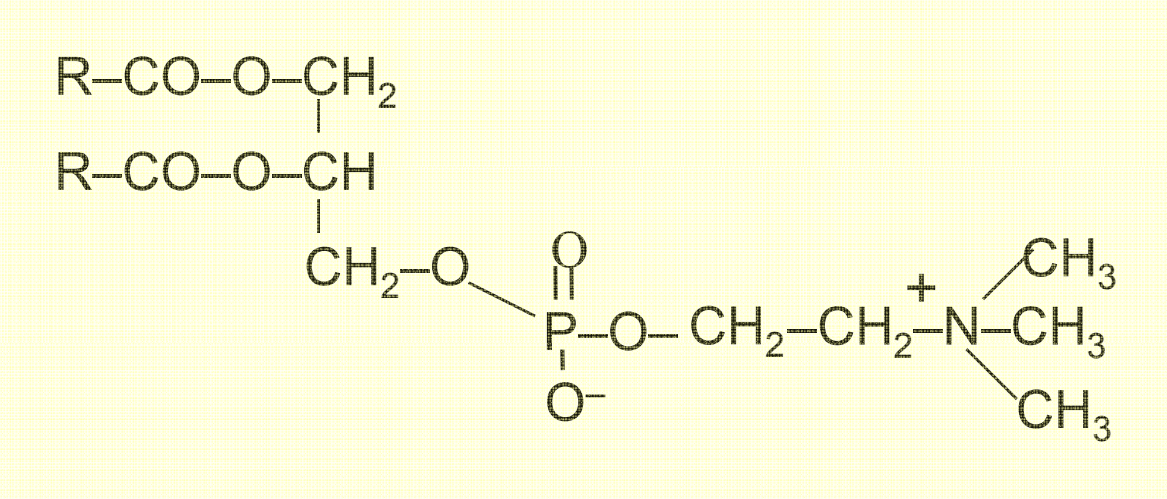
Choline phosphate + CTP → **CDP-choline** + PP_i



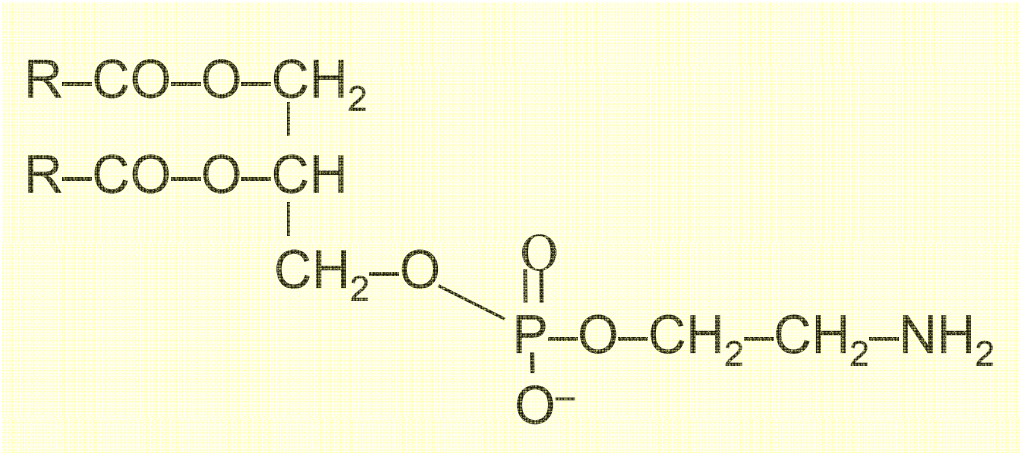
CDP-choline plays a part formally similar to that of UDP-glucose in the synthesis of glycogen.

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

1,2-diacylglycerol + CDP-choline ⟶ CMP + **phosphatidyl choline** (PC)



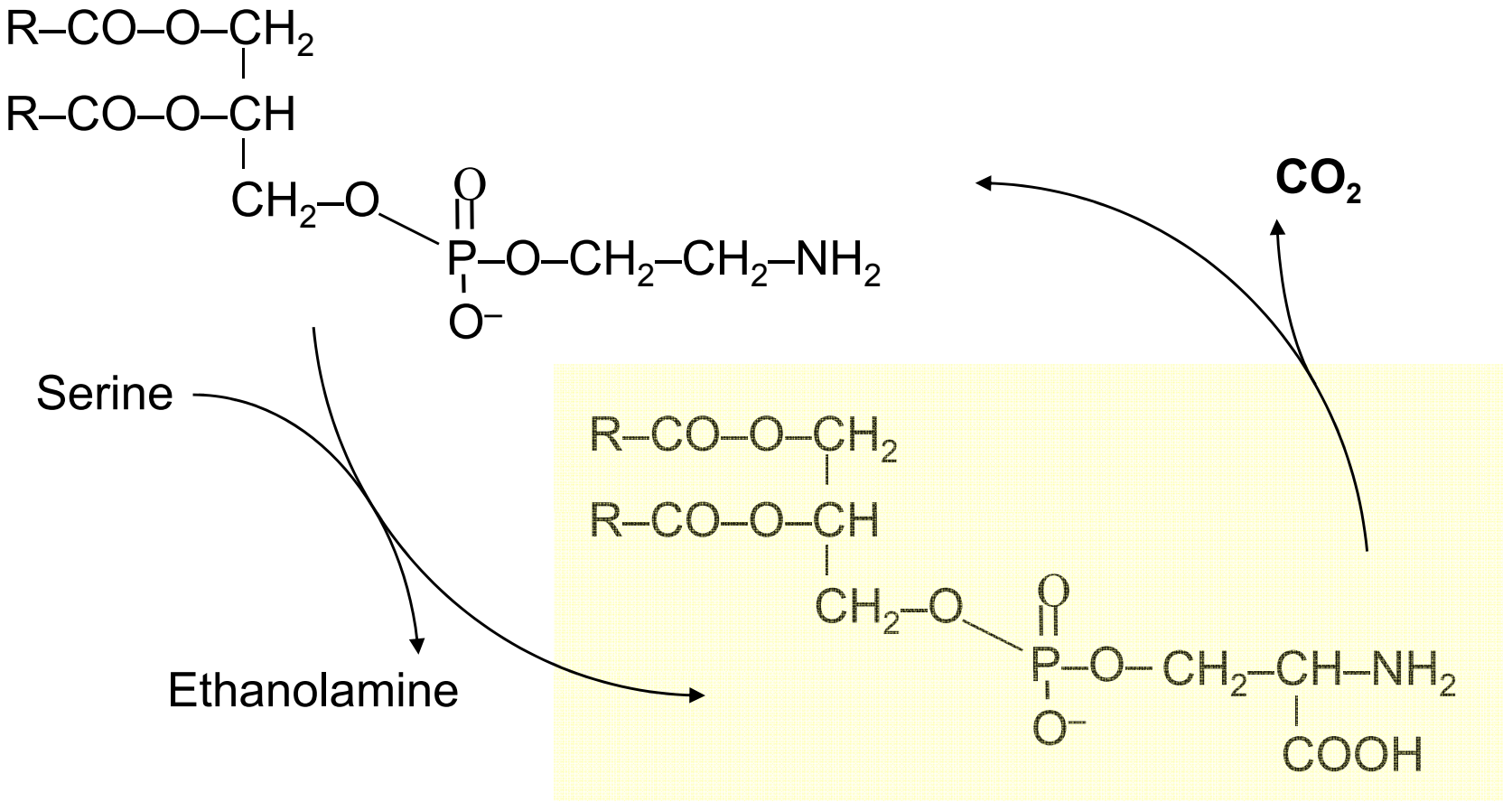
The biosynthesis of **phosphatidyl ethanolamine** (PE) is similar.



***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 ethanolamine + **serine** $\rightarrow$
phosphatidyl serine + ethanolamine

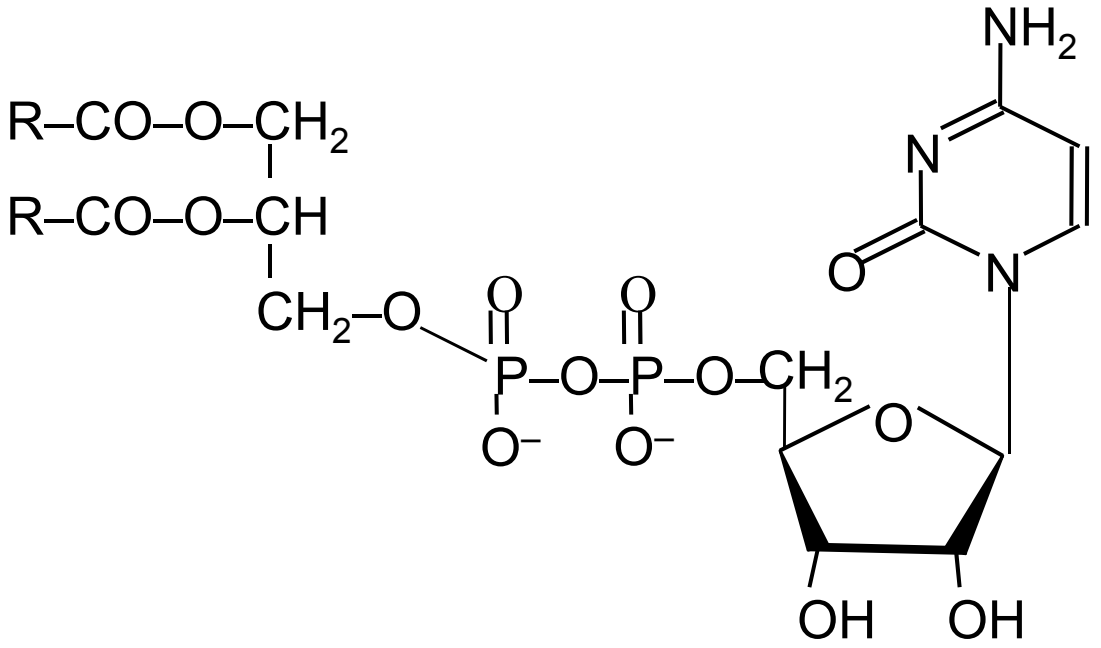


Phosphatidyl serine can be also **decarboxylated** to form **PE**.

2 Synthesis of phosphatidyl inositol and cardiolipin

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

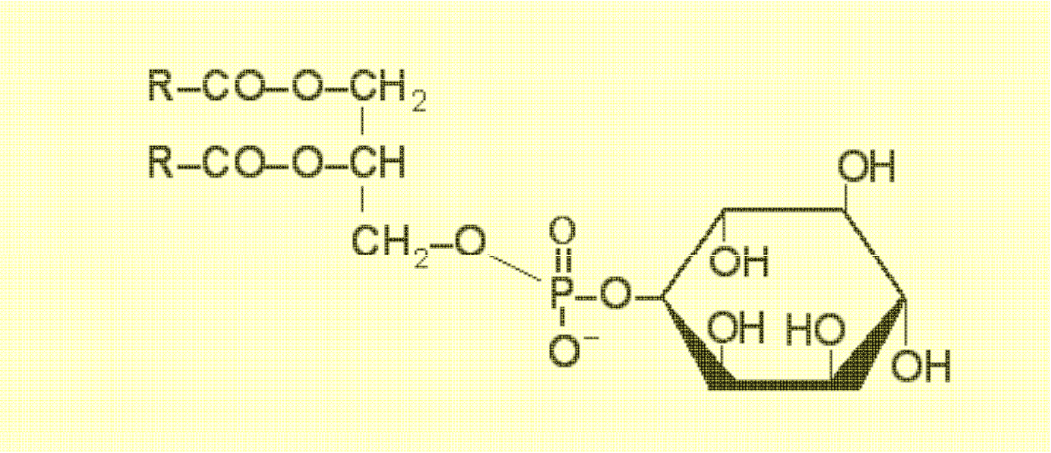
Phosphatidic acid + CTP $\rightarrow$ **CDP-diacylglycerol** + PP_i



CDP-diacylglycerol = activated phosphatidate

CDP-Diacylglycerol then reacts with free inositol to give phosphatidyl inositol (PI)

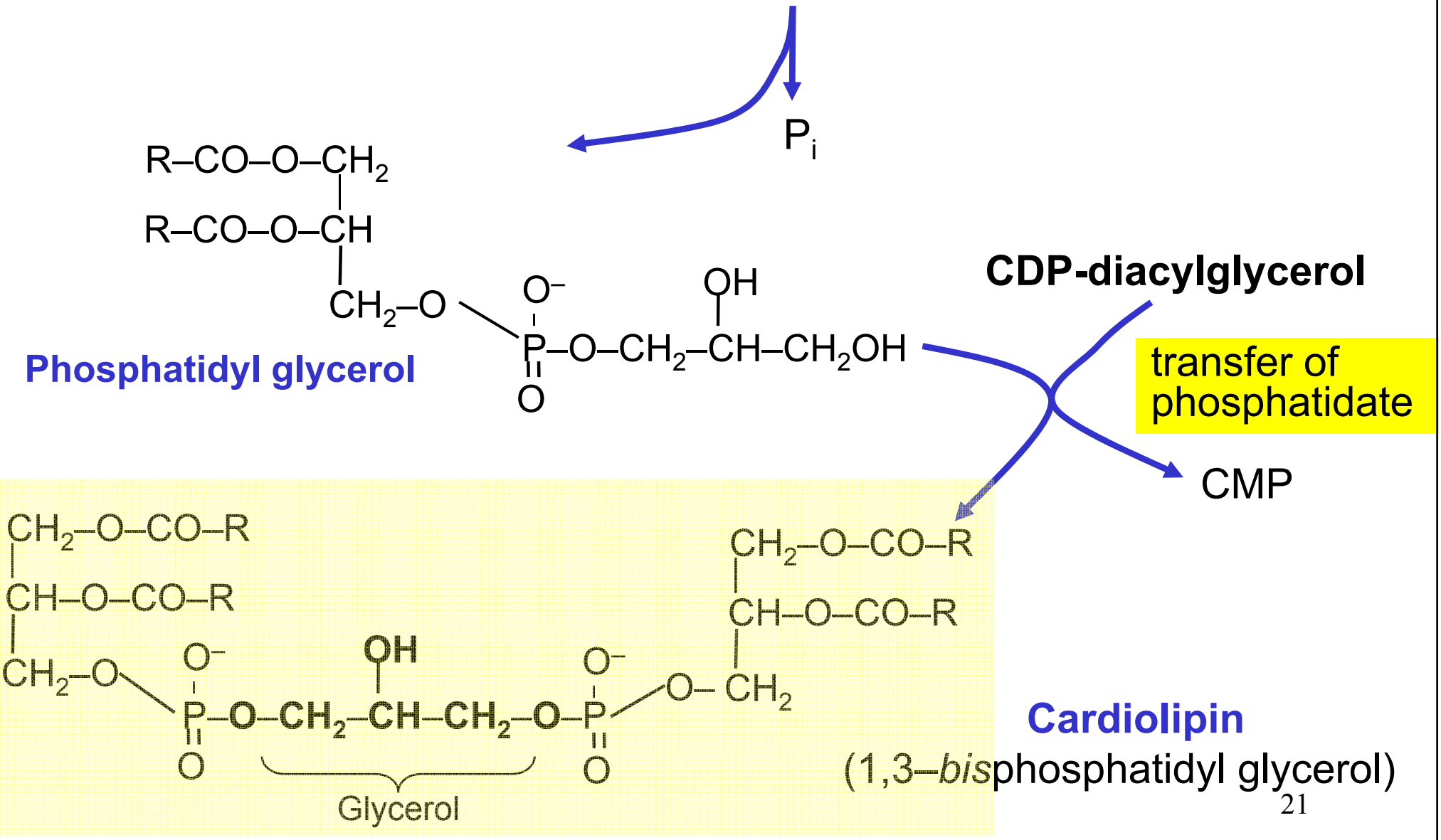
CDP-diacylglycerol + inositol $\rightarrow$ CMP + phosphatidyl inositol (PI)



Further phosphorylations of PI generate phosphatidyl inositol *bis*phosphate (PIP_2) which is an intermediate of the phosphatidyl inositol cycle generating important intracellular messengers IP_3 and diacylglycerol.

Cardiolipin (constituent of the inner mitochondrial membrane)

CDP-diacylglycerol + glycerol 3-P → phosphatidyl glycerol 3-P + CMP

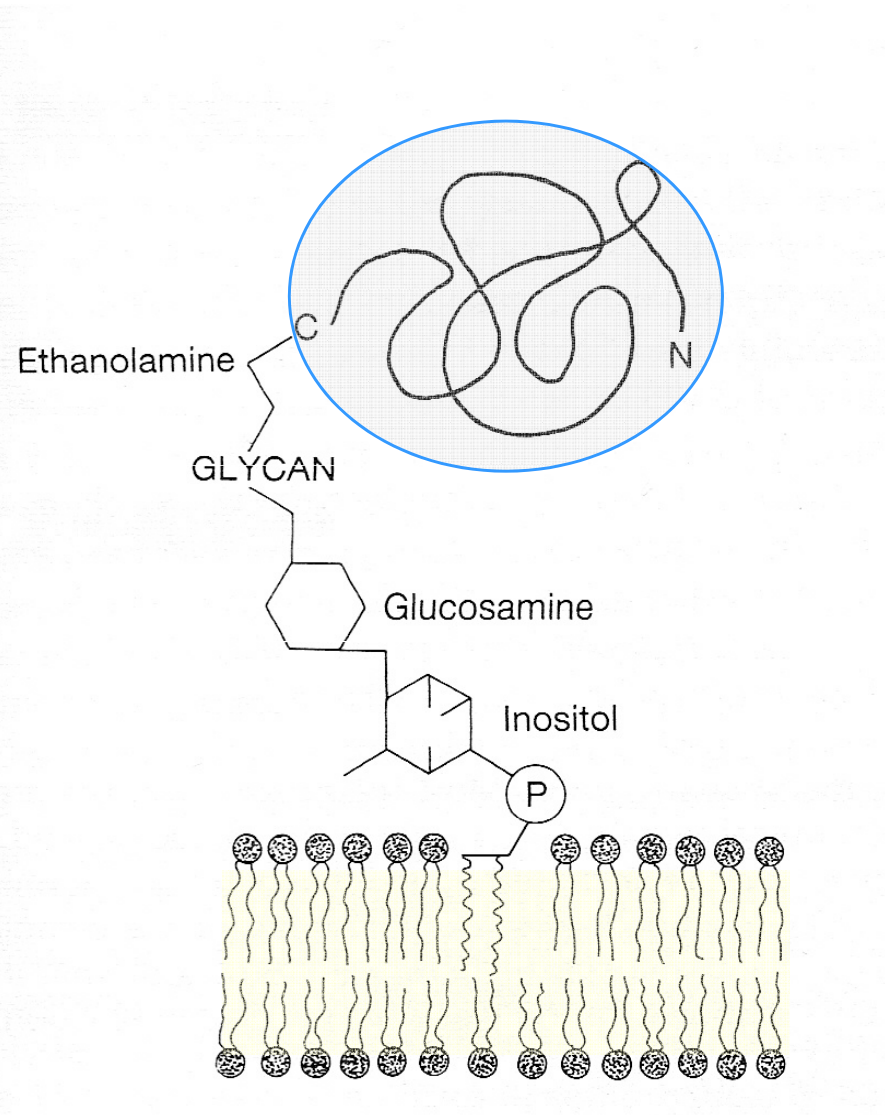


Glycerophospholipids are

- essential structural components of all biological **membranes (bilayer)**
- essential components of **lipoproteins** in blood **(monolayer)**
- 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

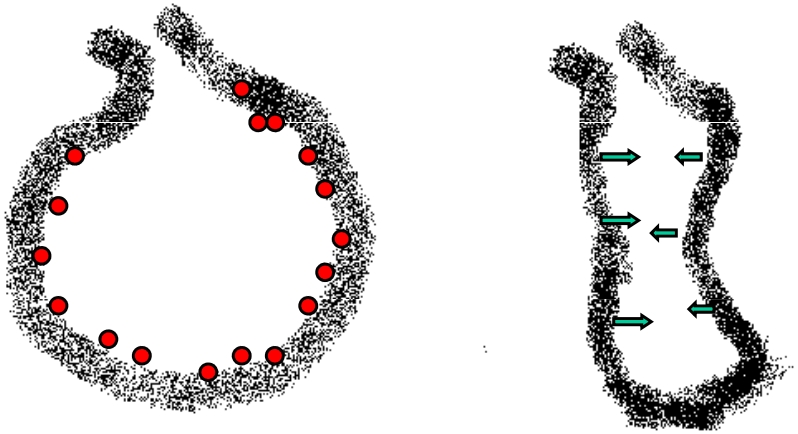
The linkage between the COOH-terminus of a protein and phosphatidylinositol fixed in the membrane lipidic bilayer exist in several ectoenzymes (alkaline phosphatase, acetylcholinesterase, some antigens).



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.

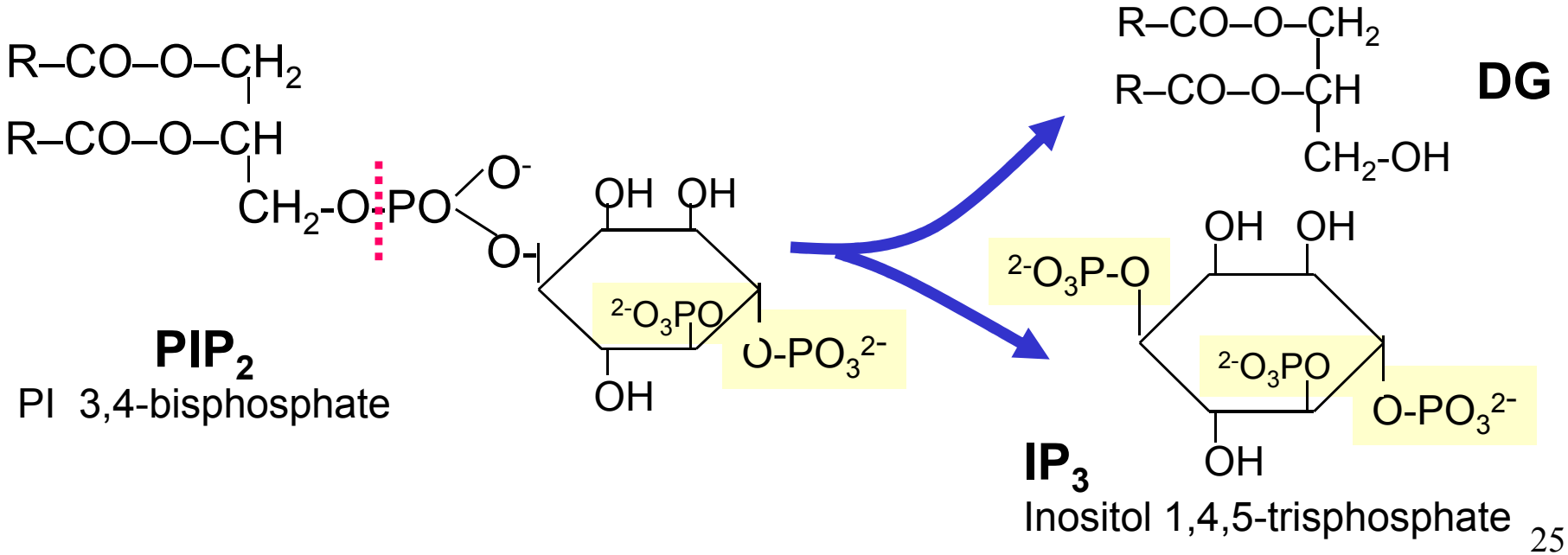


The respiratory distress syndrome (RDS) of premature infants is caused, at least in part, by a deficiency in the synthesis of lung surfactant.

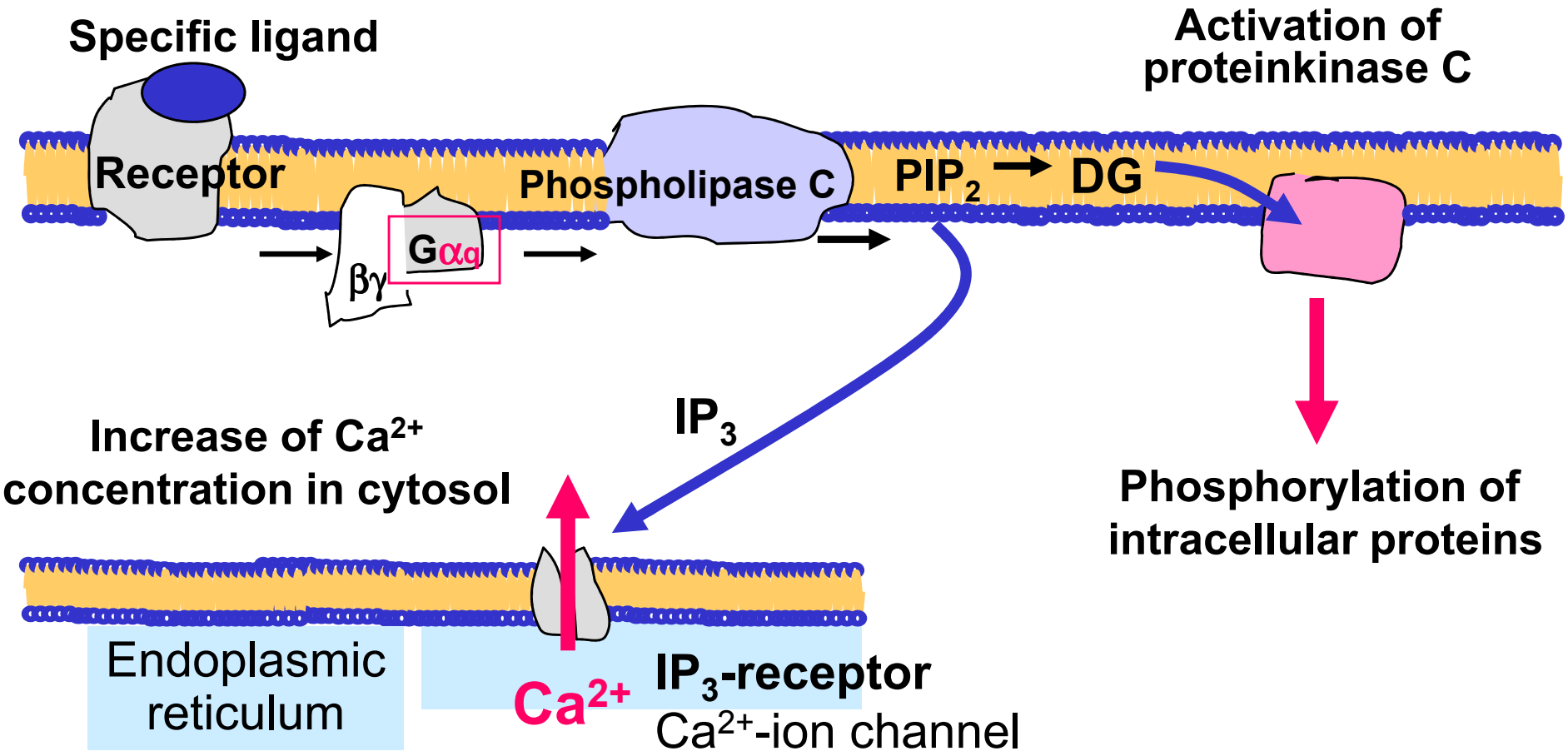
Phosphatidyl inositol phosphates

(PIP, PIP_2, PIP_3) 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_2) to **diacylglycerol** and **inositol 1,4,5-trisphosphate** (IP_3), both are **second messengers**



Phosphatidyl inositol cascade



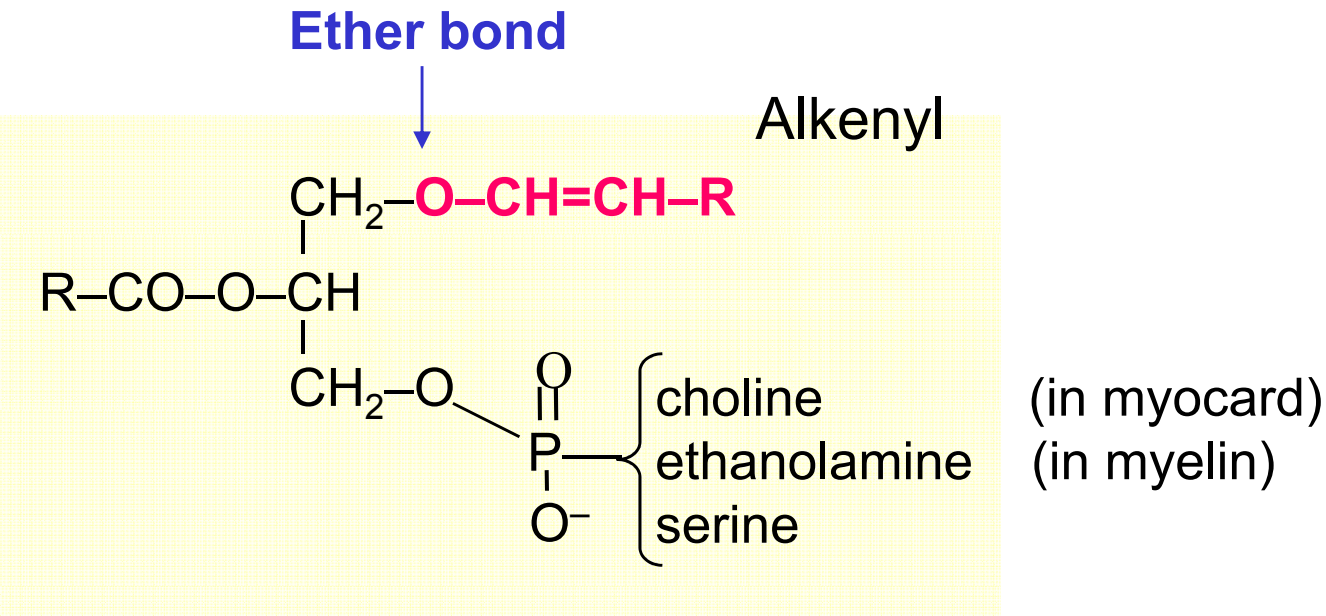
Second messengers

- are intracellular compounds
- their concentration raises after binding signal molecule (hormone) 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.
- typical example of second messenger is cAMP

Plasmalogens

are modified glycerophospholipids – called alkoxylipids or ether glycerophospholipids.

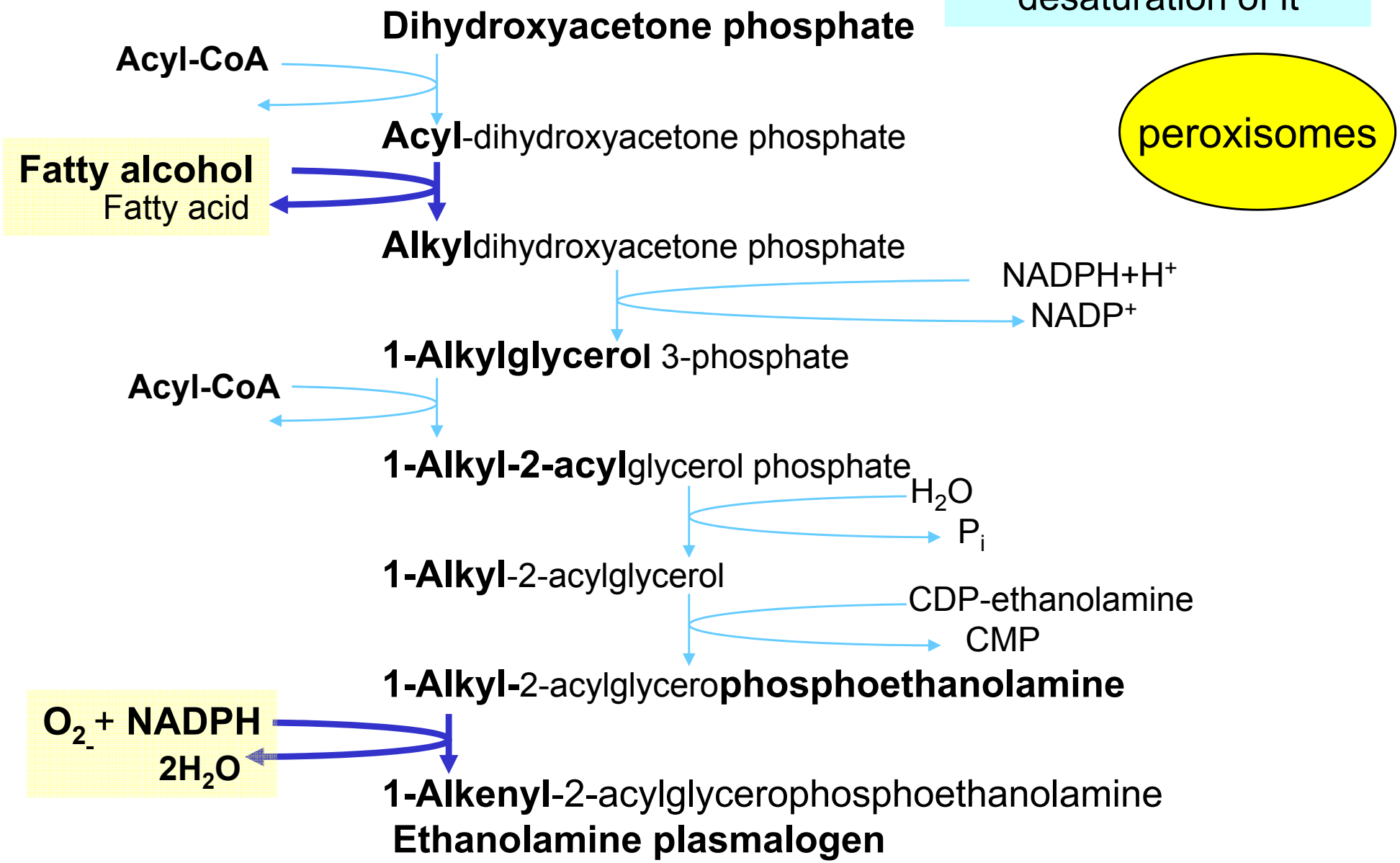
Plasmalogens represent about 20 % of glycerophospholipids. Choline plasmalogen is found in myocard, in the liver (~1 %), ethanolamine plasmalogen in myelin (~ 23 %).



Synthesis of plasmalogens

(ether glycerophospholipids, alkoxylipids)

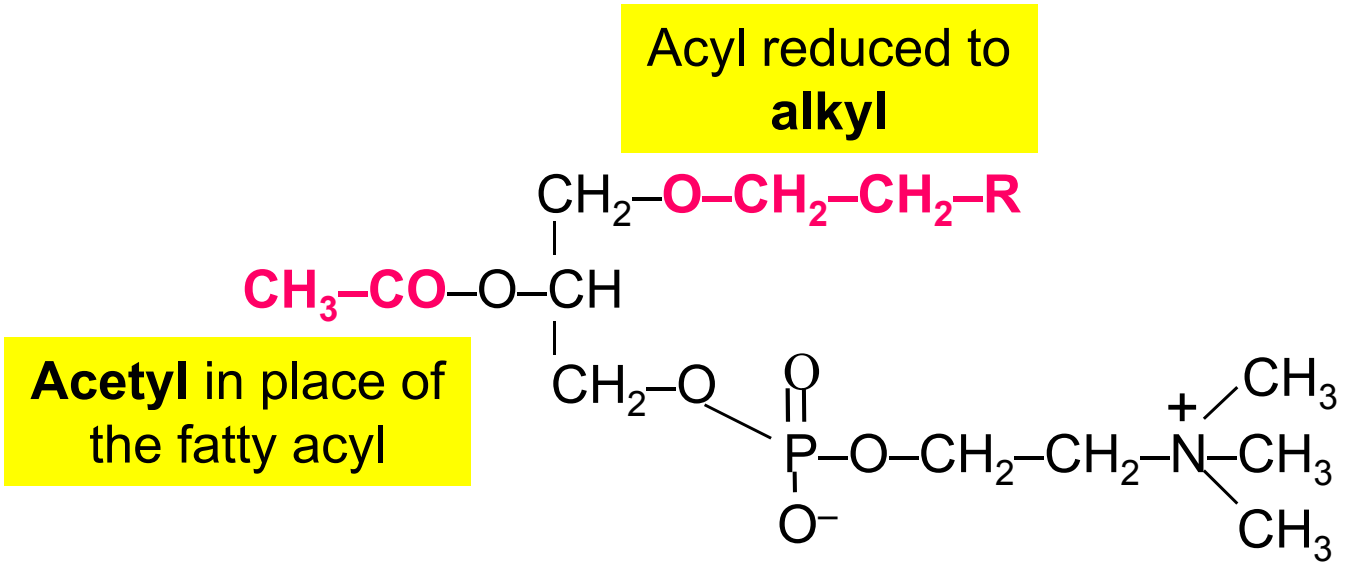
Exchange of the acyl for an alcohol and the desaturation of it



PAF (platelet activating factor)

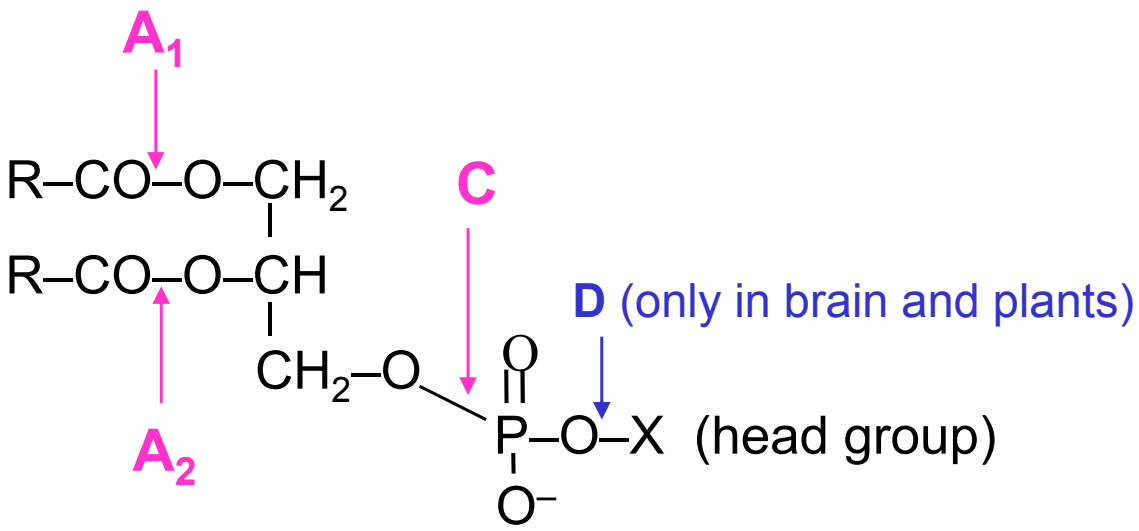
is an unusual alkoxylipid in which the alkenyl group of plasmalogens was reduced to saturated **alkyl** and the fatty acyl at C2 was exchanged for **acetyl**.

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 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:



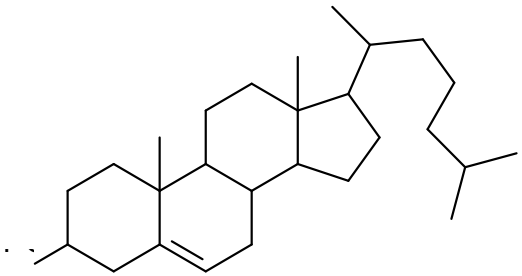
Phospholipase A_1 (PL A_1) exhibits preference for phosphatidyl ethanolamines.

Phospholipase A_2 obviously prefers phosphatidyl cholines and is of special importance because it liberates arachidonic acid as a precursor of **eicosanoids**.

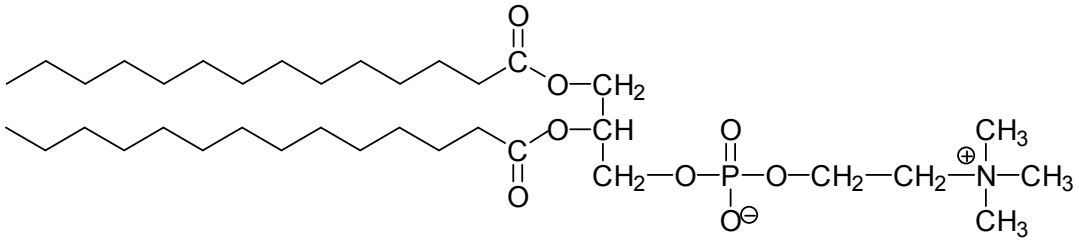
Either PL A_1 or A_2 set free only one acyl residue and leaves **lysophospholipid** The remaining acyl group is removed by the action of lysophospholipase-transacylase (formerly called phospholipase B).

Phospholipase C is stimulated by some hormonal signals and some neurotransmitters. It hydrolyses PIP_2 to IP_3 and DG – the crucial step in phosphatidyl inositol cascade.

Lysolecithine is formed also during esterification of blood cholesterol in HDL particles by the action of LCAT



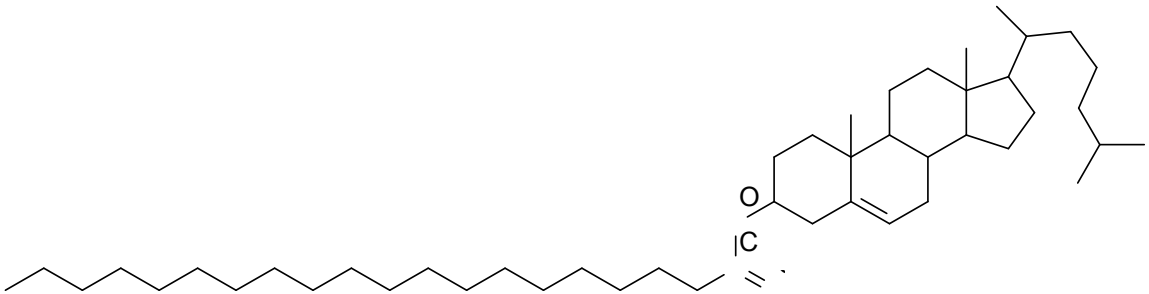
cholesterol



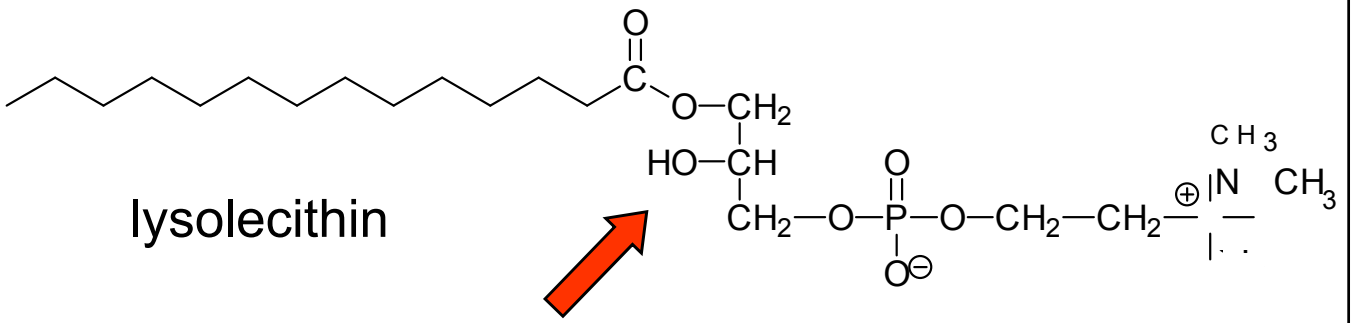
lecithin

LCAT

lecithin cholesterol acyl transferase



cholesteryl ester

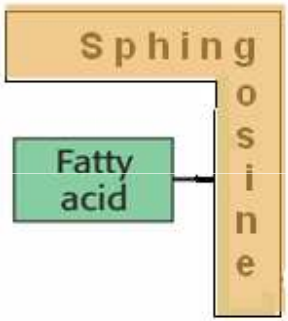


lysolecithin

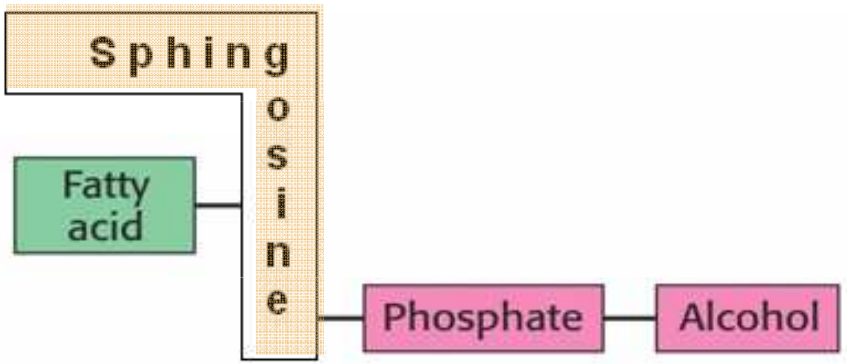
lyso = 2-deacyl

Sphingolipids – schematic structure

Ceramide
N-Acylsphingosine

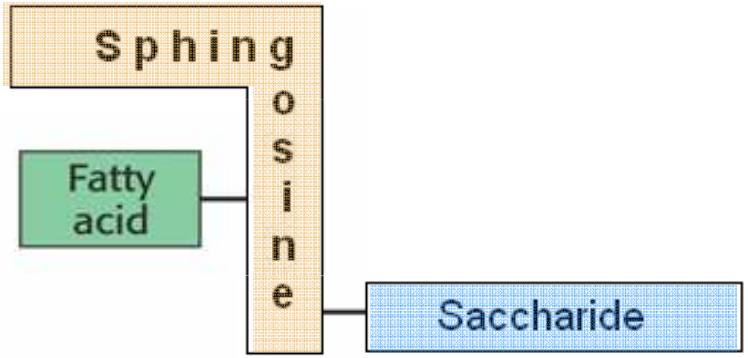


A sphingophospholipid



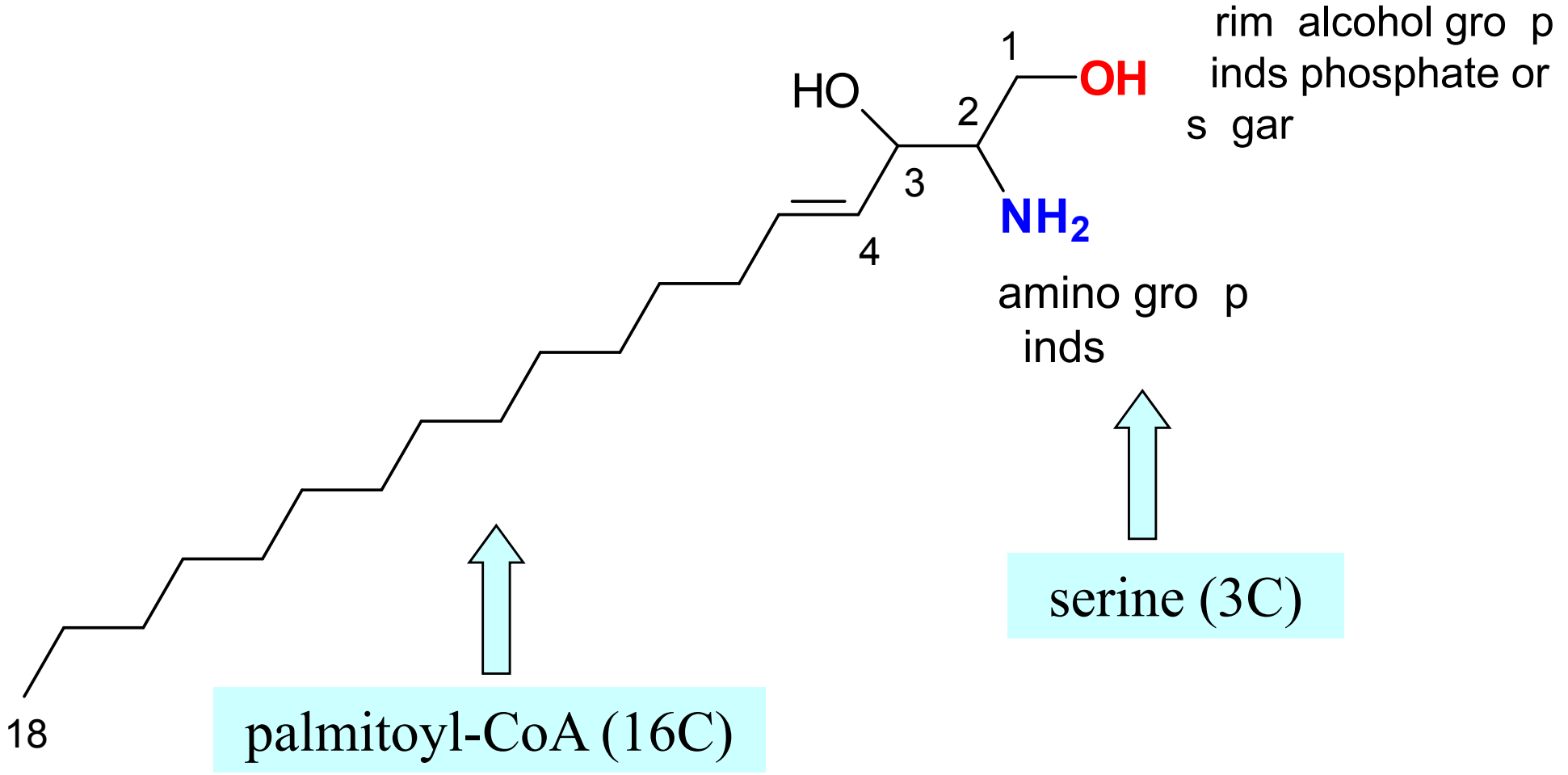
The "head" group

A glycolipid

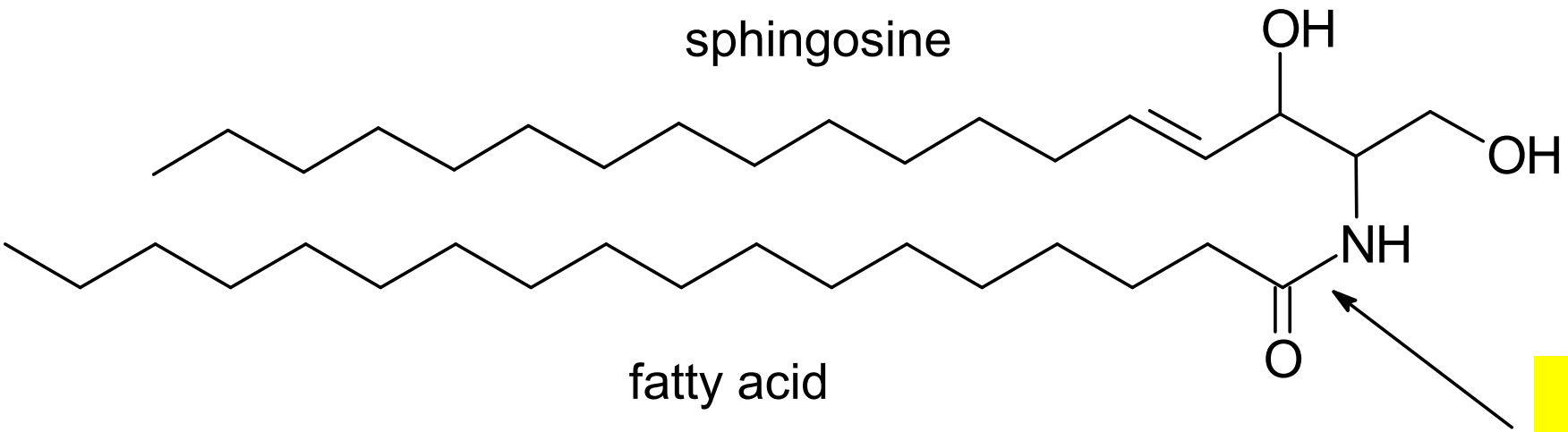


Sphingosine has 18 C (16 from palmitate, 2 from serine)

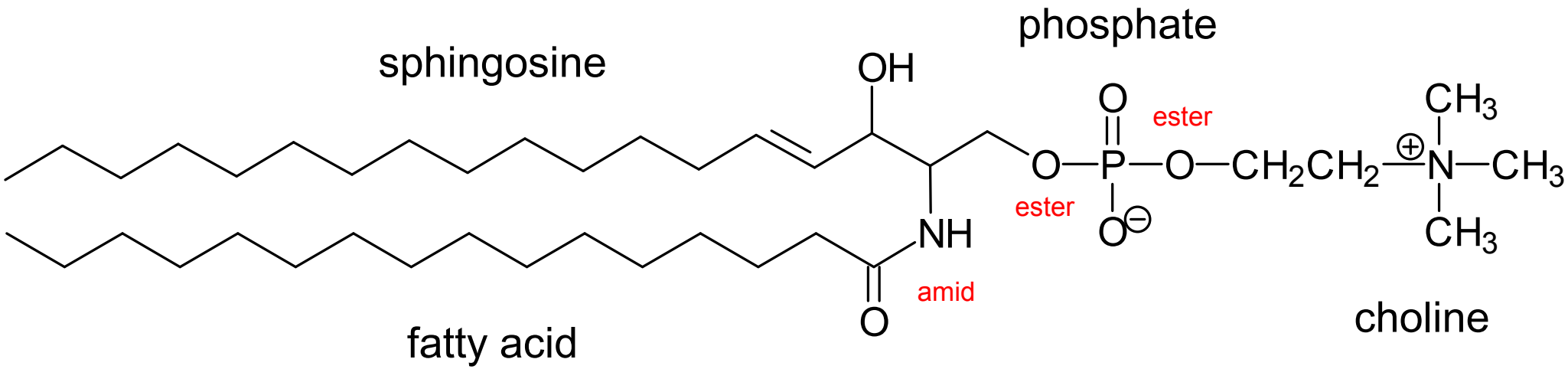
(systematic name 2-aminooctadec-4-ene-1,3-diol)



Ceramides are amides (*N*-acylated sphingosines)



Sphingomyelines

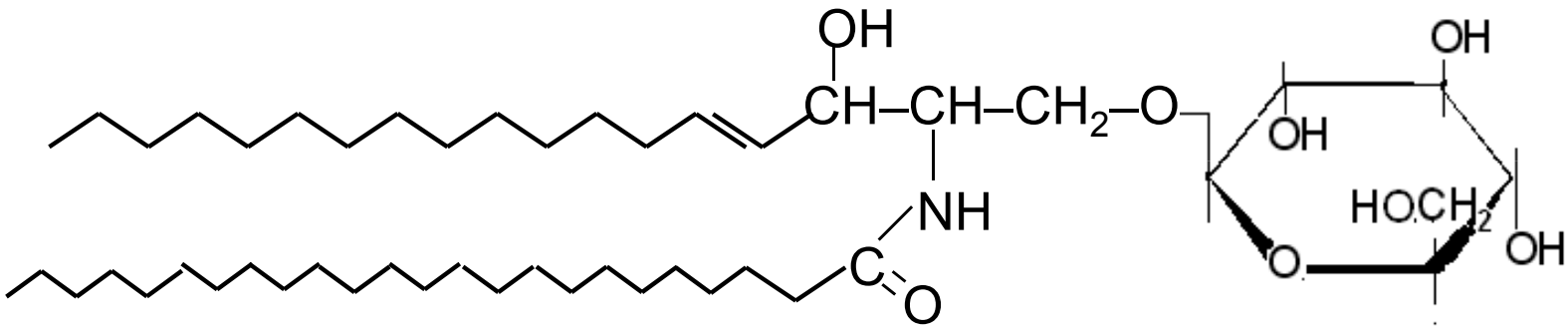


FA – lignoceric 24:0 and nervonic 24:1(15)

Glycolipids

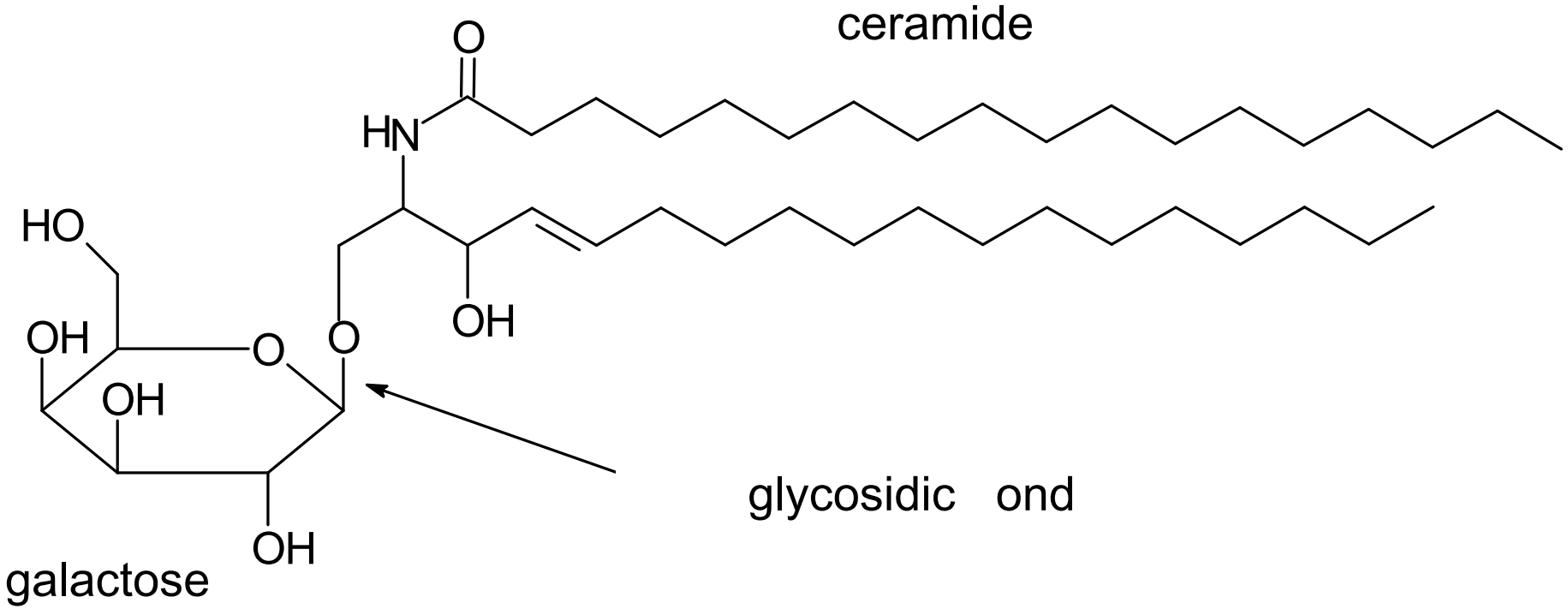
are ceramides to which a saccharidic component is attached by glycosidic bond: monoglycosylceramides – **cerebrosides**, oligoglycosylceramides, acidic sulphoglycosylceramides, and sialoglycosylceramides – **gangliosides**.

Cere roside



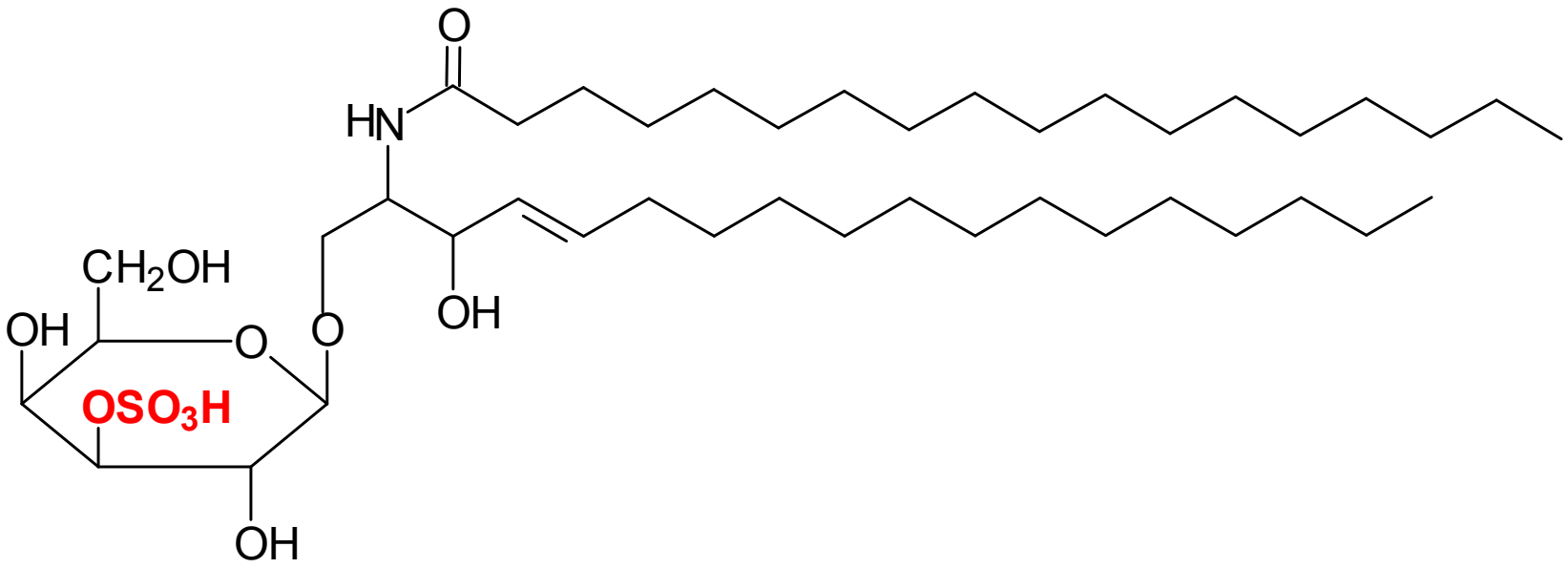
β-D-Glucopyranosyl

Cerebroside (monoglycosylceramide)



galactosylceramide

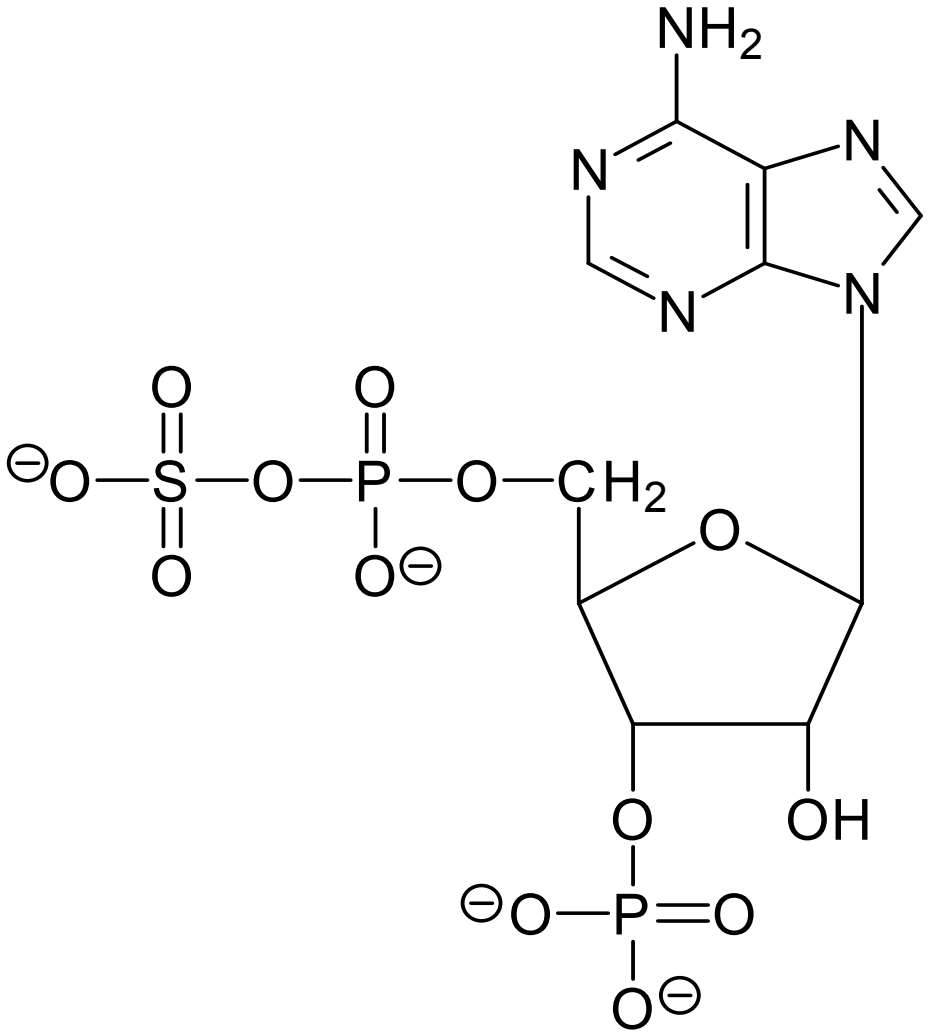
Glycolipids can be sulfated



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

PAPS (3'-phosphoadenosine-5'-phosphosulfate)

- a mixed anhydride of H_2SO_4 and H_3PO_4
- sulfation = esterification of hydroxyl groups by sulfuric acid



Saccharidic components of glycolipids - examples:

Cerebroside Ceramide–(1←1β)Glc

Oligoglycosylceramide Ceramide–(1←1β)Glc (4←1β)Gal

Sulphoglycosphingolipid Ceramide–(1←1β)Glc-3´-sulphate

Gangliosides

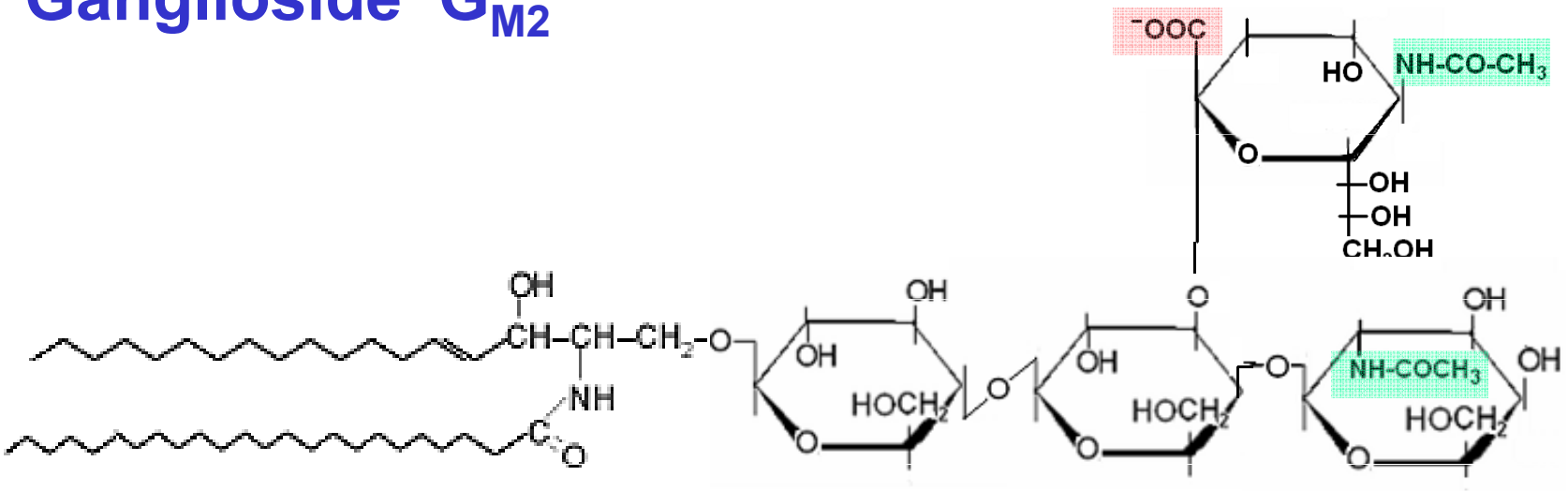
G_{M3} (monosialo ganglioside type III)

Ceramide–(1←1β)Glc-(4←1β)Gal
↑(3←2α)
NeuAc

G_{M2} Ceramide–(1←1β)Glc-(4←1β)Gal-(4←1β)GlcNAc
↑(3←2α)
NeuAc

G_{M1} Ceramide–(1←1β)Glc-(4←1β)Gal-(4←1β)GlcNAc-(3←1β)Gal
↑(3←2α)
NeuAc

Ganglioside G_{M2}

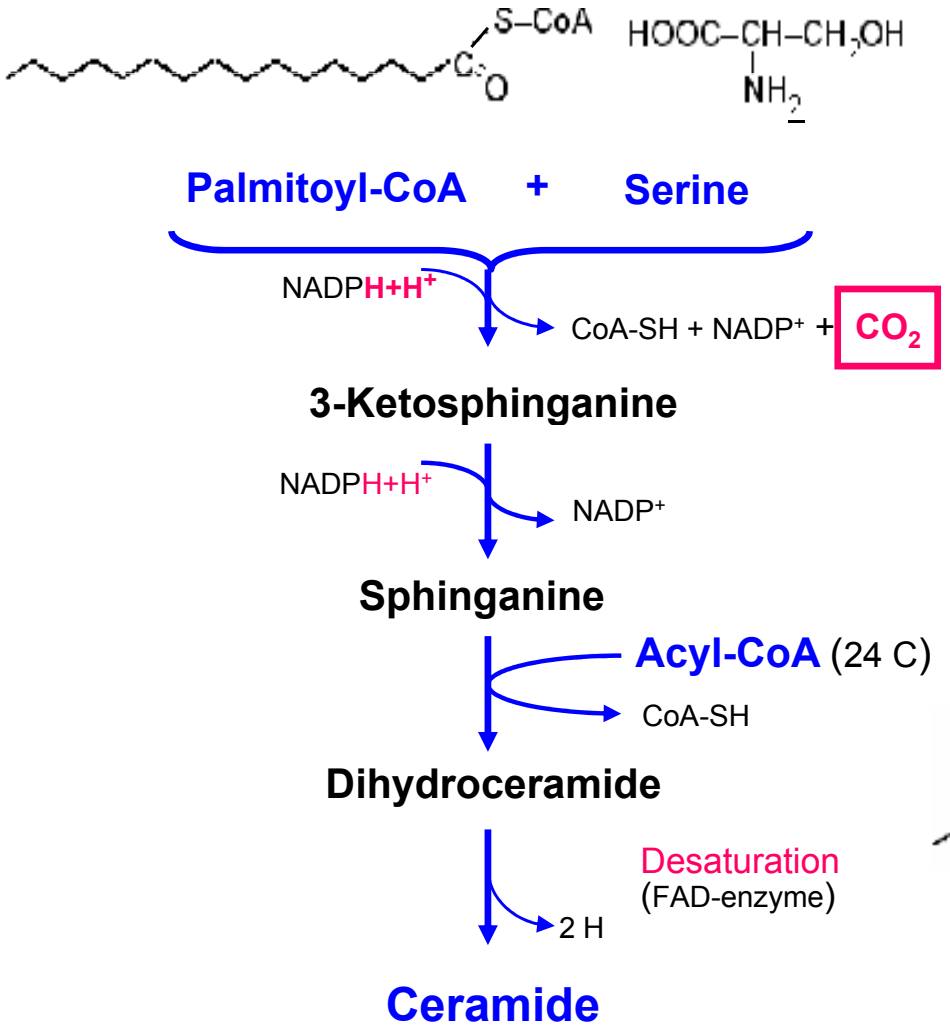


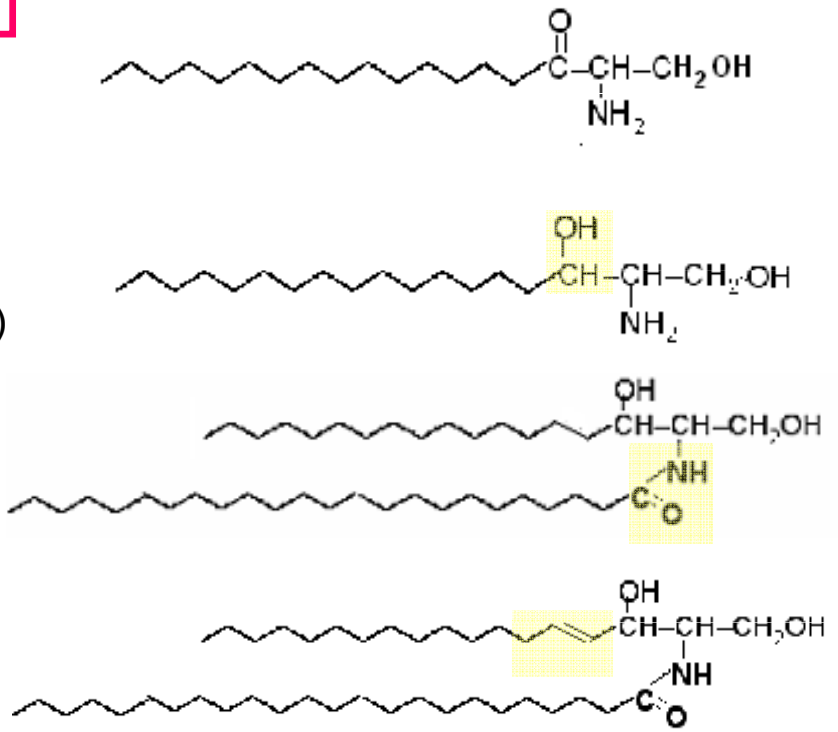
NeuAc
↓(3←2α)

Ceramide–(1←1β)Glc-(4←1β)Gal-(4←1β)GlcNAc

Biosynthesis of ceramide

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





Biosynthesis of sphingomyelin and glycolipids by attachment of activated group to free 1-hydroxyl of ceramide

Synthesis of sphingomyelin

CDP acts as a carrier of phosphoryl choline:

Ceramide + CDP-choline → Sphingomyelin + CMP

activated choline

Synthesis of glycolipids by the transfer from UDP-monosaccharide:

Ceramide + UDP-Gal → Cerebroside + UDP

activated sugar

Attachment of further glycosyls proceeds in a similar way.

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

Oligoglycosylceramide + CMP-NeuAc → Ganglioside + CMP

Degradation of sphingolipids in lysosomes

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

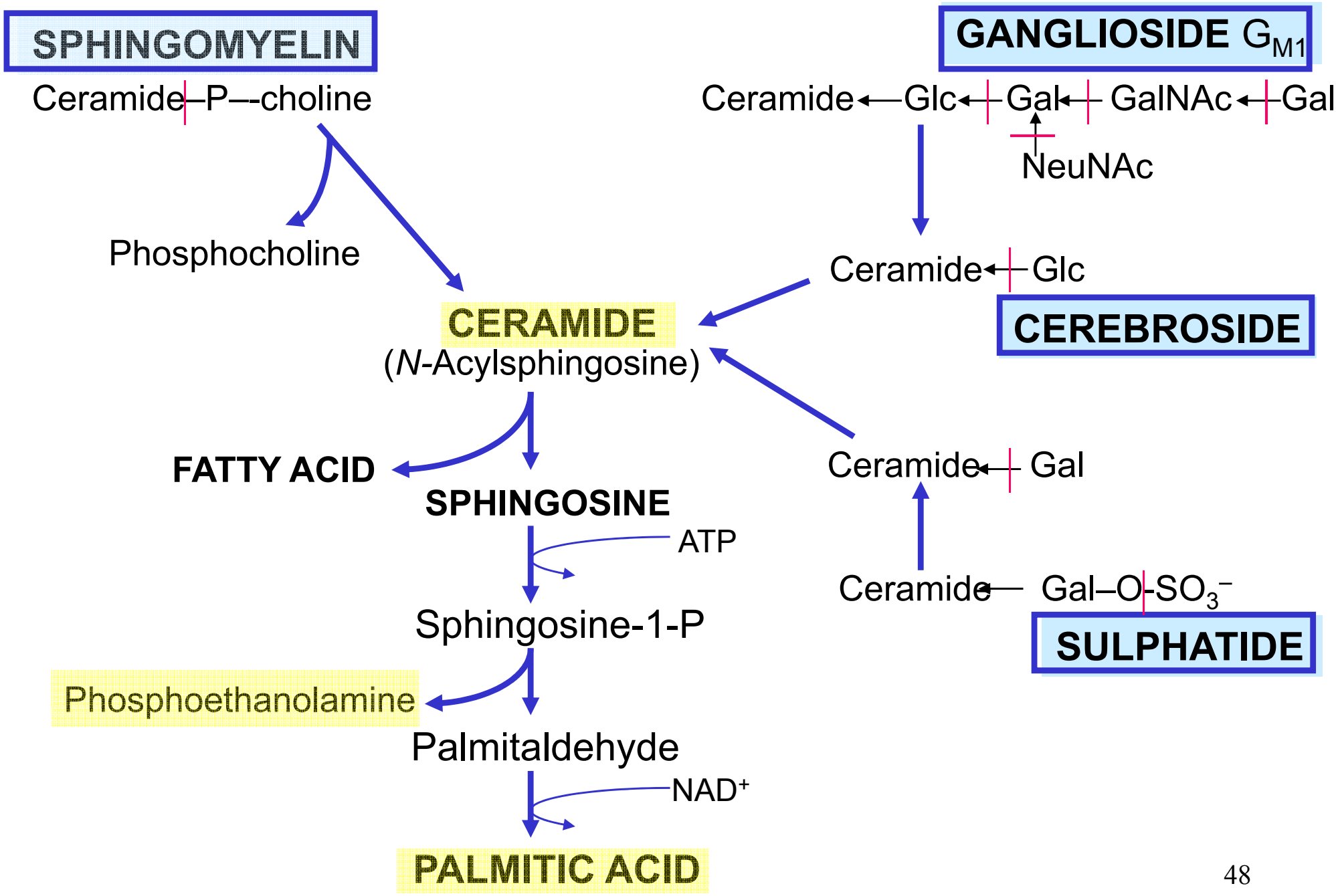
Sphingomyelins loose phosphocholine to give **ceramide**.

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



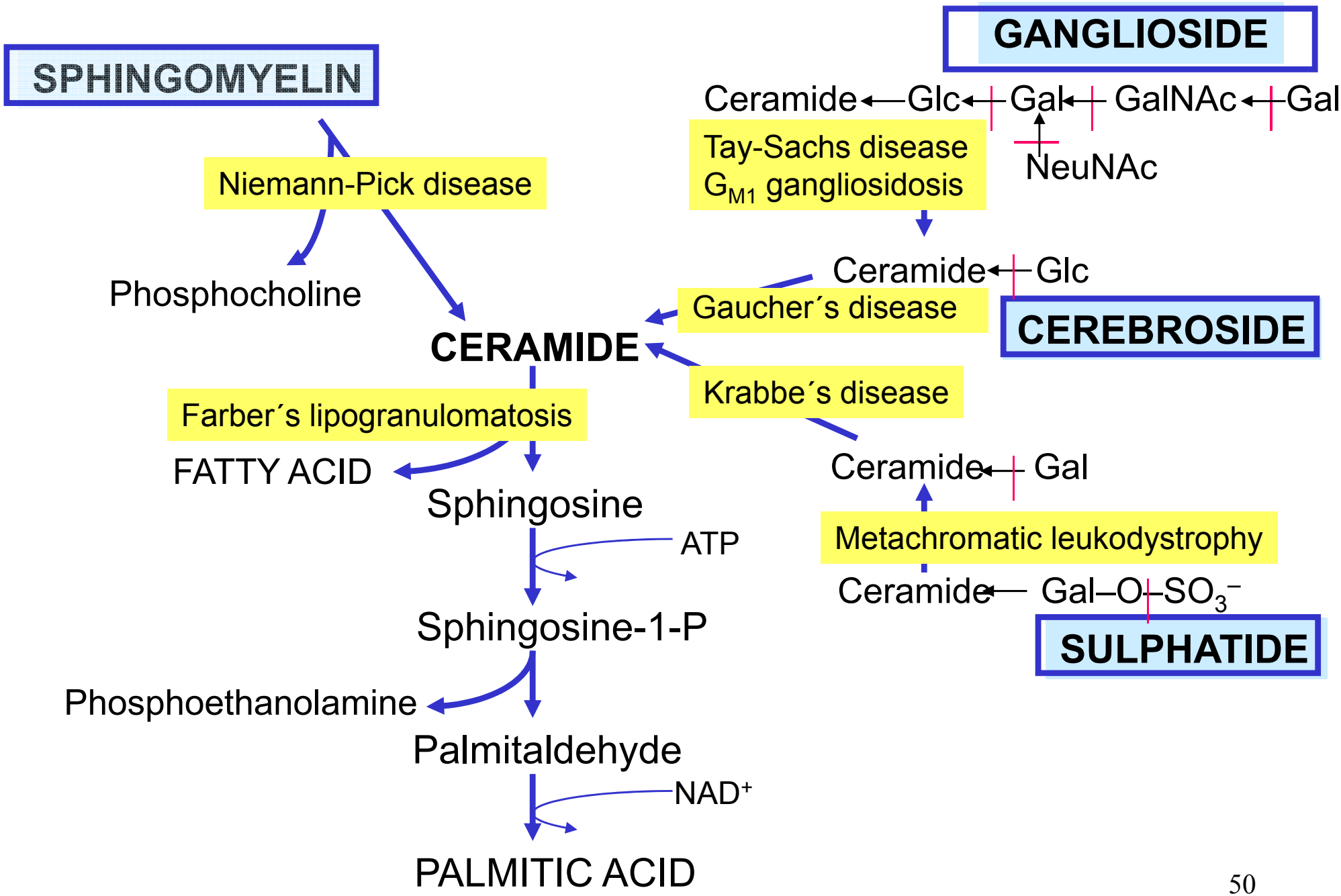
In general, the turnover of sphingolipids is very slow, particularly in brain.

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.

Sphingolipidoses – genetic defects (deficiency of lysosomal enzymes)

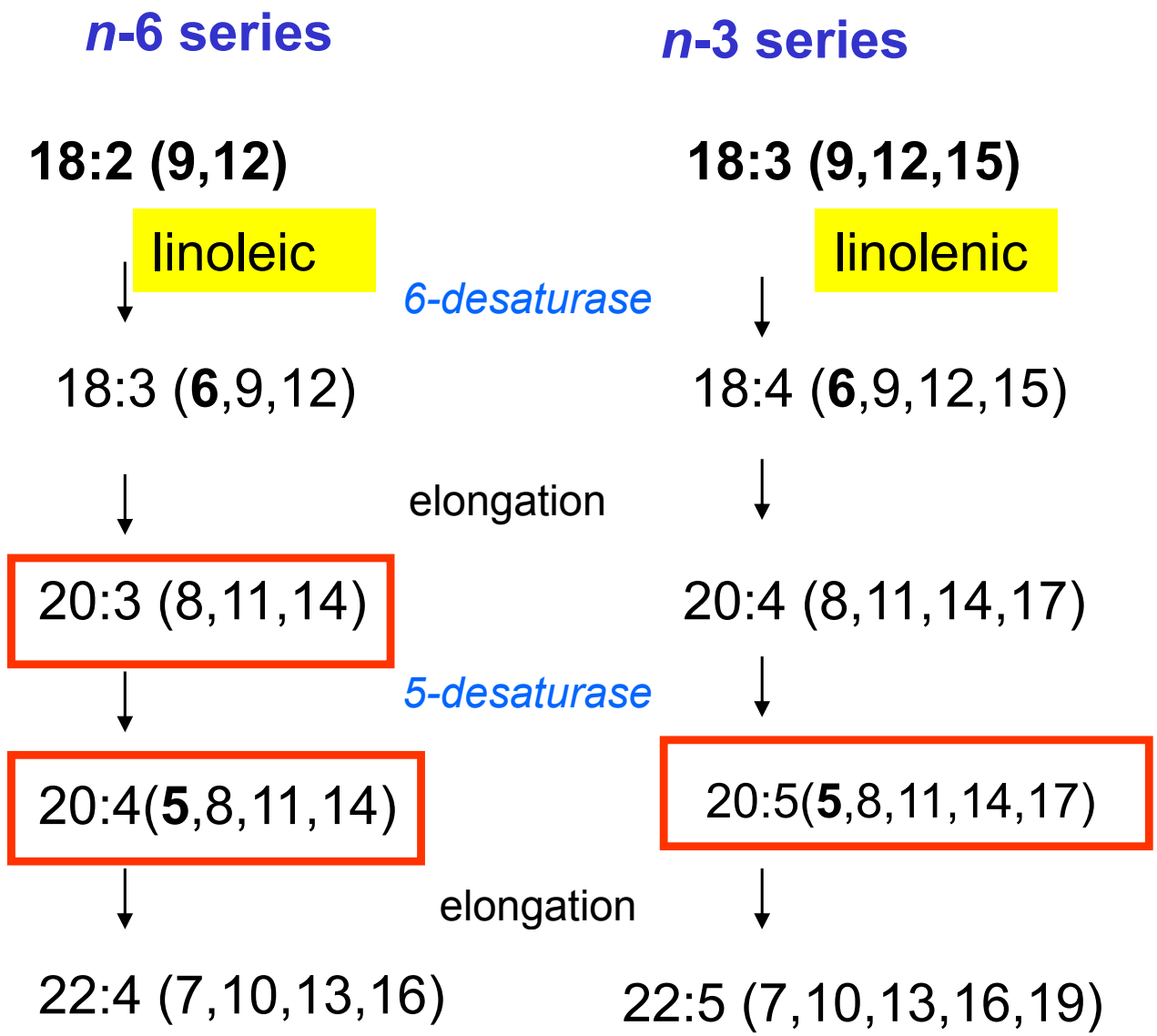


Eicosanoids

C_{20} PUFA precursors of the three eicosanoid groups

Common name Systematic name	Abbreviated Record	Origin	Eicosanoid group
Arachidonic Eicosatetraenoic	20:4 (5,8,11,14)	Membrane PL, Linoleic acid metabolism	PG_2, TX_2 ....
(EPE) Eicosapentaenoic	20:5 (5,8,11,14,17)	Linolenic acid metabolism	PG_3, TX_3 ....
Dihomo-γ-linolenic eicosatrienoic	20:3 (8,11,14)	Linoleic acid metabolism	PG_1, TX_1 ....

Desaturation and elongation of PUFA



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_2**

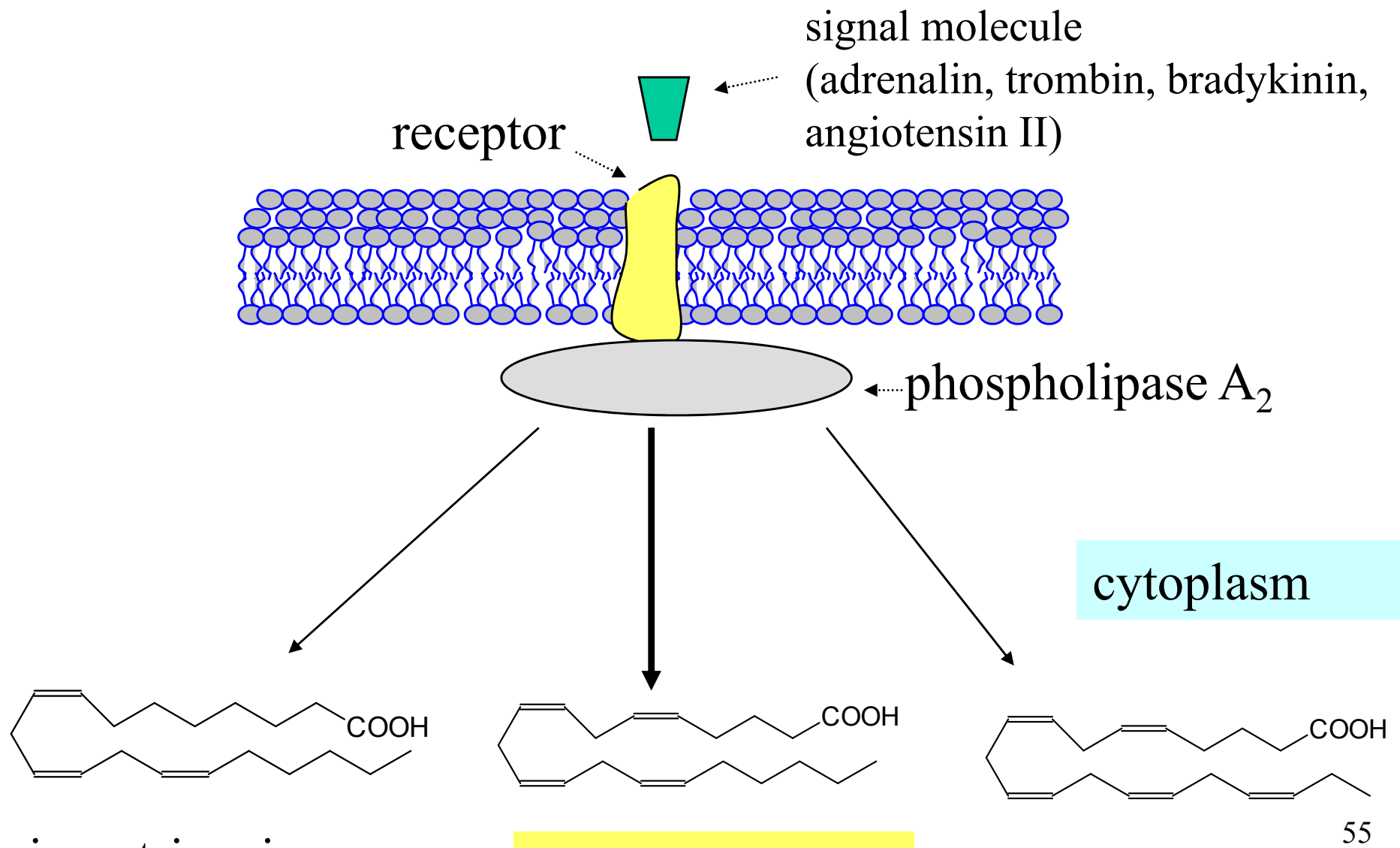
The activity of phospholipase A_2 is a process closely regulated by extracellular mediators (adrenaline, thrombin, angiotensin II, bradykinin).

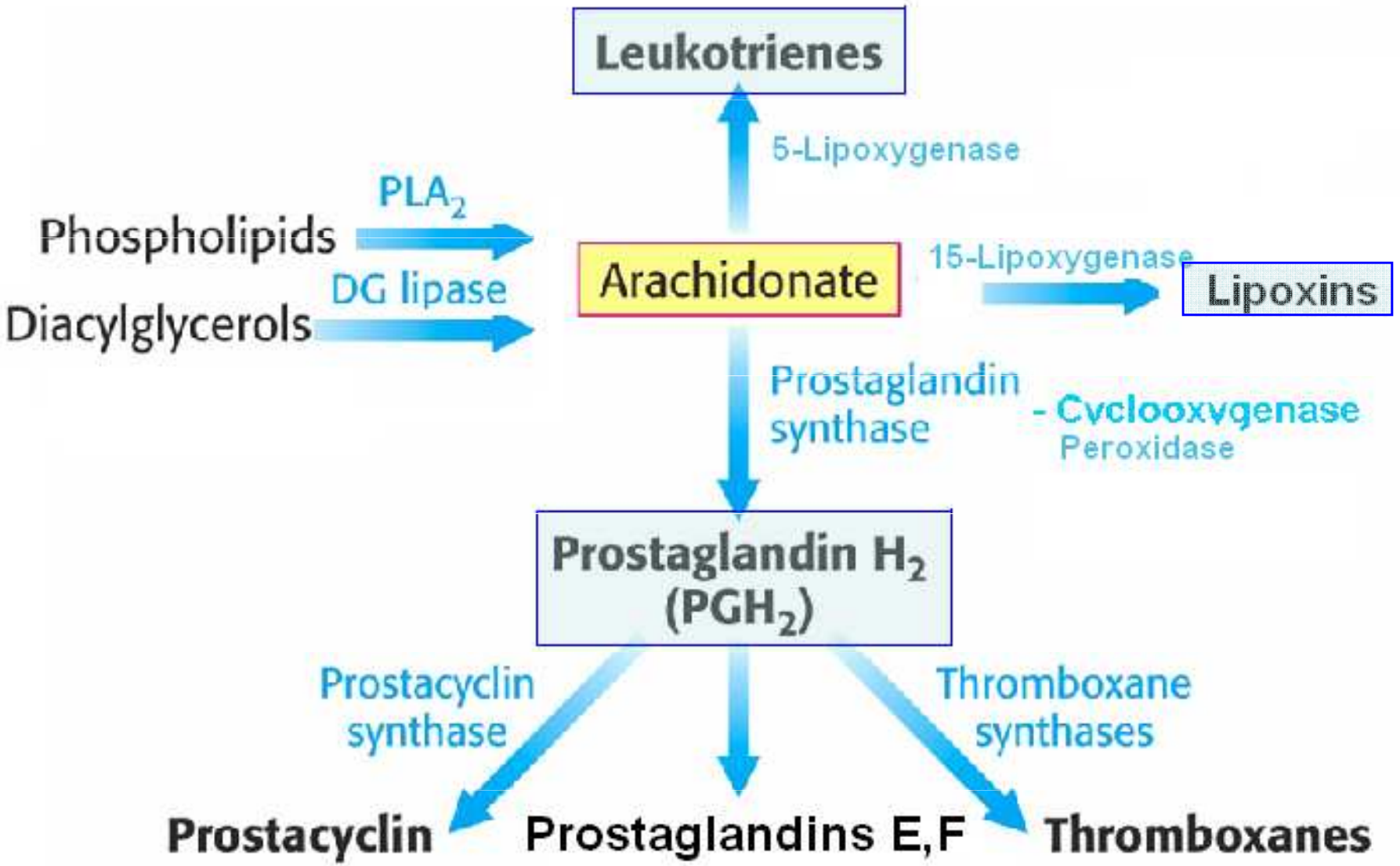
On the other hand, corticosteroids through induction of lipocortin inhibit the activity of phospholipase A_2.

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.

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




Leukotrienes
5-Lipoxygenase
PLA2
Phospholipids
DG lipase
Diacylglycerols
Arachidonate
15-Lipoxygenase
Lipoxins
Prostaglandin synthase
- Cyclooxygenase
Peroxidase
Prostaglandin H2 (PGH2)
Prostacyclin synthase
Thromboxane synthases
Prostacyclin
Prostaglandins E,F
Thromboxanes

Cyclooxygenase pathway

Synthesis of cyclic eicosanoids - prostanoids

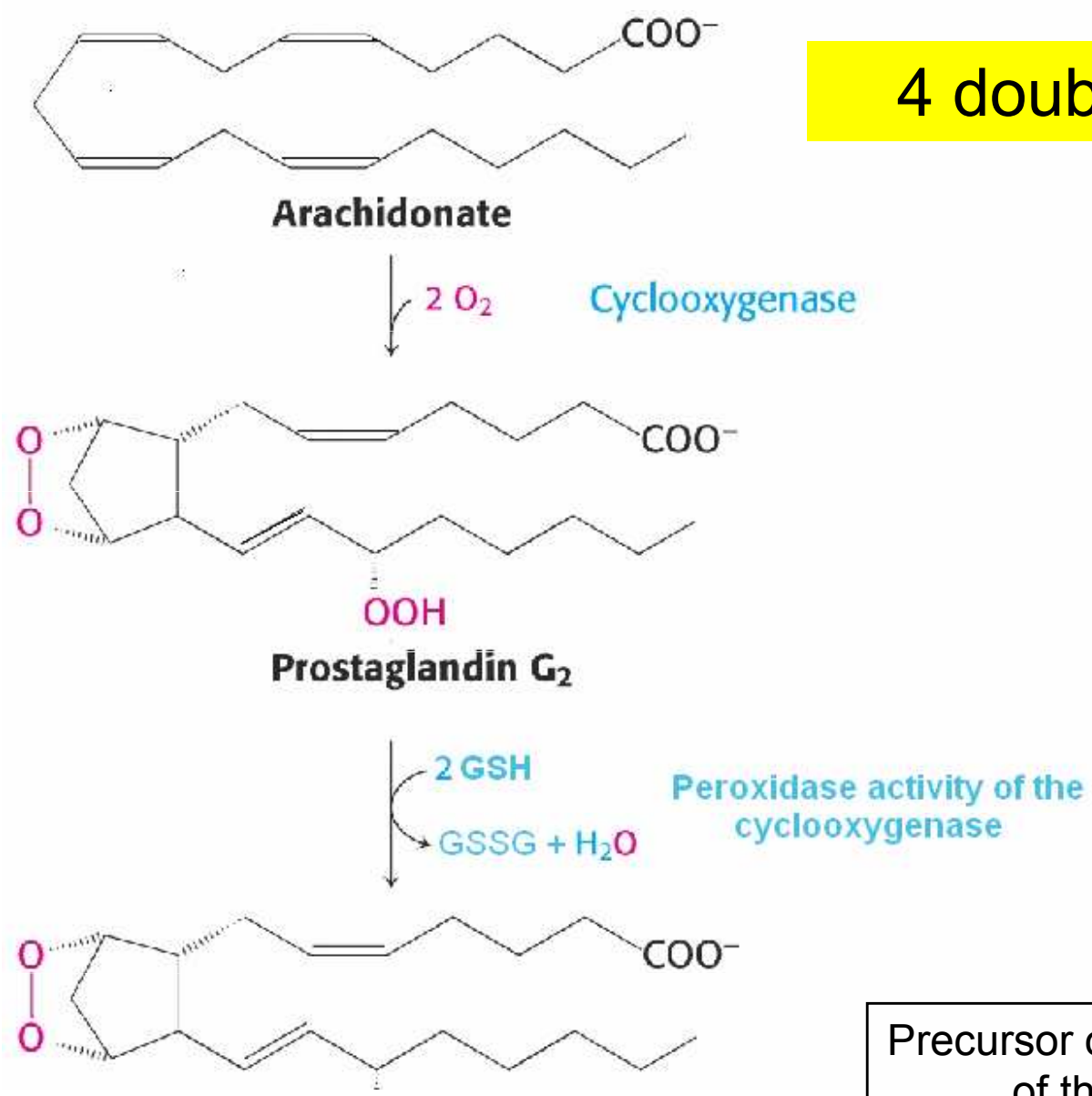
Cyclooxygenase (**COX**, prostaglandin endoperoxide synthase) is a membrane-bound enzyme, which has cyclooxygenase and peroxidase activities. It exists in two forms:

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_2** – 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_3**, the precursor of the prostanoids of the 3-series (triene prostanoids), and conversion of **eicosatrienoate** to **PGH_1**



4 double bonds

Precursor of all prostanoids of the 2-series

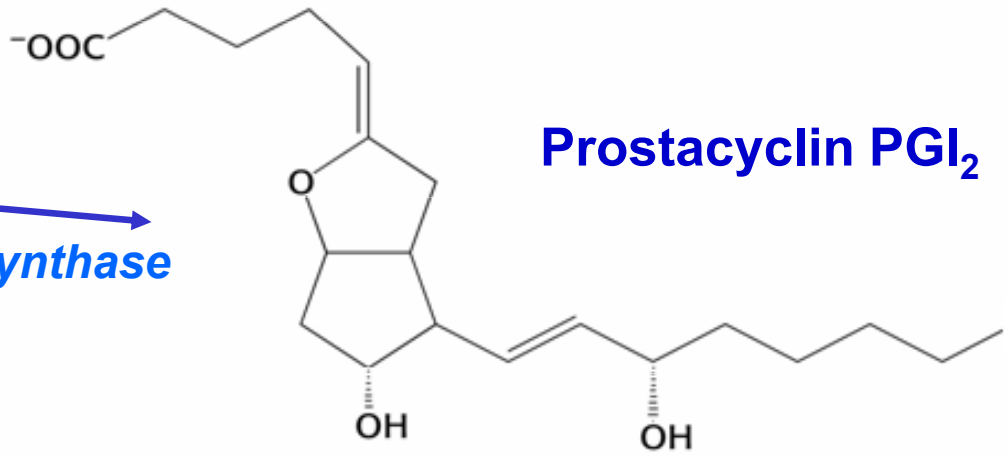
2 double bonds

Prostaglandin H_2

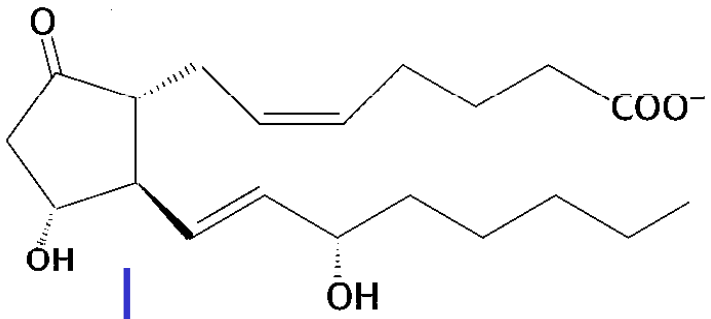
PGE synthase

PGI synthase

TXA synthase

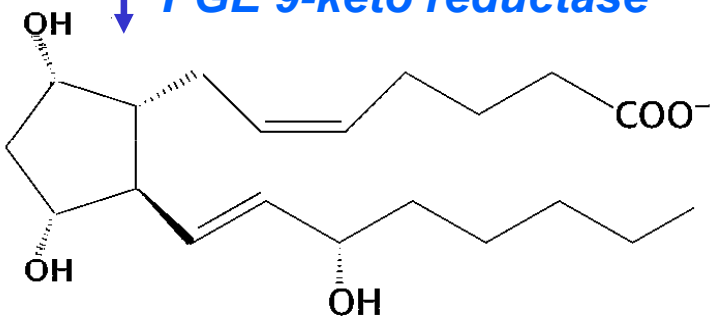


Prostacyclin PGI_2

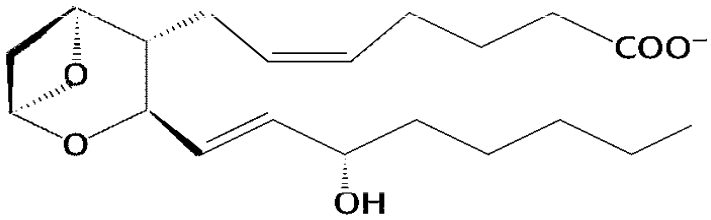


Prostaglandin PGE_2

PGE 9-keto reductase

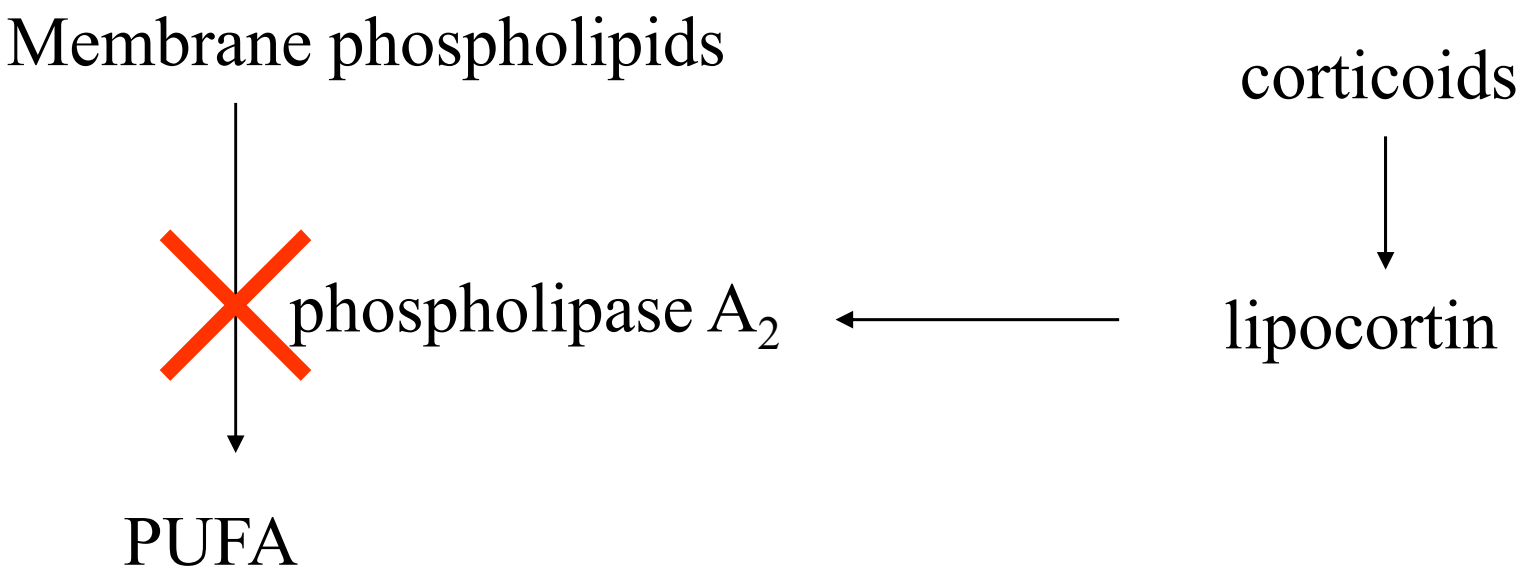


Prostaglandin $PGF_{2\alpha}$



Thromboxane TXA_2

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

Inhibition of cyclooxygenase blocks prostanoid production

Prostanoids mediate, at least partly, the inflammatory response.

Advisable effects of supressed prostanoid production:

the anti-inflammatory effect,
relief of pain, mitigation of fever.

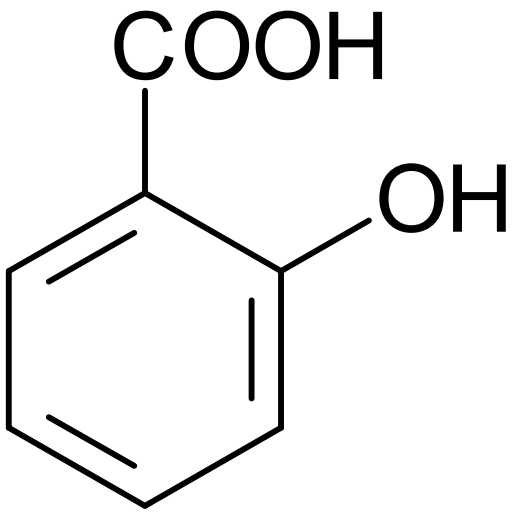
On the contrary, there may be some **undesirable effects** of blocked prostanoid production, e.g. decline in blood platelet aggregation, decreased protection of endothelial cells and of gastric mucosa.

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

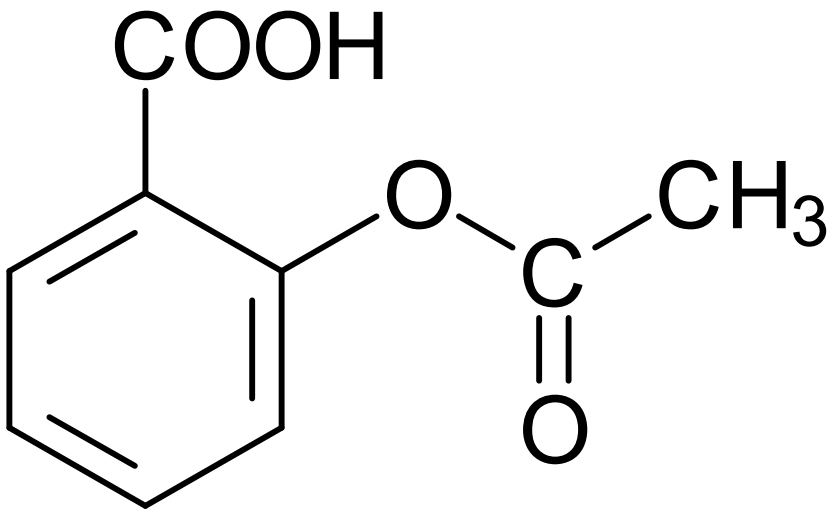
- **acetylsalicylic acid** (Aspirin) – inhibits both COX-1 and COX-2 irreversibly by acetylation the enzyme at its active site
- **acetaminophen (paracetamol), ibuprofen** – reversible COX inhibitors.

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.

Acetylsalicylic acid (Aspirin) has acidic and ester group



salicylic acid

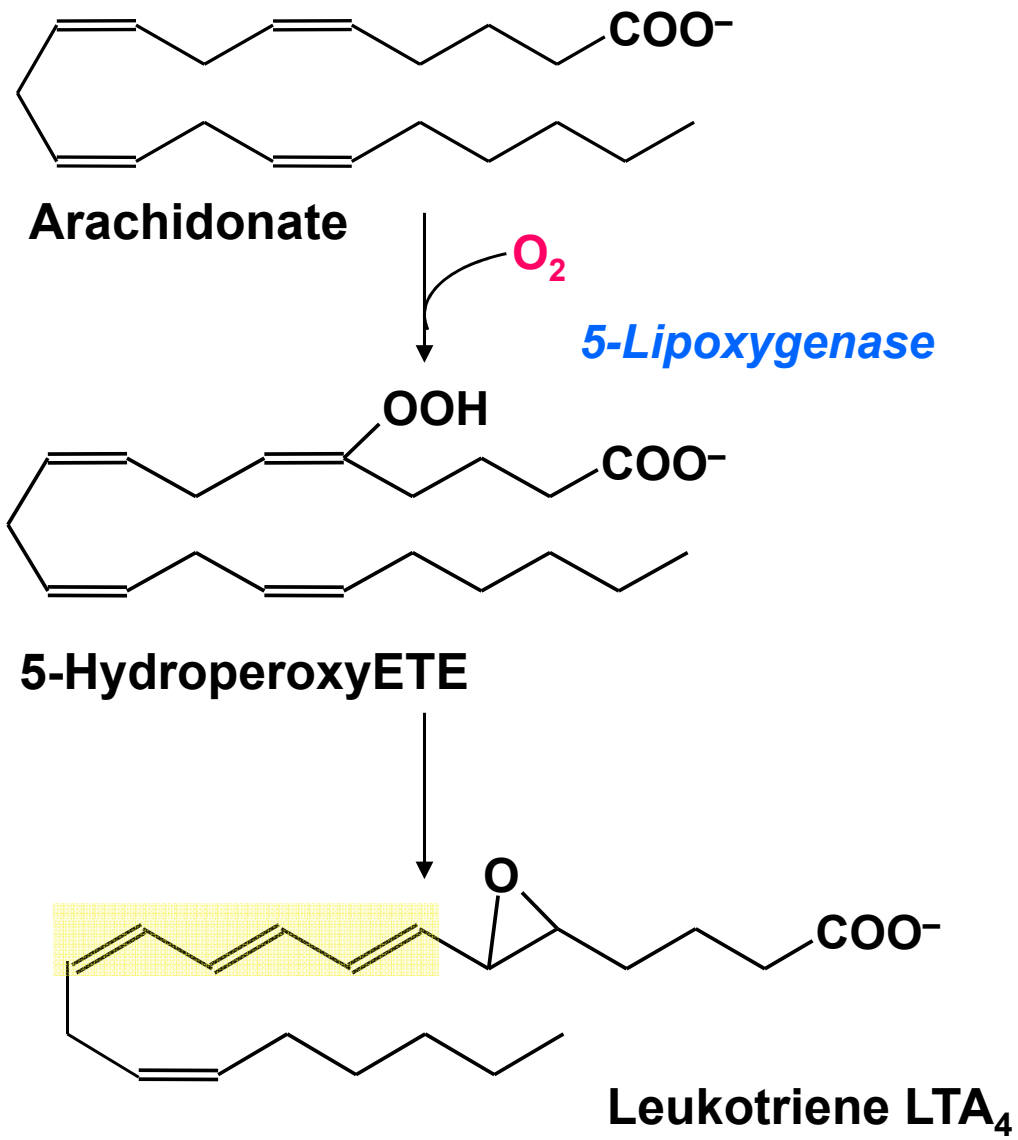


acetylsalicylic acid

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

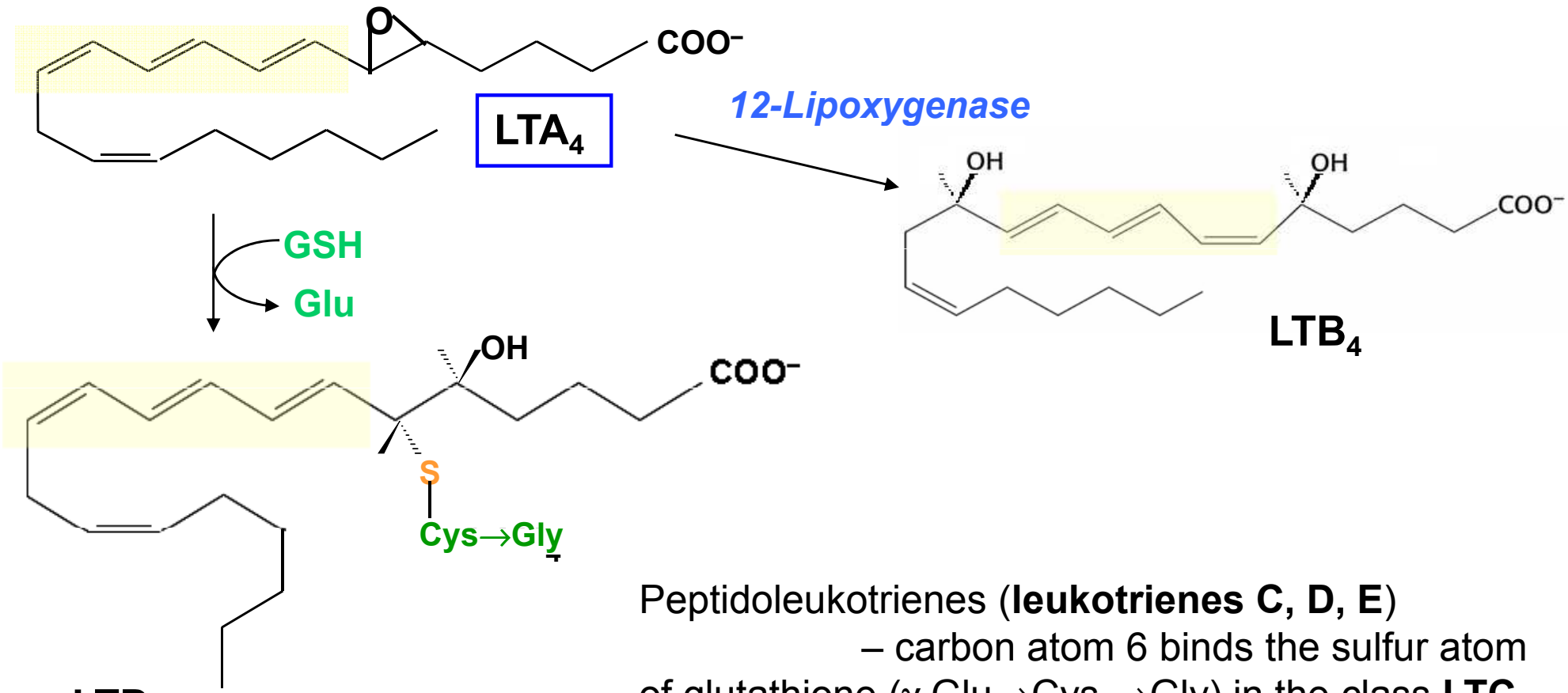
Lipoxygenase pathway

Synthesis of leukotrienes



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.



LTD_4
Slow-reacting substance
of anaphylaxis (SRS-A)

Peptidoleukotrienes (**leukotrienes C, D, E**)
– carbon atom 6 binds the sulfur atom
of glutathione (γ-Glu→Cys →Gly) in the class **LTC**,
of cysteinyl-glycine in the class **LTD**, and
of only cysteine in the class **LTE**.

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.

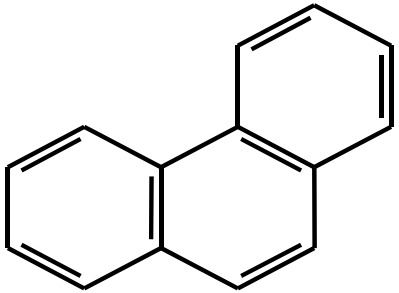
The **endothelial cells** of blood vessels synthesize only prostacyclins.

Catabolism of eicosanoids is rapid.
The biological half-life of prostanoids $t_{½}$ was found to be in the range from seconds to few minutes.

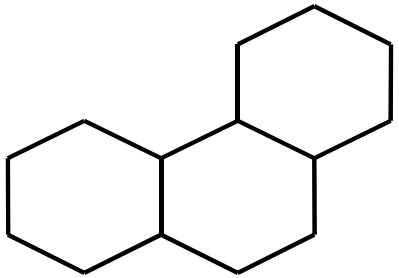
Eicosanoids

Example	Structural group	Synthesized in	The most remarkable effect
PGE_2	prostaglandin E	nearly all cell types	inflammatory reaction, vasodilation, inhibition of HCl secretion
$PGF_{2\alpha}$	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
TXA_2	thromboxane	blood platelets	platelet aggregation, vasoconstriction
LTD_4	leukotriene	leukocytes, mast cells	bronchoconstriction, vasoconstriction
LXA_4	lipoxin	various cell types	bronchoconstriction, vasodilation

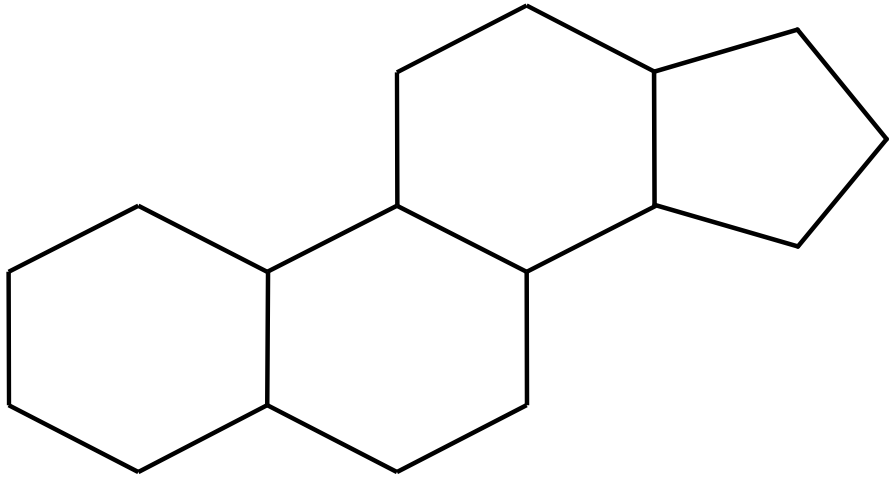
Synthesis of Cholesterol



phenanthrene
(fused aromatic benzene rings)

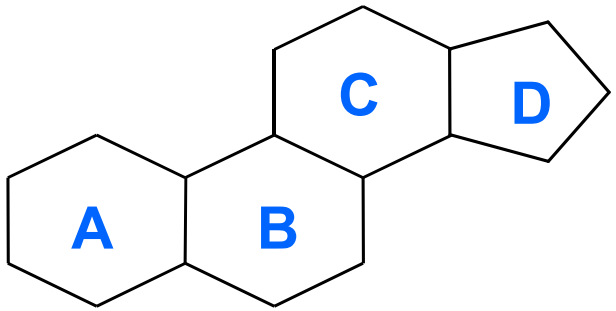


perhydrophenanthrene
(fused cyclohexane rings)

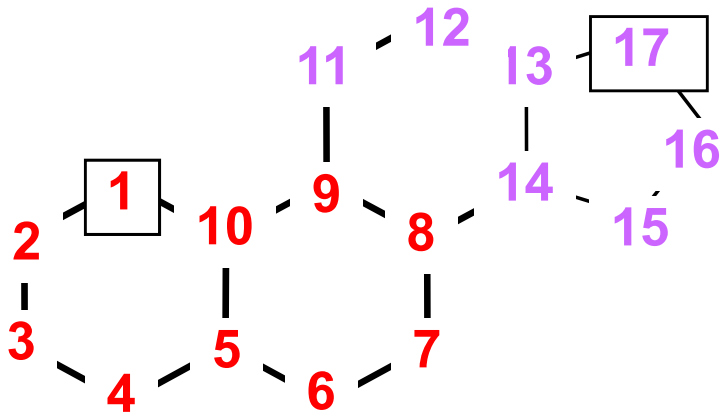


cyclopentanoperhydrophenanthrene
(**sterane**)

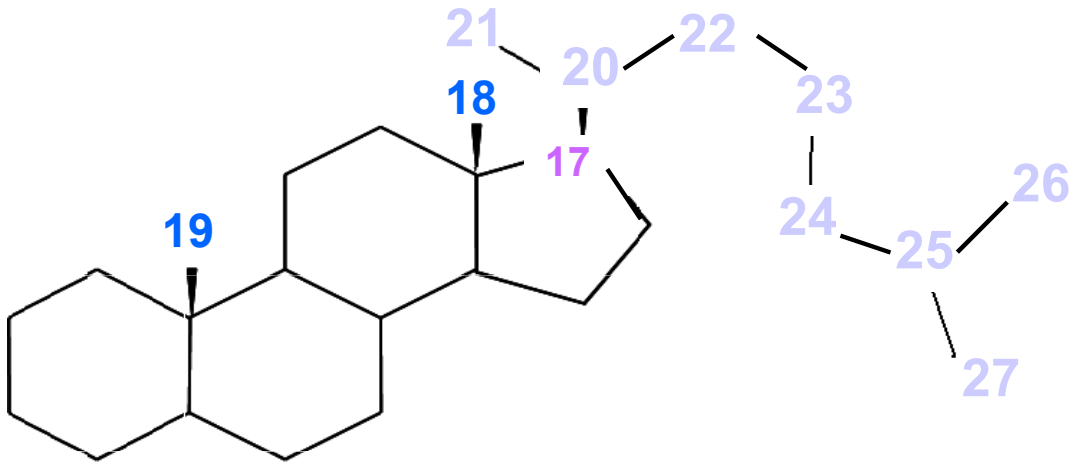
The rings in steroids are denoted by the letters A, B, C, and D:



Carbon atoms in steroids are numbered:

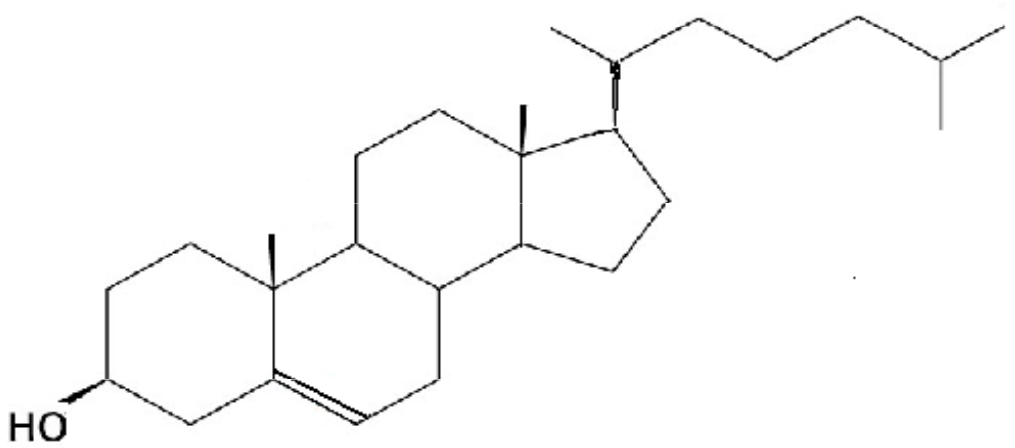


Carbons outside the rings:



Cholesterol

(**Cholest-5-ene-3β-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.

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 occurs in all nucleated cells

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.**

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.

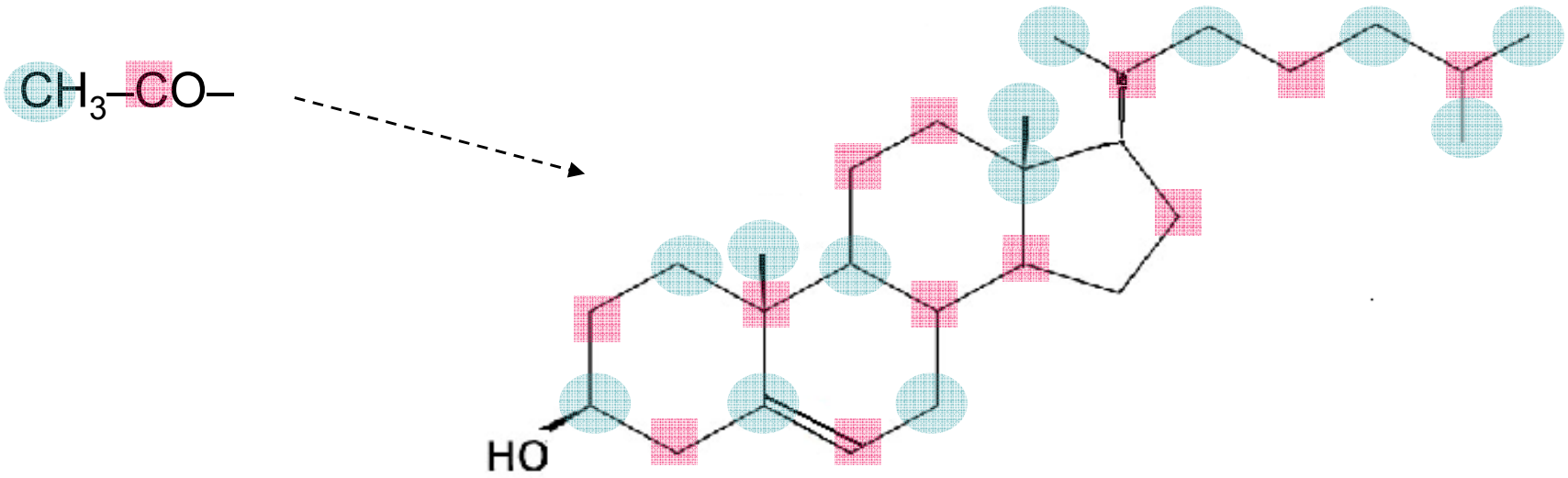
2 The conversion of two mevalonates to two **activated isoprene units** that are the key building blocks of cholesterol.

3 The condensation of six molecules of activated isoprenes to form **squalene.**

4 The **cyclization** of squalene and c**onversion** of the four-ring steroid system into cholesterol.

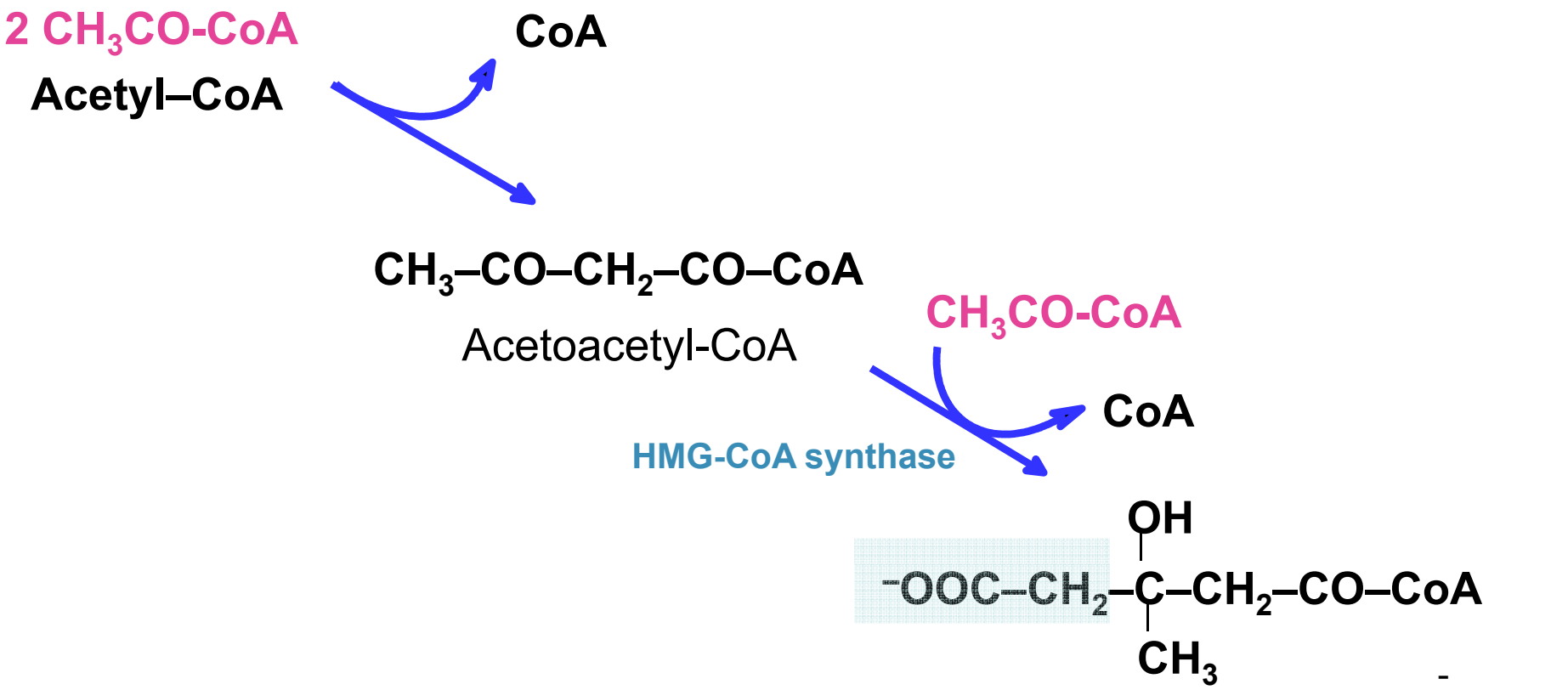
15 **Acetyl-CoA** ----------(tens of reactions)----------▸ **cholesterol**

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



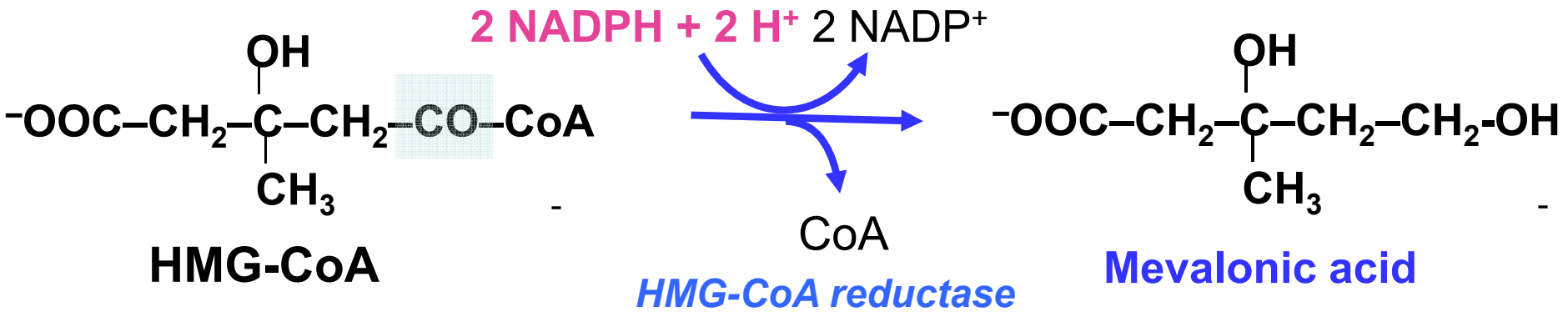
3-Hydroxy-3-methylglutaryl-CoA

(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):

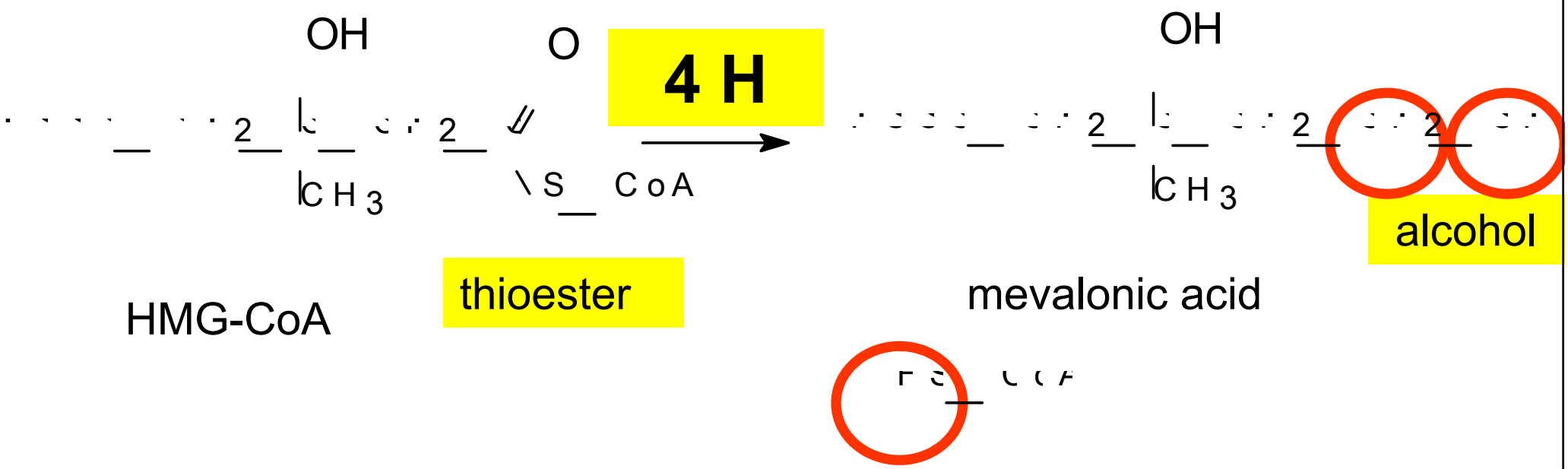
Cytosol



This reduction of MHG-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).

Reductive cleavage of thiester bond



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

Inhibition

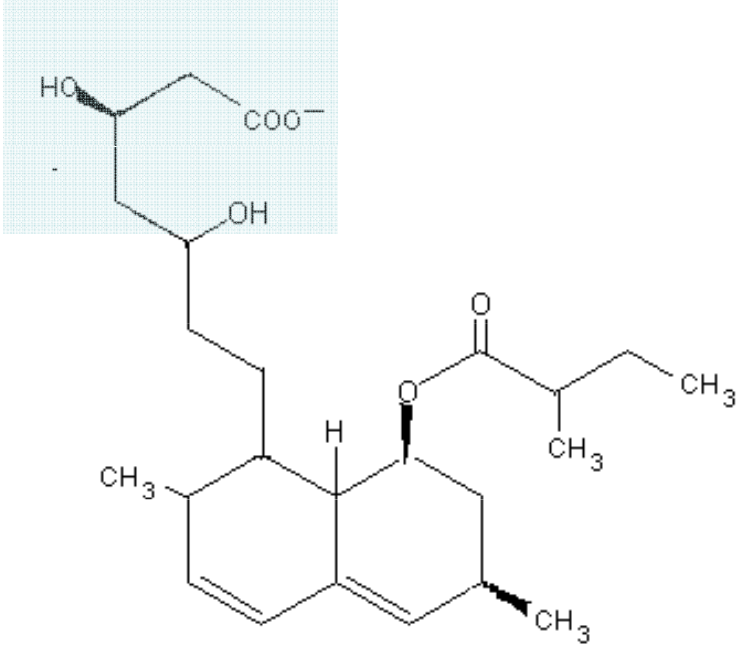
– by cytosolic free cholesterol (repress transcription of HMG-CoA reductase via SREBP – sterol regulatory element-binding protein) (feed-back control)

– by reversible phosphorylation of the enzyme

– by drugs called statins.

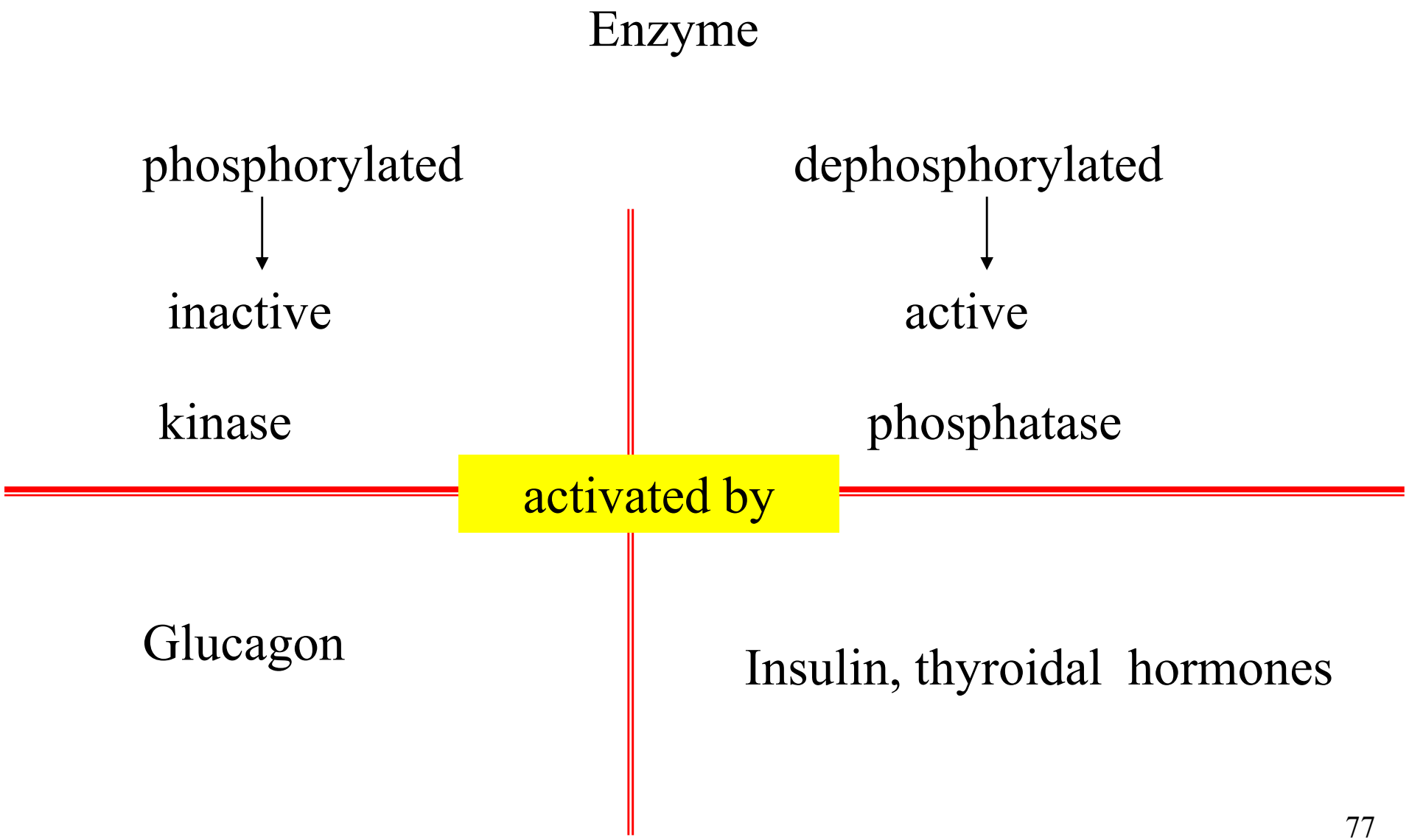
Statins are competitive inhibitors of HMG-CoA reductase, either fungal products (e.g. lovastatin), or quite synthetic compounds (3rd generation of statins, e.g. cerivastatin).

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

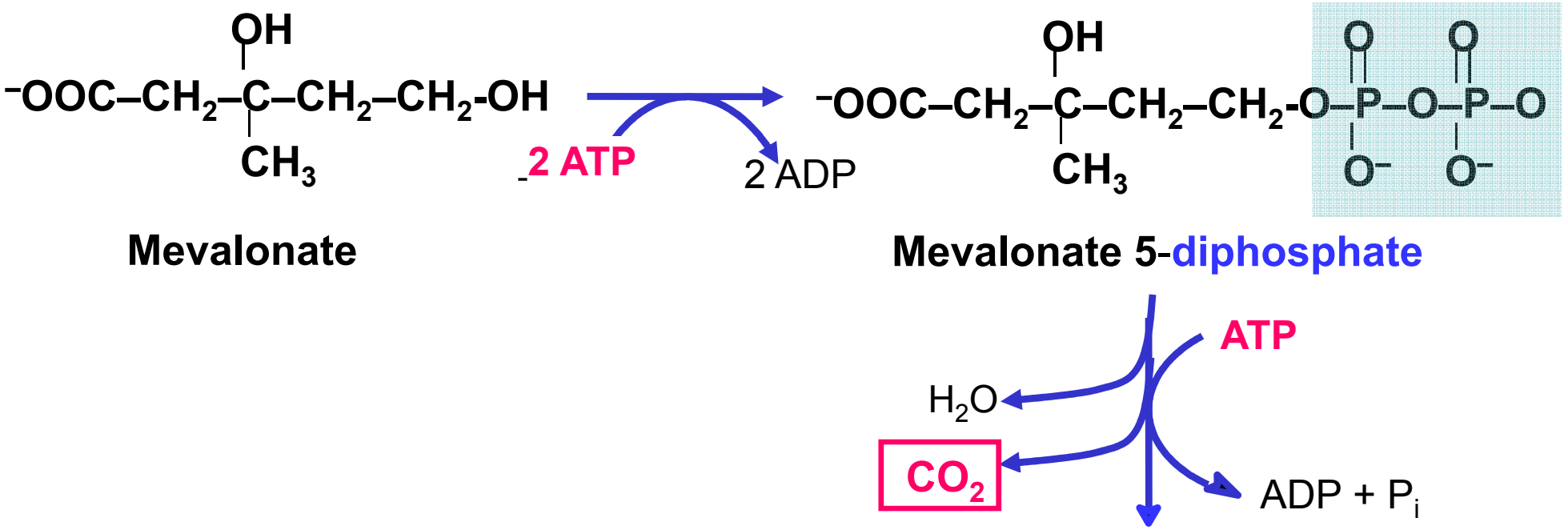


Lovastatin

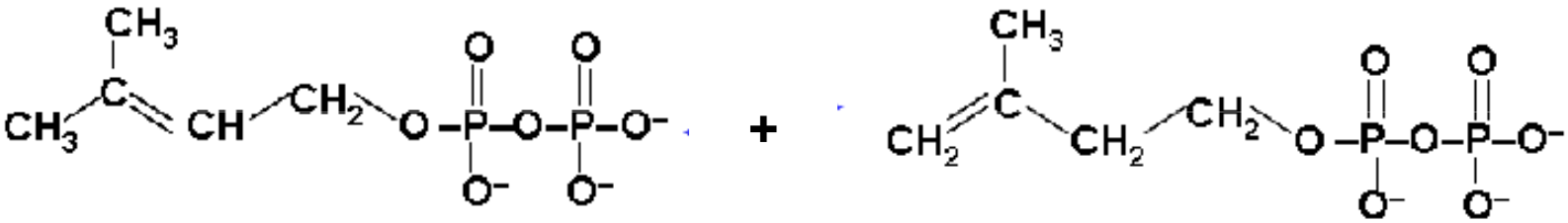
Regulation of HMG-CoA reductase by de/phosphorylation



2 The conversion of mevalonate to activated isoprene units

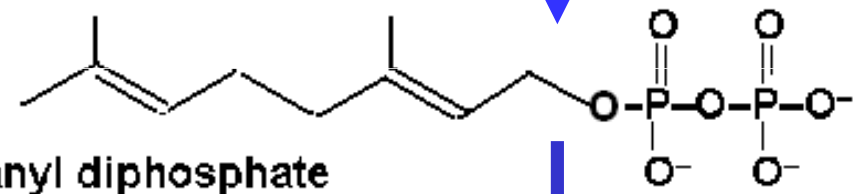


3 The condensation of molecules of activated isoprenes to form squalene (30 C):



3,3-Dimethylallyl diphosphate + Isopentenyl diphosphate

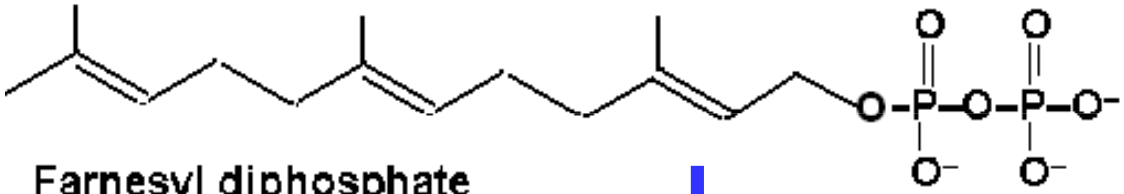
5 C + 5 C



Geranyl diphosphate + diphosphate

10 C + 5 C

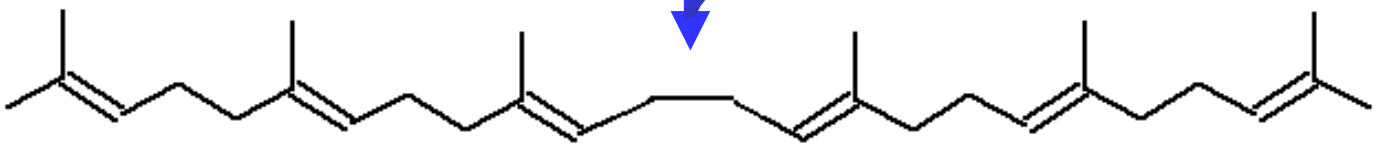
+ Isopentenyl diphosphate



Farnesyl diphosphate + diphosphate

15 C + 15 C

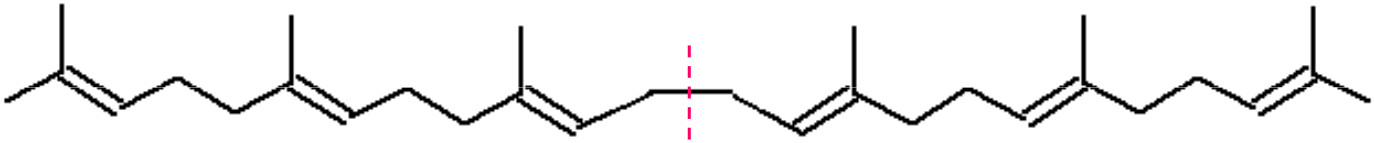
+ farnesyl diphosphate + NADPH + H^+



SQUALENE **30 C**

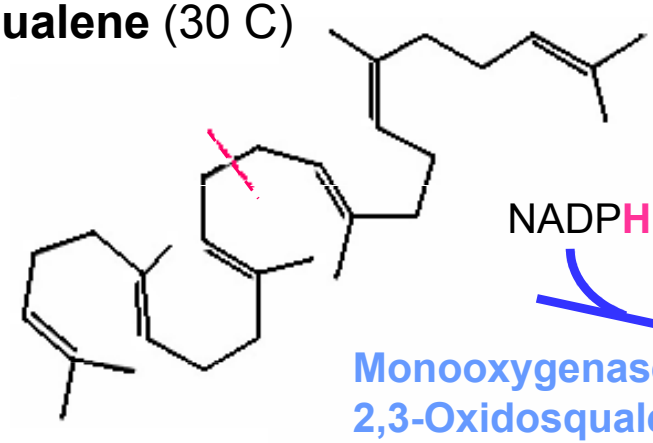
+ 2 diphosphate + $NADP^+$

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:

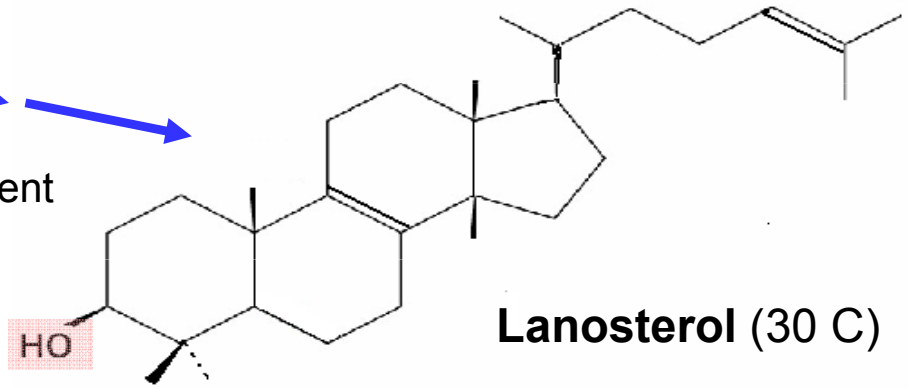
Squalene (30 C)



$NADPH+H^+ + O_2$

H_2O

Monooxygenase
2,3-Oxidosqualene cyclase
and spontaneous rearrangement



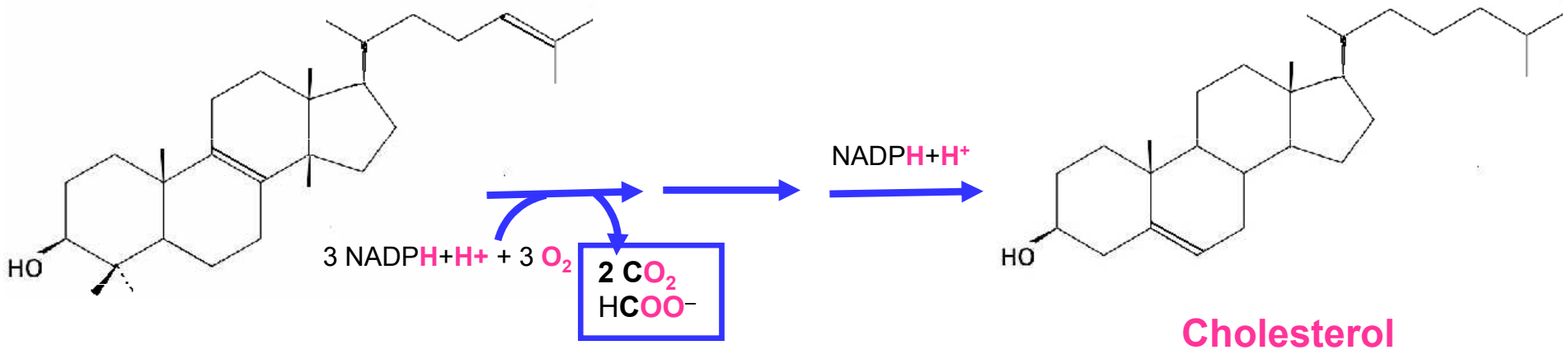
Lanosterol (30 C)

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 $-CH_3$ groups
(catalysed by a monooxygenase) as 2 CO_2 and $HCOO^-$,
- rearrangement of double bonds,
- reduction (saturation) of one of the two double bonds.

Lanosterol (30 C)



Cholesterol
(27 C; **cholest-5-ene-3β-ol**)

Squalene (30 C) → → → **lanosterol** (30 C) → → → **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.