Integration of metabolism pathways

Mitochondria

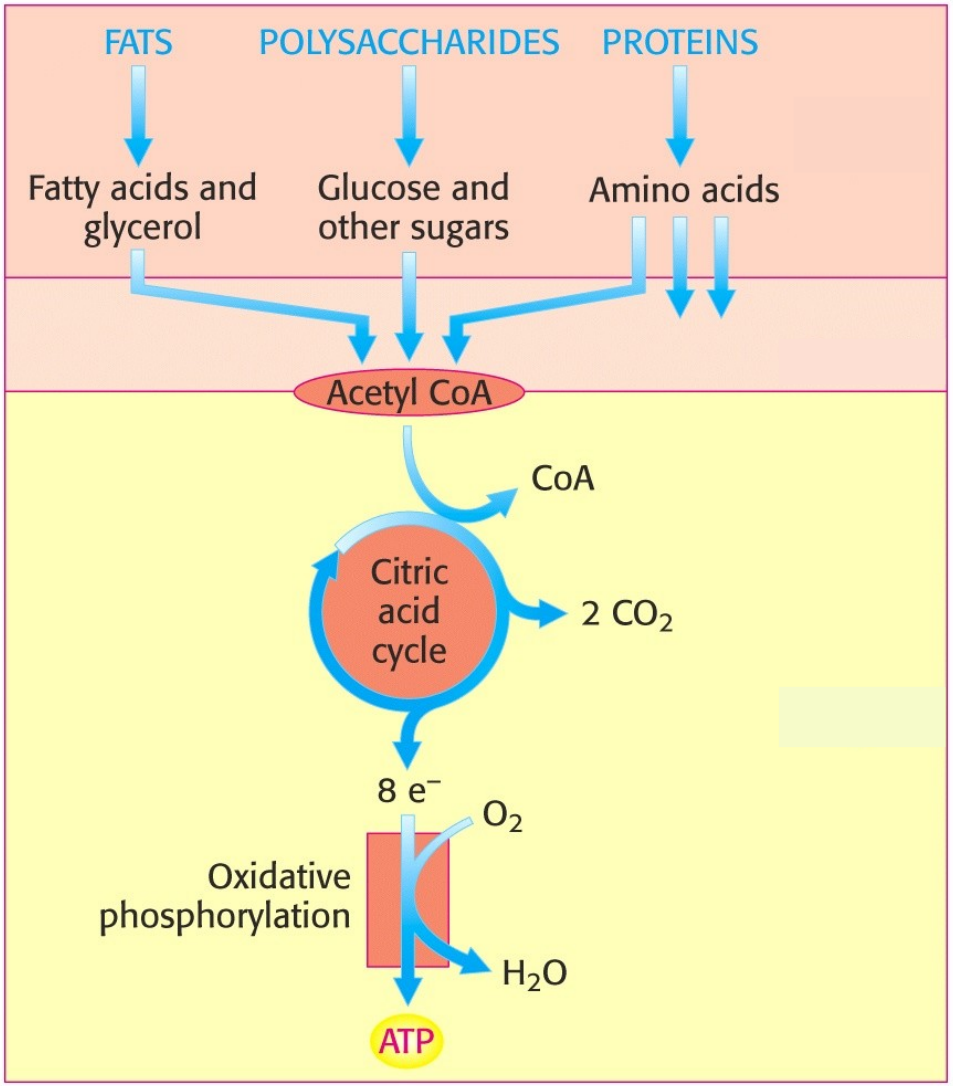
The citric acid cycle

Biosynthesis of haem

Biochemistry I
Lecture 10

2008 (J.S.)

Stages in the extraction of energy from foodstuffs

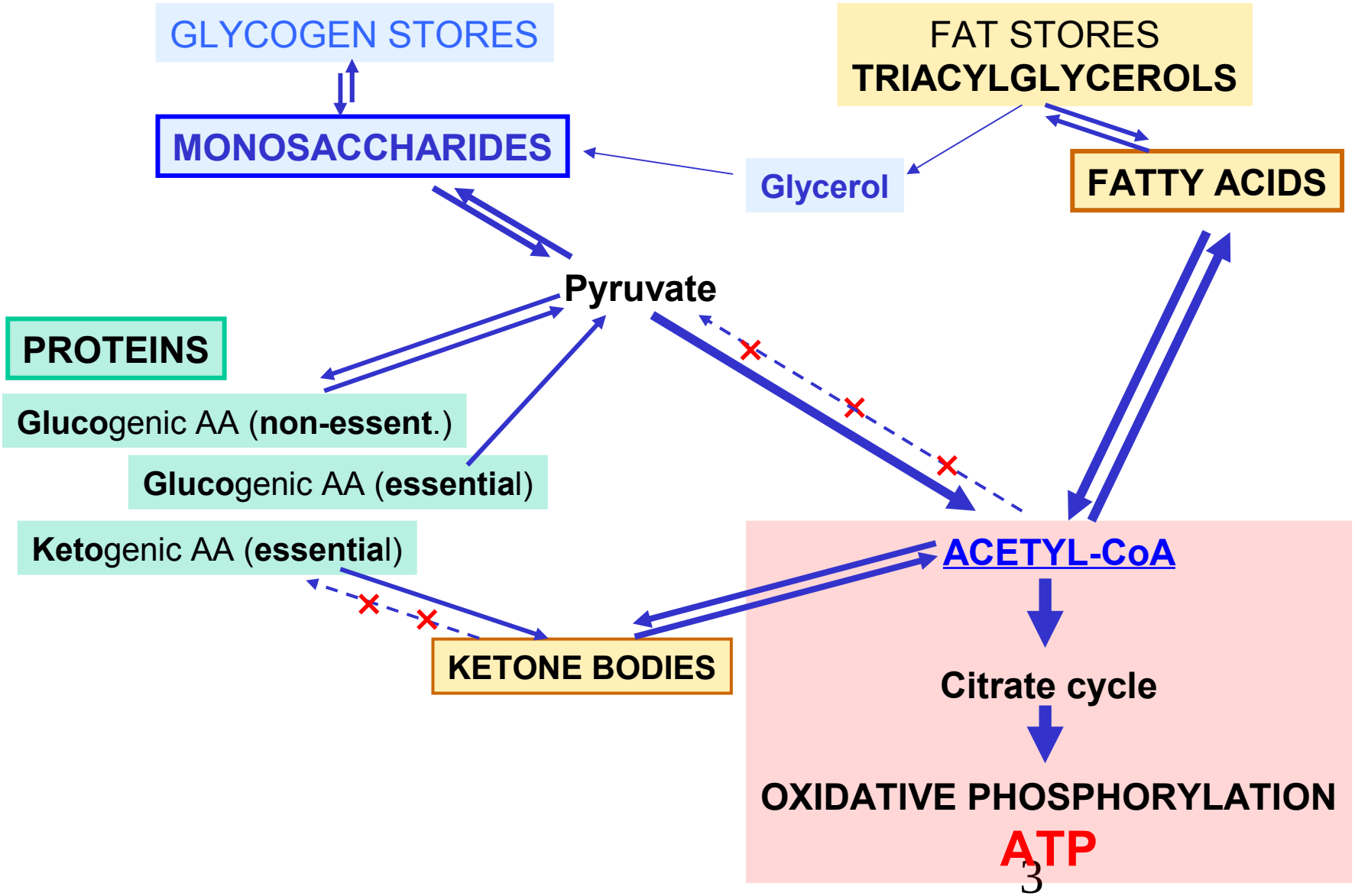


The first stage of catabolism
Large molecules in food are broken down into smaller units

Stage II
Degradation to a few amphibolic intermediates

Stage III
The final common pathways – most of the ATP is produced from the complete oxidation of the acetyl unit of acetyl CoA

Relationships among the major energy metabolism pathways



Summary of the previous picture

Saccharides are the most **universal nutrients –**
the overdose is transformed in the fat stores,
carbon skelet of non-.essential amino acids may originate from saccharides.

Triacylglycerols exhibit the highest **energetic yield** –
but **fatty acids cannot convert into saccharides** or the skelet of amino acids.

Amino acids represent the unique **source of nitrogen** for proteosynthesis
that serves as fuel rather when the organism is lacking in other nutrients -
glucogenic amino acids can convert into glucose,
a overdose of diet protein may be transformes in fat stores.

The metabolism of nutrients is sophistically controlled with different mechanisms

in the **well-fed state** (absorptive phase),

short fasting (post-absorptive phase), and in

prolonged starvation.

It also depends on **energy expenditure** (predominantly muscular work) –
either of maximal intensity (anaerobic, of short duration only)
or aerobic work of much lower intensity (long duration).

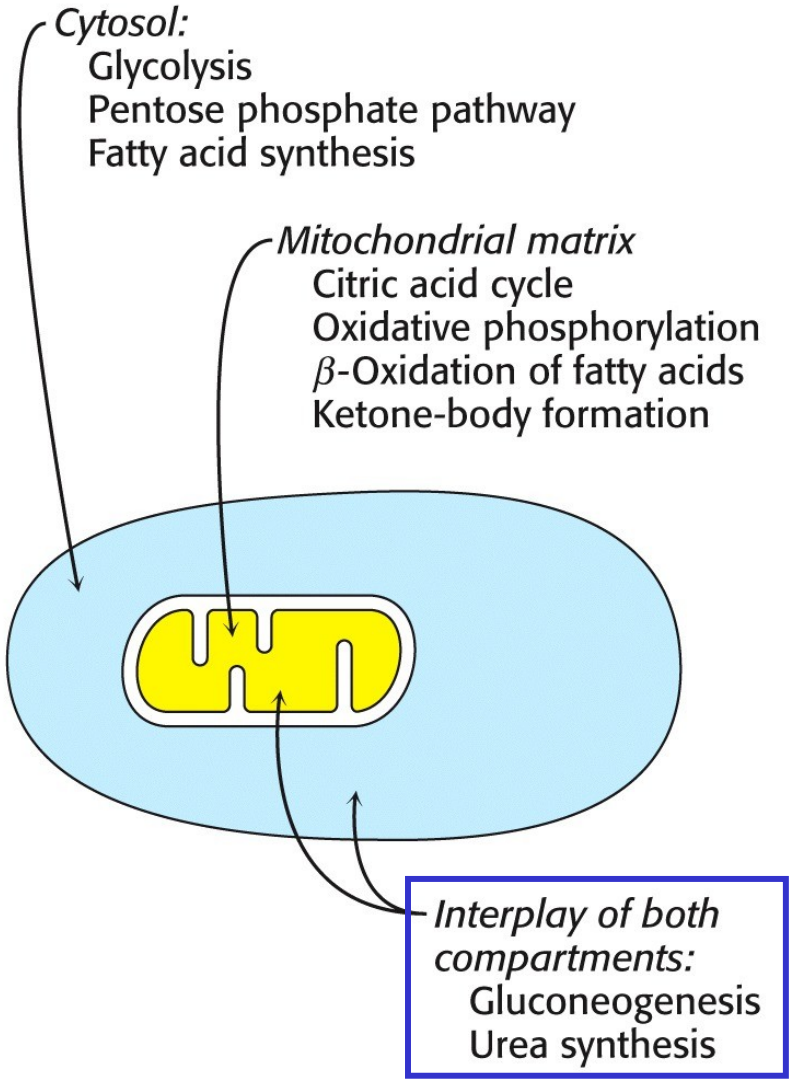
The tissues differ in their enzyme equipment:

Pathway	Liver	Kidney	Muscle	CNS	RBC	Adipose tissue
Glycolysis	+	+	+	**+**	**+**	+
FA β-oxidation	**+**	+	**+**	0	0	0
Utilization of ketone bodies	**0**	+	**+**	(+)	0	+
Ketogenesis	**+**	0	0	0	0	0
Gluconeogenesis	**+**	+	0	0	0	0
FA synthesis	**+**	±	±	±	0	**+**

Cellular compartmentation of the major metabolic pathways

Plasma membrane	Transport in and out of cells, signal transduction
Nucleus	DNA replication, RNA synthesis (DNA transcription)
Cytosol	Glycolysis, pentose phosphate pathway, FA synthesis, proteosynthesis on ribosomes, etc.
Mitochondrion	Citrate cycle, FA β-oxidation, aerobic oxidation of α-ketoacids, oxidative phosphorylation
Endoplasmic reticulum	Lipid and glycoprotein synthesis, FA desaturation, hydroxylation of xenobiotics, etc..
Golgi complex	Protein glycosylation, intracellular sorting of proteins, secretion vesicles
Lysosome	Degradation of biopolymers by hydrolysis
Peroxisome	Oxidations, production and degradation of H_2O_2

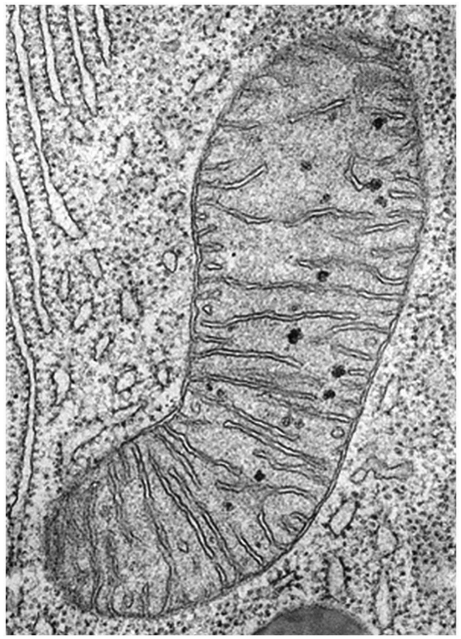
Compartmentation of the major pathways of metabolism

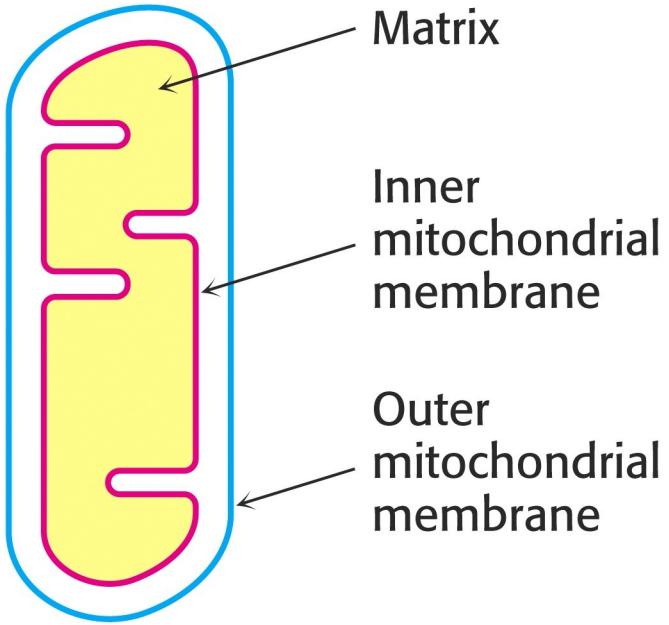


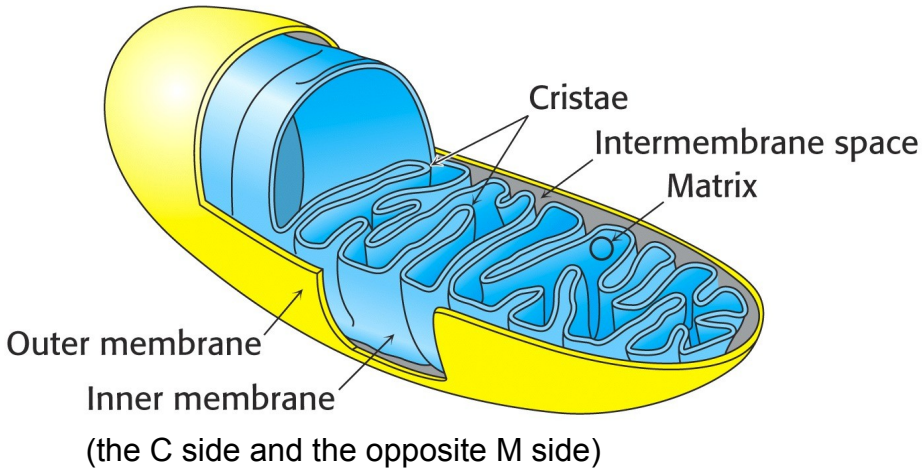
Mitochondria

Mitochondria are semiautonomous organelles that live in an endosymbiotic relation with the host cell.

Oxidative phosphorylation (terminal respiratory chain producing a proton gradient that drives the phosphorylation of ADP) in eukaryotes takes place in mitochondria. These organells also contain the enzymes of the **citric acid cycle**, **β-oxidation** of fatty acids, and other important metabolic pathways.





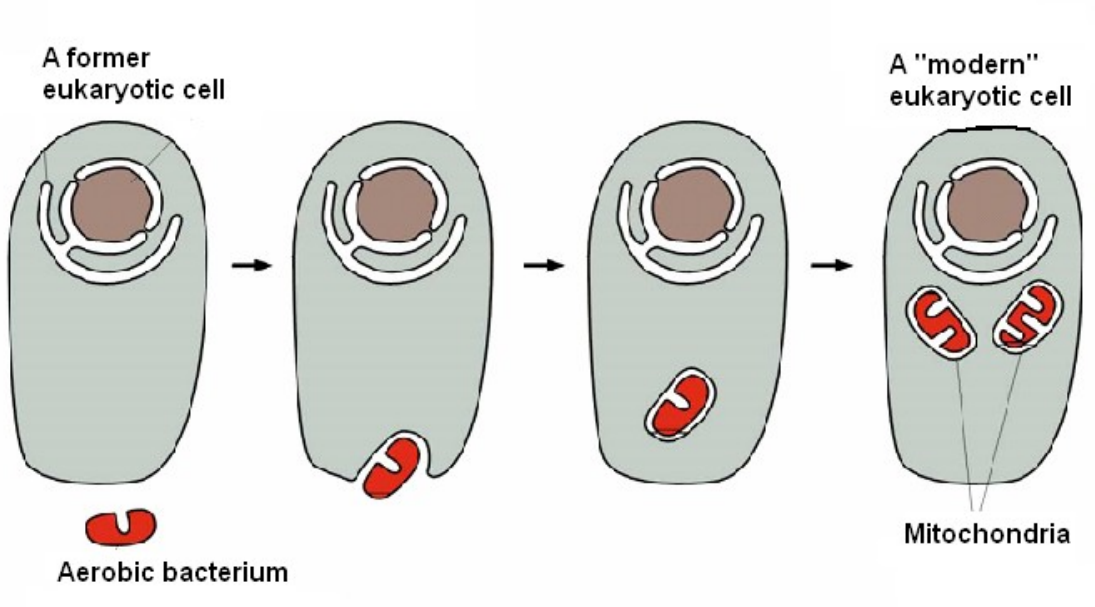


The outer membrane is quite permeable for small molecules and ions – it contains many copies of **mitochondrial porin** (voltage-dependent anion channel, VDAC).

The inner membrane is intrinsically impermeable to nearly all ions and polar molecules, but there are many **specific transporters** which shuttles metabolites (e.g. pyruvate, malate, citrate, ATP) and protons across the membrane.
The external side of this membrane is called *cytosolic* (C side, also P side because of the positive membrane potential), the inner side of the inner mitochondrial membrane is the *matrix side* (M side, also N from the negative membrane potential).

Mitochondria are the result of endosymbiosis

– a free-living organism capable of oxidative phosphorylation was engulfed by another cell.



These organelles – have the double membrane,

– cardiolipin, the typical phospholipid of bacteria, is constituent of the inner membrane,

– mitochondria contain their own circular DNA and the mitochondrial-specific transcription and translation machinery.

The citric acid cycle

(also known as the **tricarboxylic acid** (TCA) **cycle** or **Krebs cycle**)

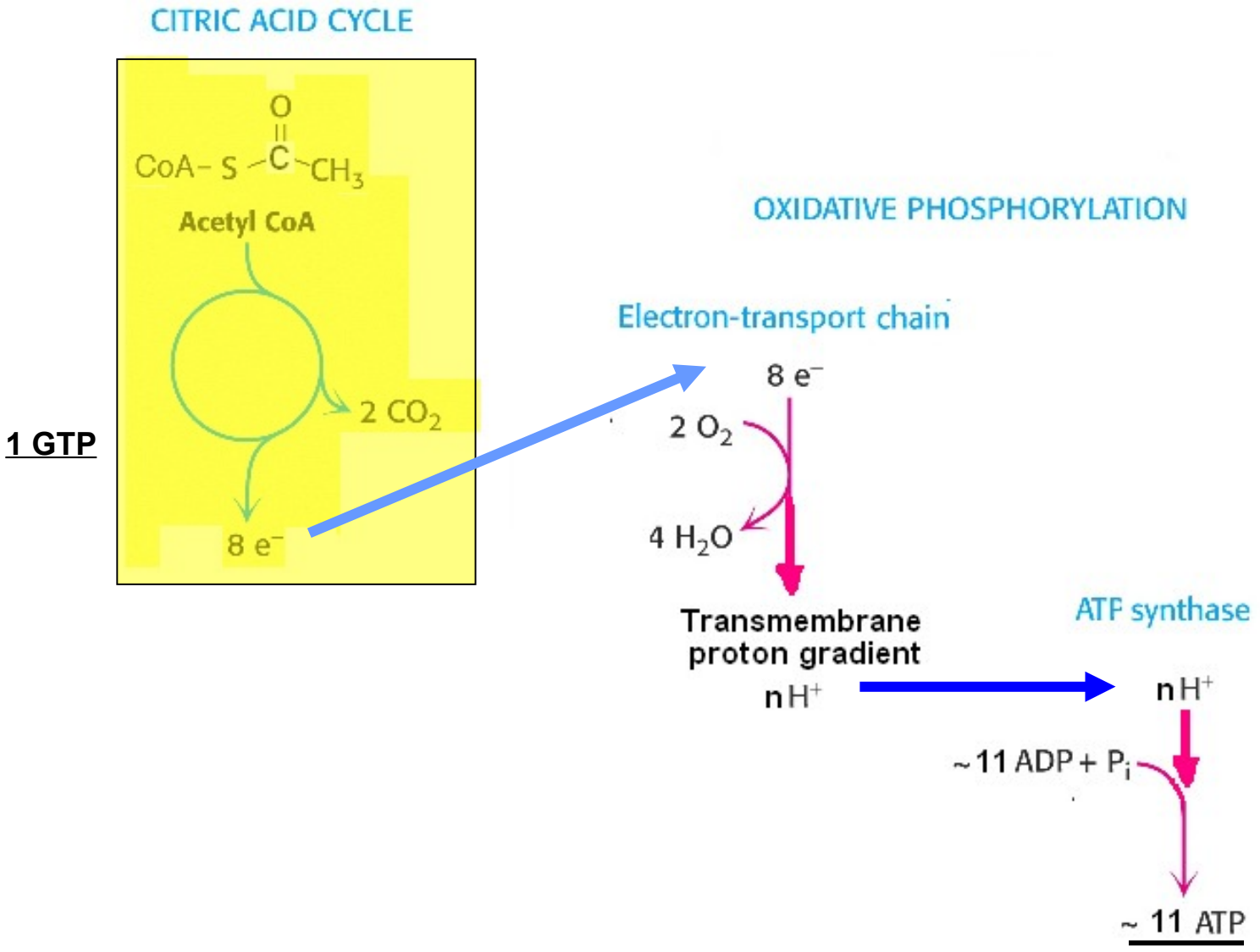
is the **final common pathway for the oxidation of nutrients** – saccharides, fatty acids, and amino acids.
Most of the intermediates enter the cycle as **acetyl-CoA**.

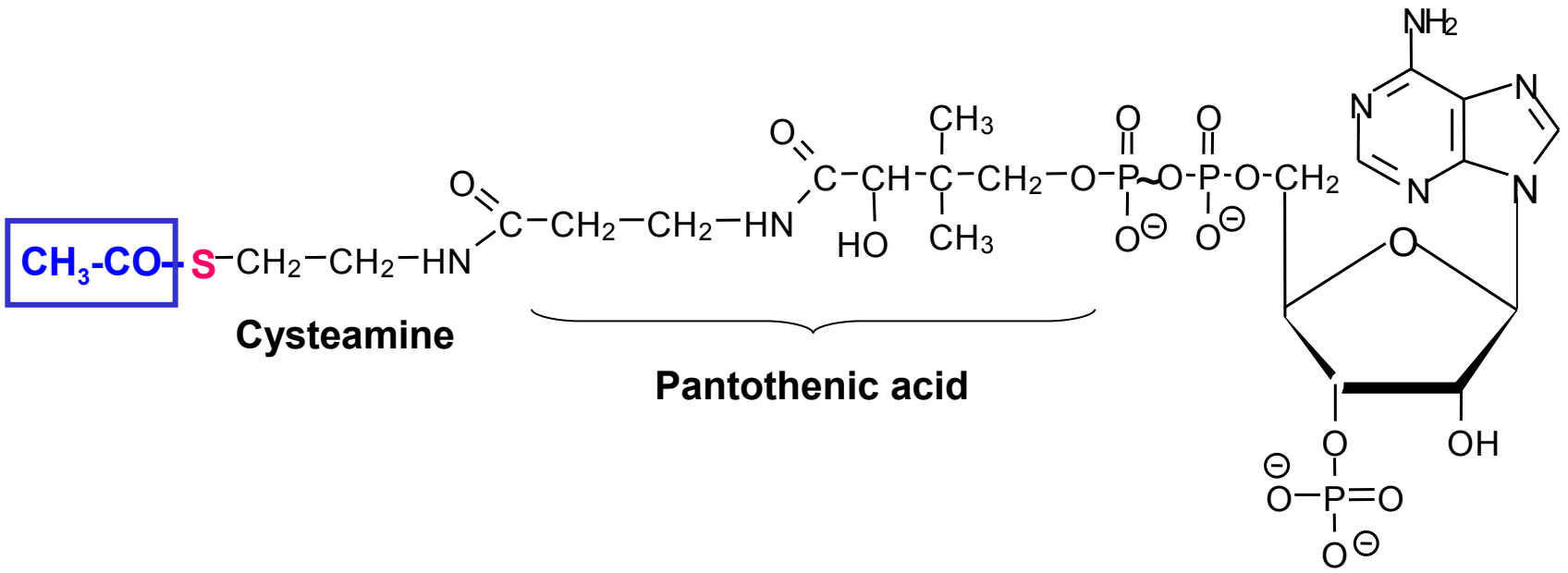
The overall result of this cycle can be summarized in the following simplified form:

The acetyl group of acetyl-CoA is oxidized – two molecules of carbon dioxide leave the cycle and eight electrons gained (in four dehydrogenations, represented as 8 H* in the equation) **serve to form 3 molecules of NADH + H^+ and a molecule of $FADH_2$.**

$$CH_3\text{-}CO\text{-S-CoA} + 3\ H_2O \longrightarrow 2\ CO_2 + 8\ H^* + \text{CoA-SH}$$

Carbon dioxide is expired. The four molecules of reduced coenzymes serve as substrates for terminal respiratory chain.
The **direct** energy yield is not large – oxidation of one acetyl-CoA in the cycle yields **only one molecule of GTP** formed by substrate-level phosphorylation of GDP.


CITRIC ACID CYCLE
O
CoA– S – C – CH3
Acetyl CoA
2 CO2
8 e–
1 GTP
OXIDATIVE PHOSPHORYLATION
Electron-transport chain
8 e–
2 O2
4 H2O
Transmembrane proton gradient
nH+
ATP synthase
nH+
~11 ADP + Pi
~ 11 ATP



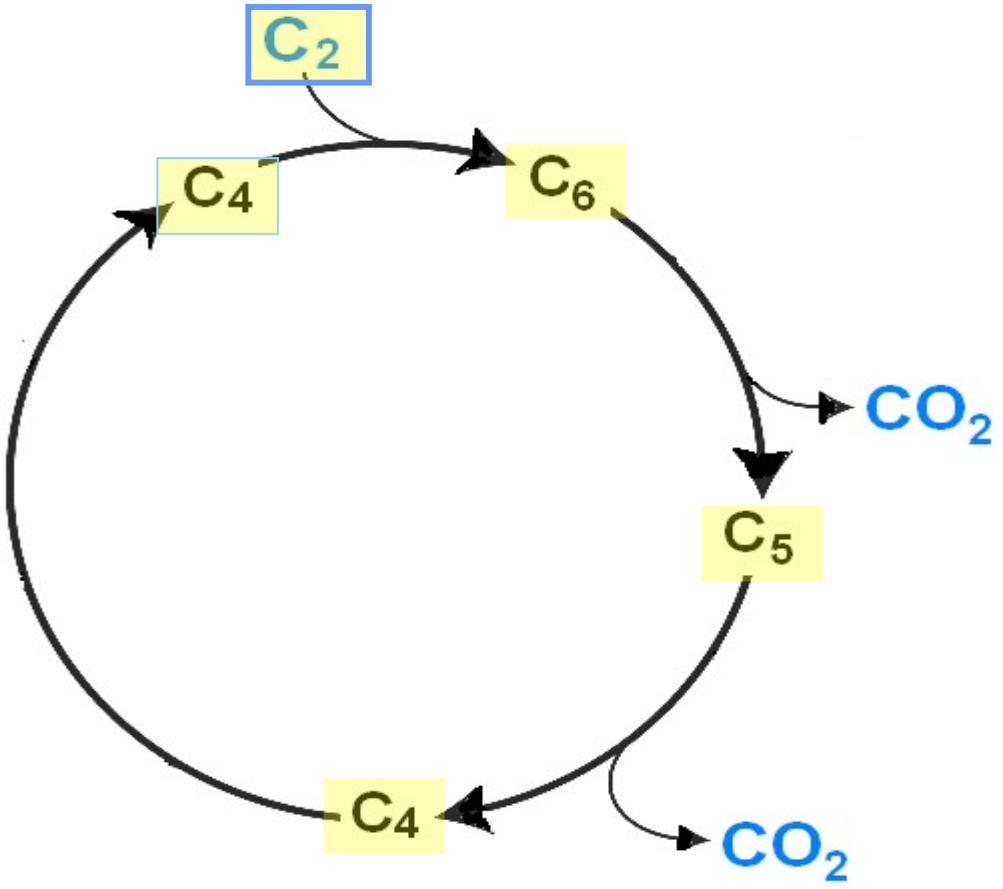
Acetyl-CoA, the substrate for the citric acid cycle, is formed from
the breakdown of

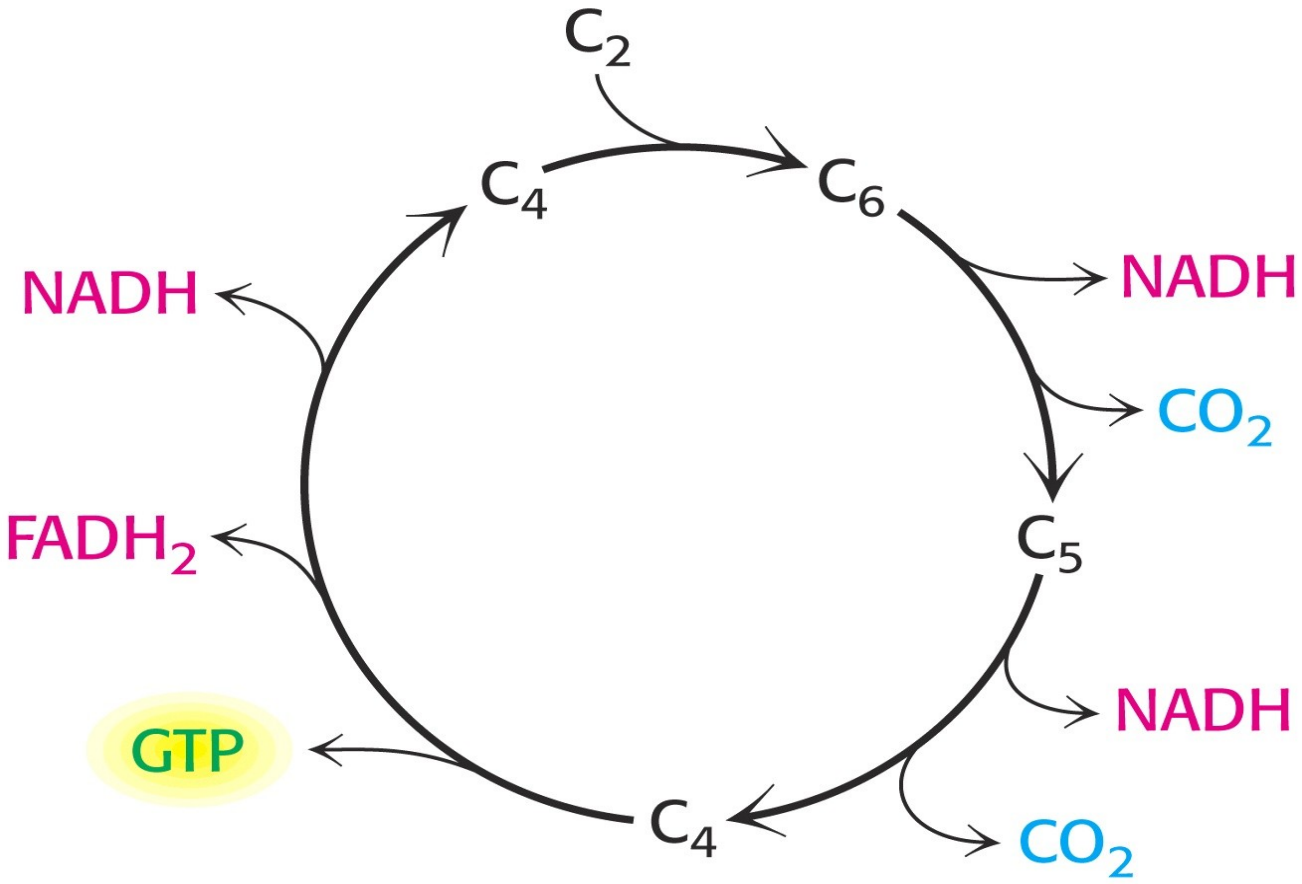
– saccharides (oxidative decarboxylation of pyruvate),

– fatty acids (β-oxidation) and ketone bodies, and

– many amino acids.

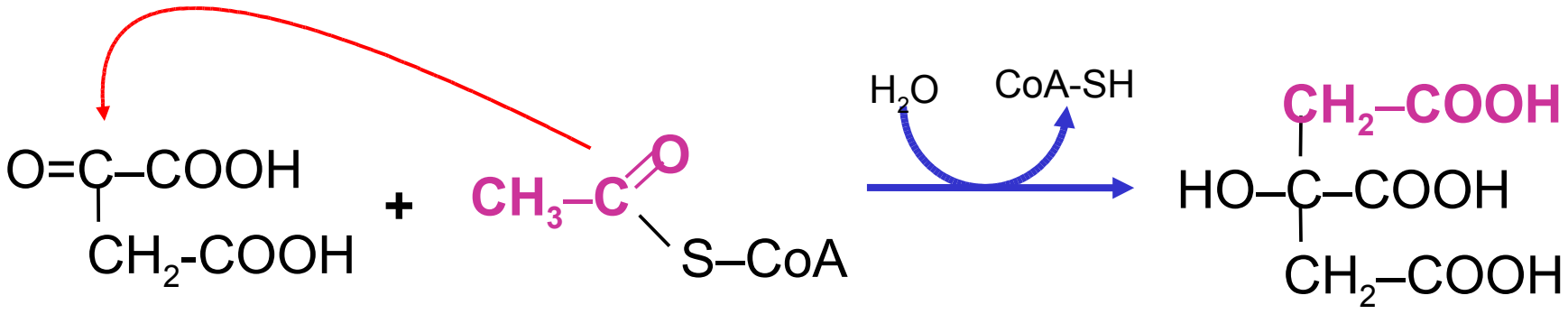
Two carbon atoms enter the cycle in the condensation of an **acetyl unit** (from acetyl CoA) **with oxaloacetate**.
Two carbon atoms leave the cycle in the form of CO_2, oxaloacetate is the end-product.




C2
C4
C6
NADH
CO2
C5
NADH
CO2
C4
GTP
FADH2
NADH

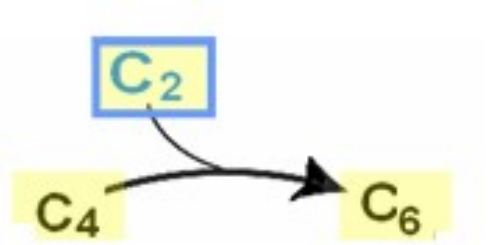
1 Condensation of acetyl CoA and oxaloacetate

is catalysed by **citrate synthase**:



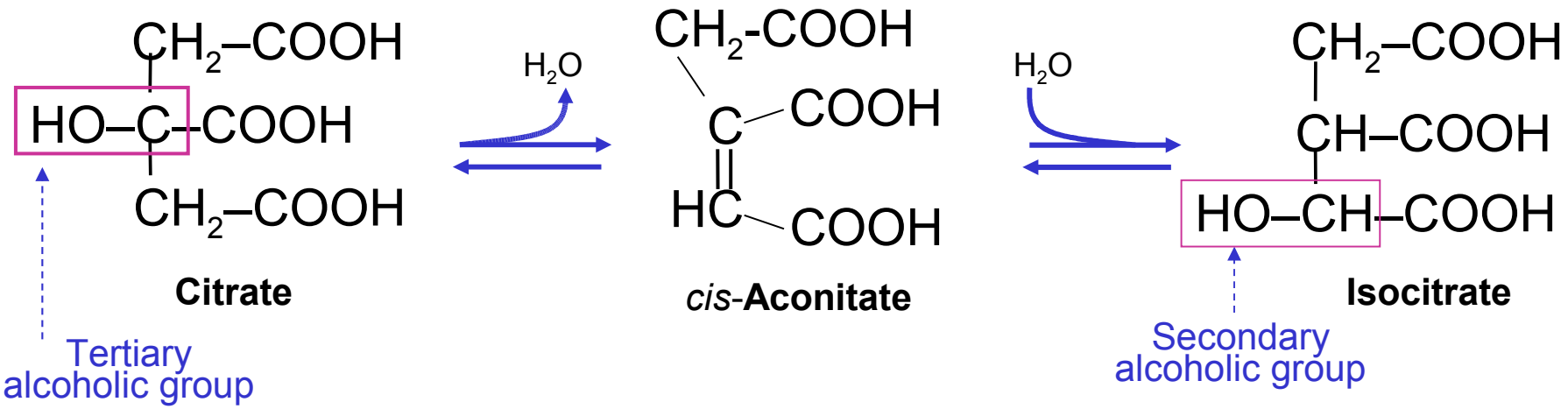
Oxaloacetate **Acetyl coenzyme A** **Citrate**

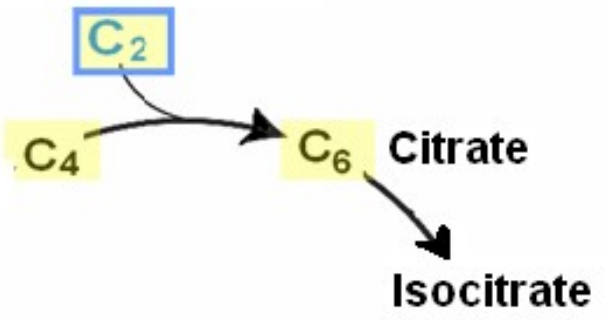
The reaction is an aldol condensation
and is **irreversible in mitochondrial matrix.**



2 Isomerization of citrate into isocitrate

is catalysed by **aconitase** (cofactor FeS-protein). The isomerization of citrate is accomplished by a **dehydratation** step followed by a **hydratation**:

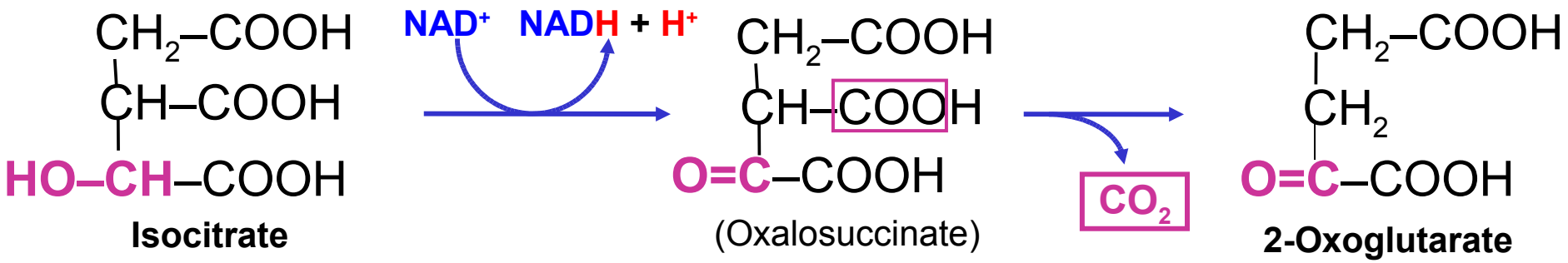




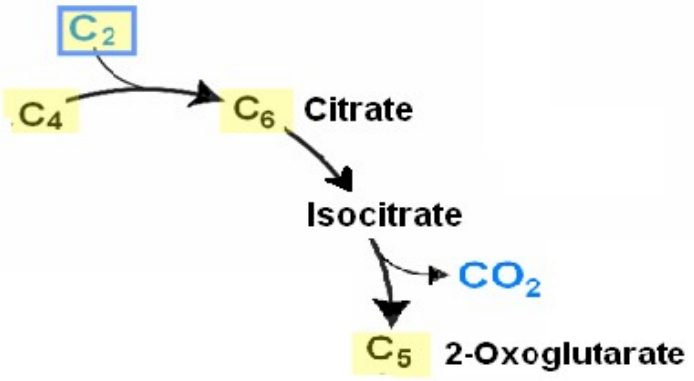
3 Isocitrate is oxidized and decarboxylated to 2-oxoglutarate

The **first of four oxidation reactions** in the citrate cycle is catalysed by **isocitrate dehydrogenase**, the cofactor is **NAD^+**.

The intermediate in this reaction is unstable oxalosuccinate which **loses CO_2**, while bound to the enzyme, to form 2-oxoglutarate.

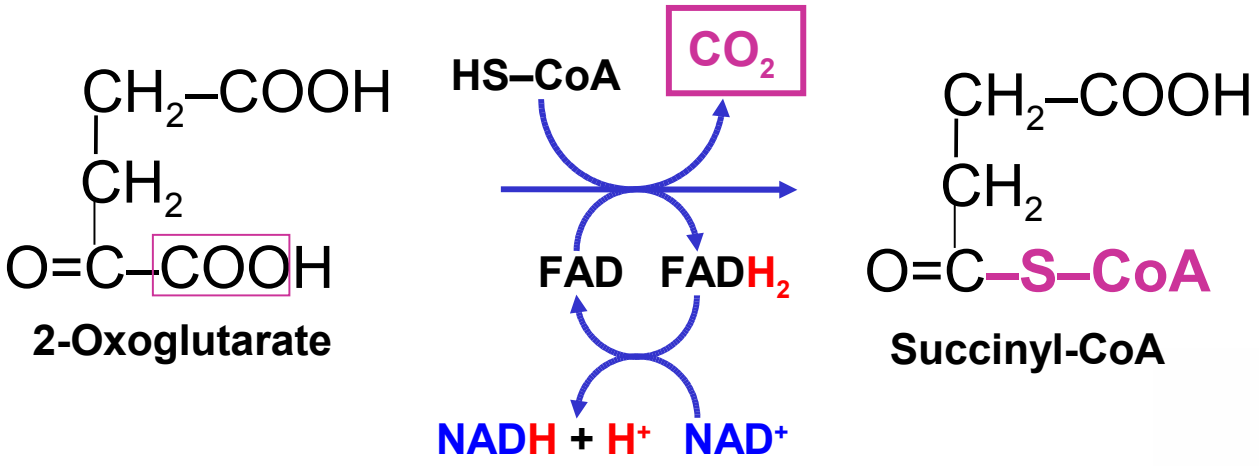


The reaction is **irreversible**.
The rate of this reaction is **important in determining the overall rate of the cycle.**

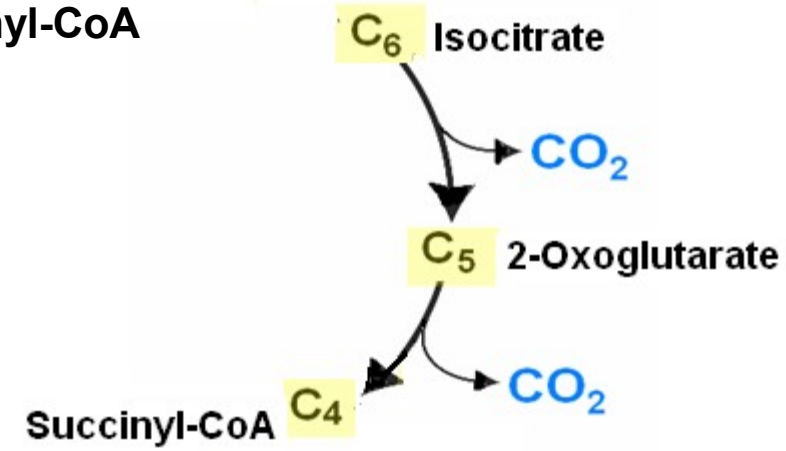


4 Oxidative decarboxylation of 2-oxoglutarate to succinyl-CoA

The **second oxidative step** and decarboxylation in the cycle is closely analogous to the oxidative decarboxylation of pyruvate.
The **2-oxoglutarate dehydrogenase complex** requires also the same five cofactors – TDP, lipoate, coenzyme A, FAD and NAD^+.



Succinyl-CoA is the high-energy thioester compound.

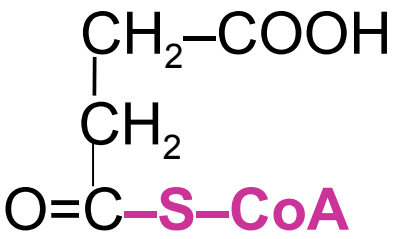


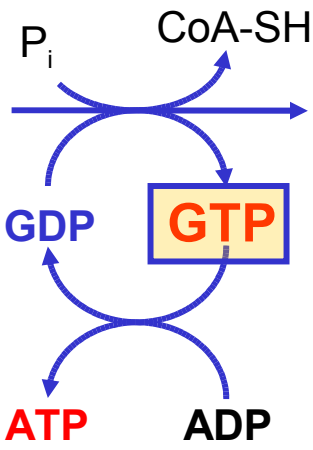
5 The cleavage of succinyl-CoA is coupled to the phosphorylation of GDP

In the reaction catalysed by **succinyl-CoA synthetase** (succinate thiokinase), the energy inherent in the thioester molecule is transformed into phosphoryl-group transfer.

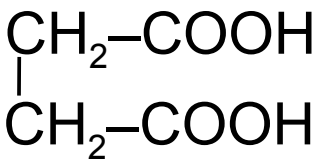
This substrate-level phosphorylation is the only step in the citrate cycle that directly yields a high-energy compound.

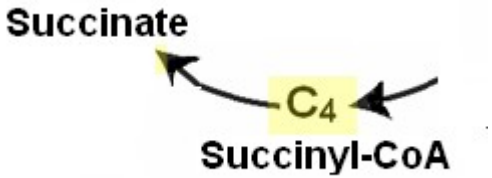
Succinyl-CoA





Succinate

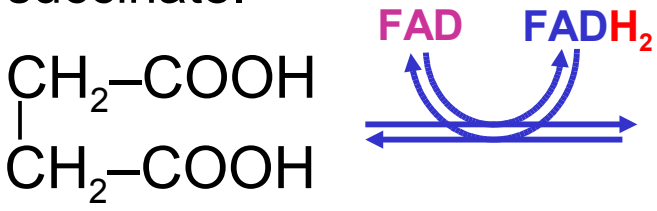




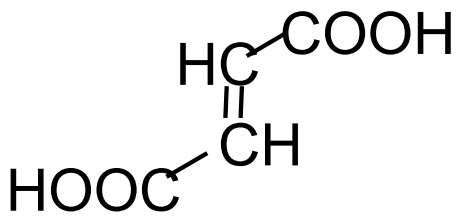
GDP is phosphorylated to GTP n three steps, succinyl phosphate and phosphohistidyl residue of the enzyme are the intermediates. The mechanism has got the nickname "passing a hot potato".

6 Oxidation of succinate to fumarate

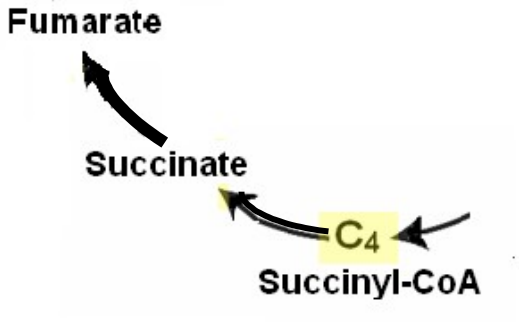
is the **third oxidative step**, catalysed by **succinate dehydrogenase**. The prosthetic group **FAD** accepts two atoms of hydrogen from succinate.



Succinate



Fumarate

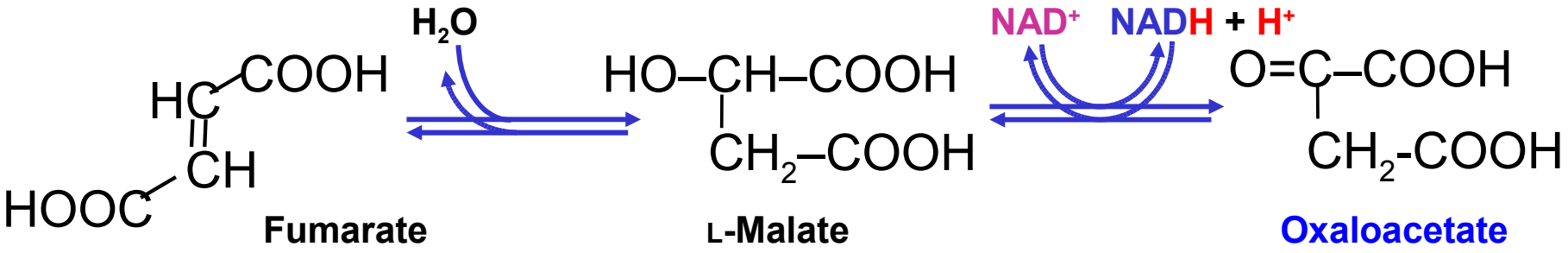


Succinate dehydrogenase differs from other enzymes in the citrate cycle in being **embedded in the inner mitochondrial membrane**.

The enzyme is directly associated with the terminal respiratory chain as the **component of the complex II**, which transfers a reducing equivalent (in the form of two electrons) to ubiquinone.

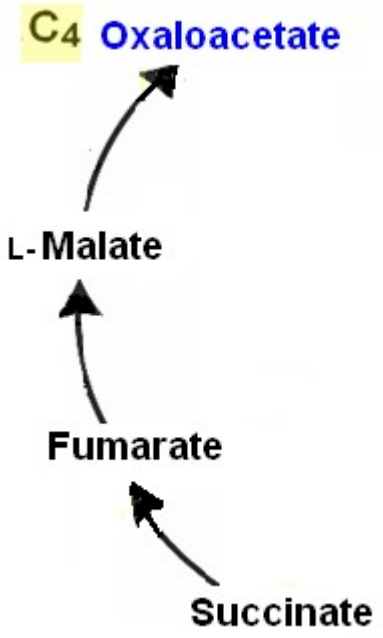
Succinate dehydrogenase, like aconitase, is a non-haem iron protein. In addition to the flavin prosthetic group, it contains three different types of Fe-S clusters that také part in the electron transport.

7 – 8 Oxaloacetate is regenerated by hydratation of fumarate and oxidation of malate

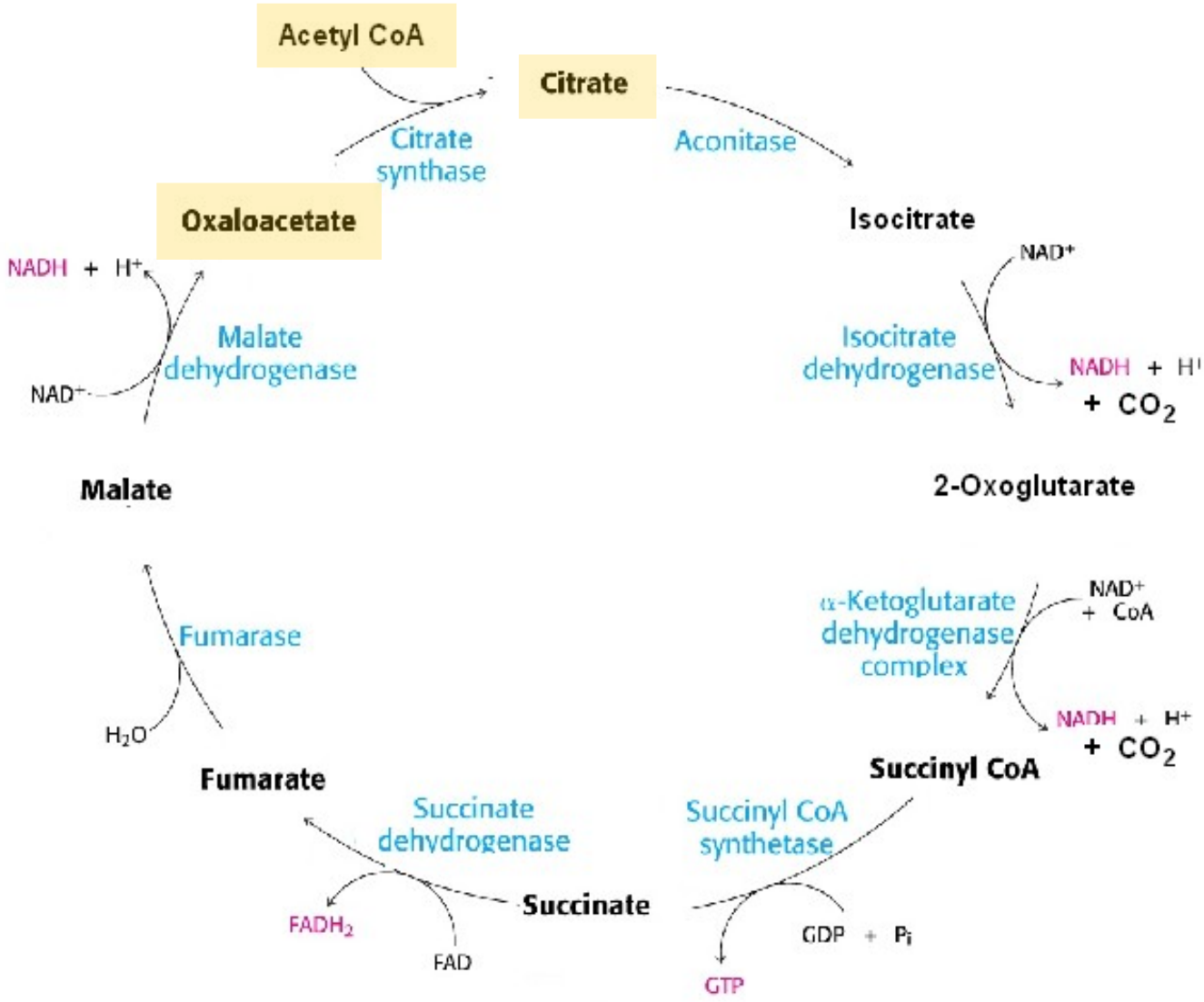


Fumarase catalyses a stereospecific *trans* addition of water, only the L-enantiomer of malate is formed.

The dehydrogenation of malate catalysed by **malate dehydrogenase** is **the fourth oxidative step** in the cycle. It is driven by the utilization of the products – oxaloacetate by citrate synthase and NADH by the terminal respiratory chain .



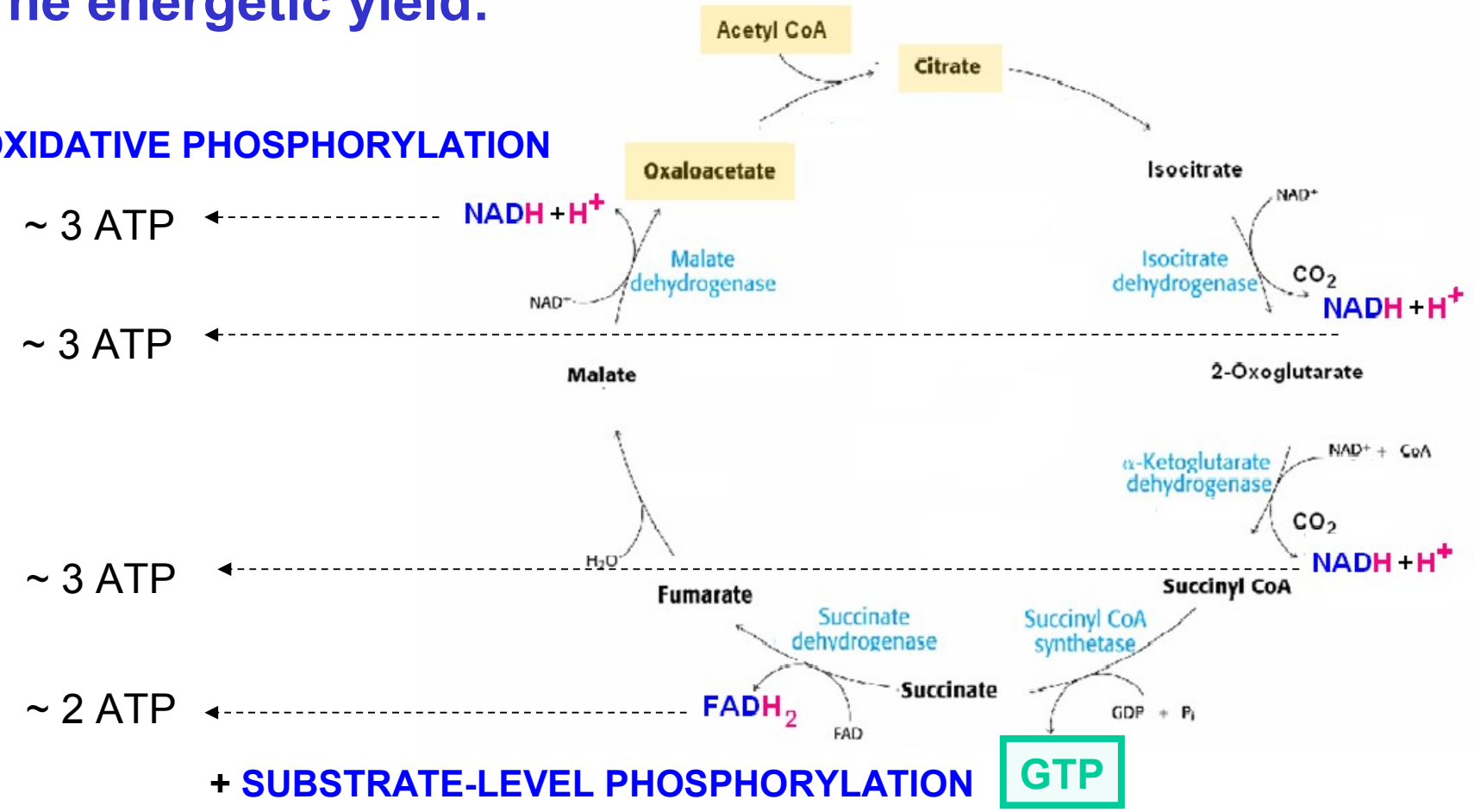
Recapitulation:



Citric acid cycle

Step	Reaction	Enzyme	Prosthetic group	$\Delta G^{\circ\prime}$ kJ mol^{-1}
1	Acetyl CoA + oxaloacetate + $H_2O \longrightarrow$ citrate + CoA + H^+	Citrate synthase		−31.4
2a	Citrate $\rightleftharpoons$ *cis*-aconitate + H_2O	Aconitase	Fe–S	8.4
b	*cis*-Aconitate + $H_2O \rightleftharpoons$ isocitrate	Aconitase	Fe–S	−2.1
3	Isocitrate + $NAD^+ \rightleftharpoons$ α-ketoglutarate + CO_2 + NADH	Isocitrate dehydrogenase		−8.4
4	α-Ketoglutarate + NAD^+ + CoA $\rightleftharpoons$ succinyl CoA + CO_2 + NADH	α-Ketoglutarate dehydrogenase complex	Lipoic acid, FAD, TPP	−30.1
5	Succinyl CoA + P_i + GDP $\rightleftharpoons$ succinate + GTP + CoA	Succinyl CoA synthetase		−3.3
6	Succinate + FAD (enzyme-bound) $\rightleftharpoons$ fumarate + $FADH_2$ (enzyme-bound)	Succinate dehydrogenase	FAD, Fe–S	0
7	Fumarate + $H_2O \rightleftharpoons$ L-malate	Fumarase		−3.8
8	L-Malate + $NAD^+ \rightleftharpoons$ oxaloacetate + NADH + H^+	Malate dehydrogenase		+29.7

The energetic yield:

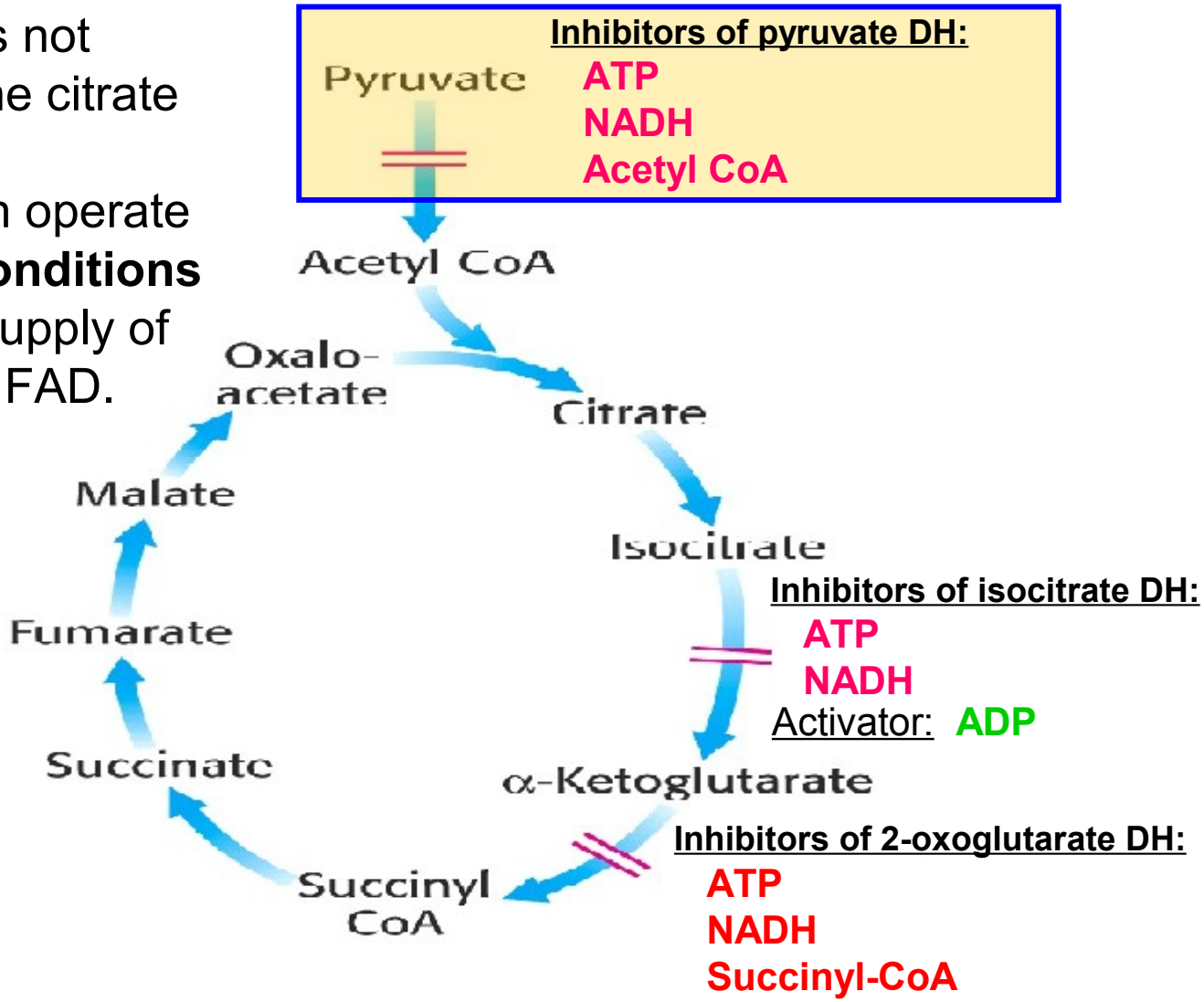


Total approx.12 molecules ATP from the oxidation of 1 acetyl-CoA

- about 11 ATP due to reoxidation of reduced coenzymes in the terminal respiratory chain,
- 1 GTP direct yield through a substrate-level phosphorylation in the citrate cycle.

Regulation of the citrate cycle

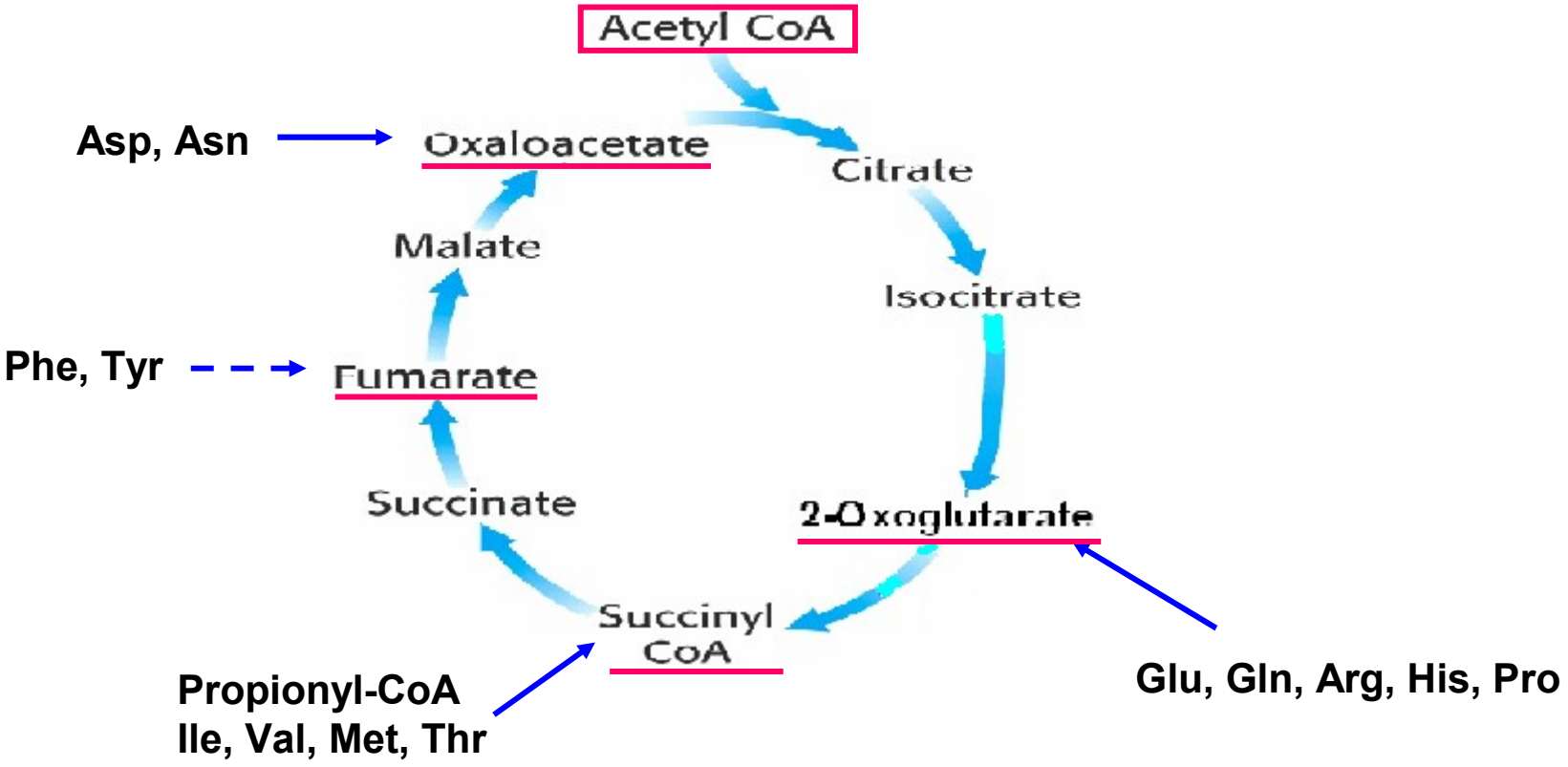
Molecular oxygen does not participate directly in the citrate cycle.
However, the cycle can operate **only under aerobic conditions** because it requires a supply of (re)oxidized NAD^+ and FAD.



The catabolic role of the citrate cycle

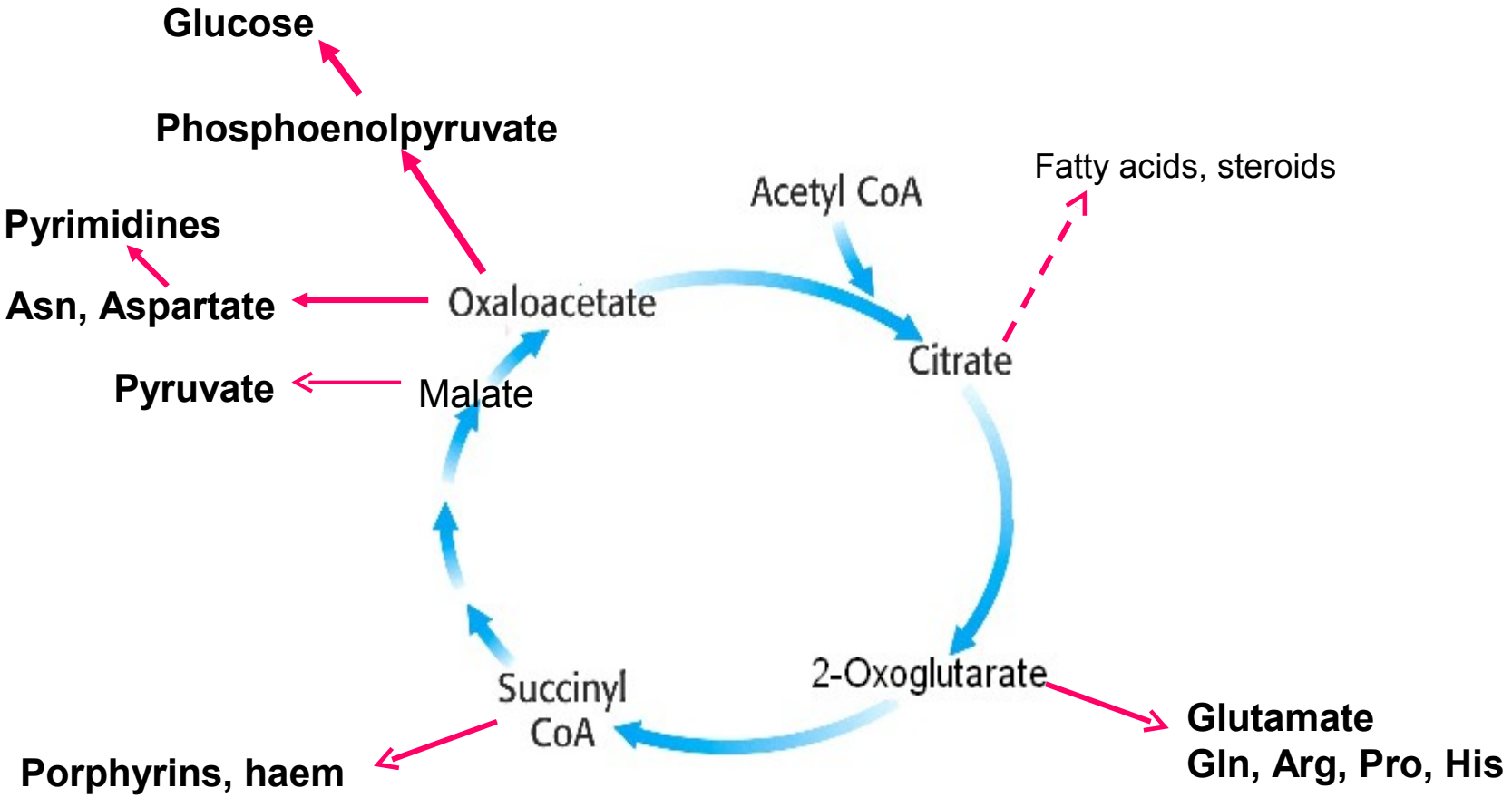
Not only acetyl-CoA is oxidized in the citrate cycle, but **also other compounds**, which are metabolized to the cycle intermediates, **can also serve as substrates** of the cycle.

The entries into the cycle:



The anabolic role of the citrate cycle

The citric acid cycle also **provides intermediates for biosyntheses** – thus it exhibits an **amphibolic character.**

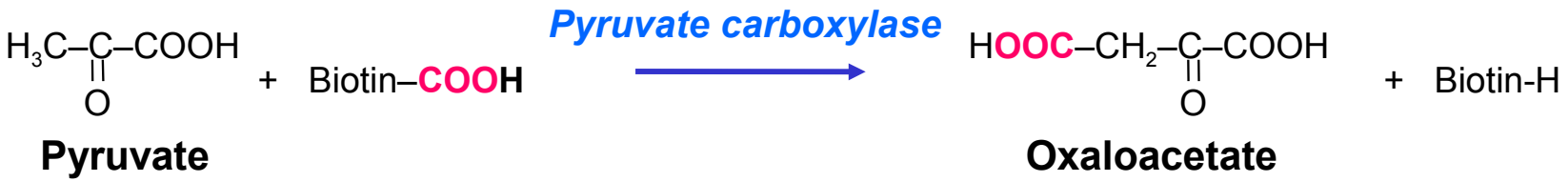


Intermediates drawn off for biosyntheses are replenished by the **anaplerotic reactions**.

Anaplerotic reactions

lead to the net synthesis, or replenishment, of pathway components.

The most important of them is the
formation of new oxaloacetate by carboxylation of pyruvate,
a crucial step in gluconeogenesis.

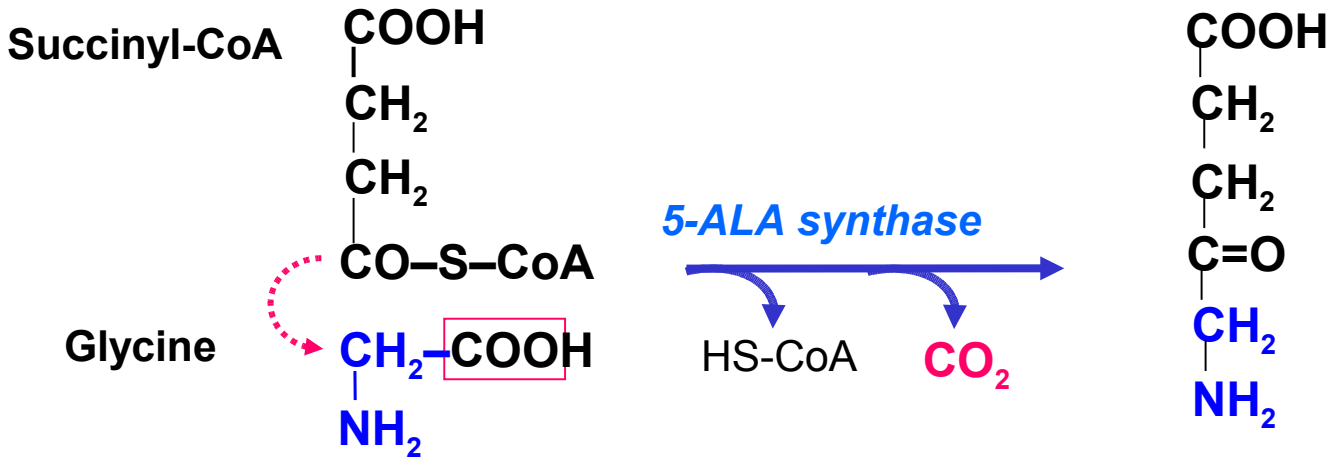


If the energy charge of the cell is low, oxaloacetate replenishes the citric acid cycle.
If the energy charge is high, oxaloacetate is converted into glucose.

There are also other anaplerotic reactions of less importance, e.g.
reductive carboxylation of pyruvate to malate,
transamination of aspartate that gives oxaloacetate,
transamination of glutamate to 2-oxoglutarate,
as well as the other reaction drawn in the picture 27
(the catabolic role of the cycle).

Biosynthesis of haem

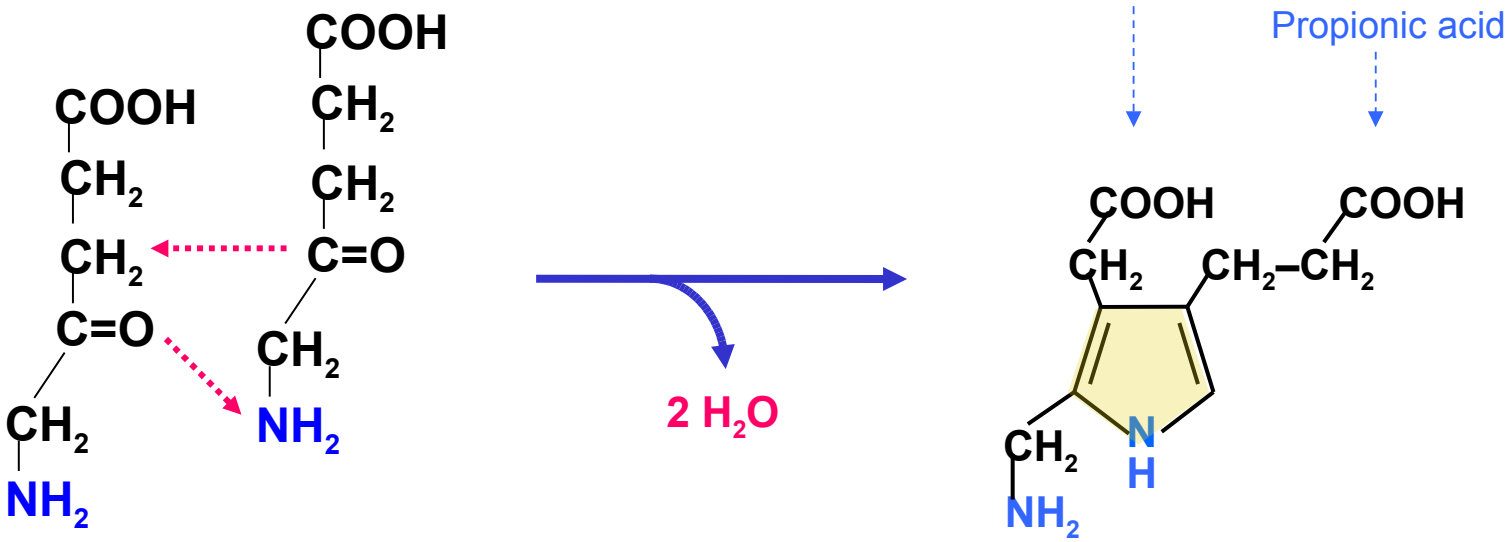
The first reaction in the biosynthesis of porphyrins is the condensation of succinyl-coenzyme A and glycine **in mitochondria**:



5-Aminolaevulinate
(5-amino-4-oxobutanoic acid)

The enzyme has pyridoxal phosphate as a prosthetic group.
5-aminolaevulinate (5-ALA, δ-aminolaevulinate) is transported **into the cytosol**.

In the cytosol, **two molecules** of 5-aminolaevulinate undergo the condensation to form a pyrrole derivative – **porphobilinogen:**

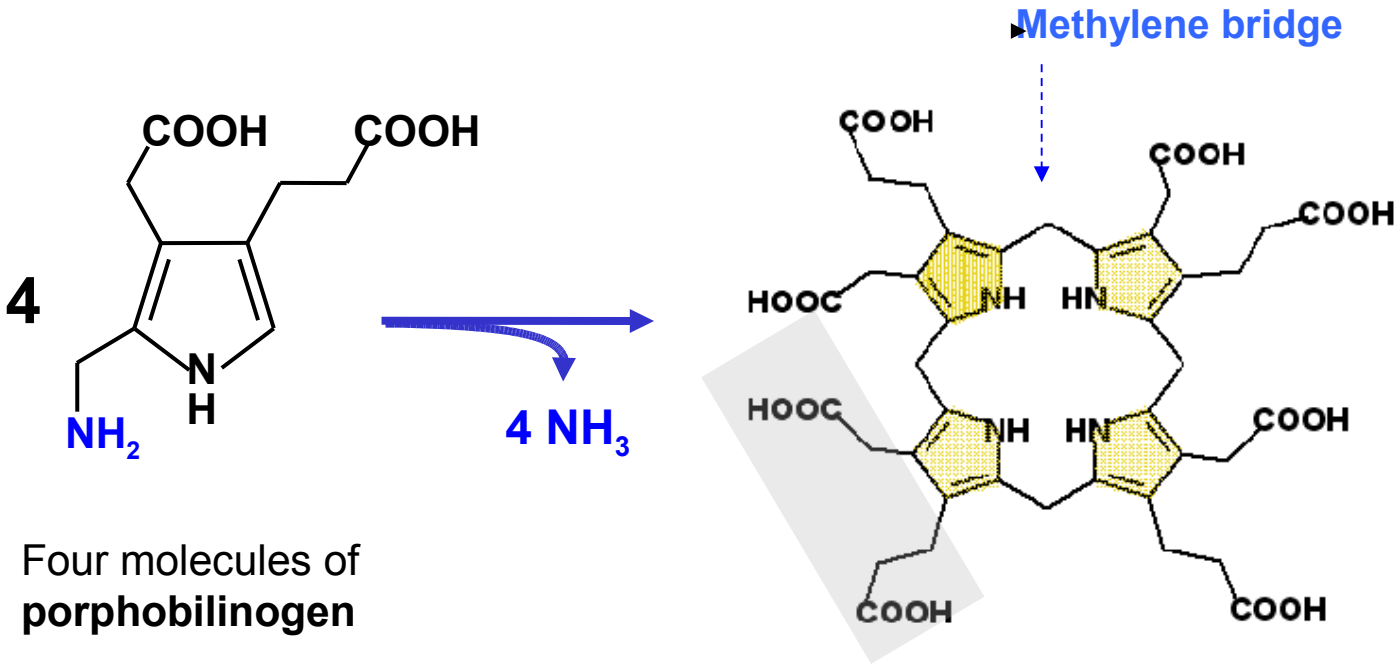


Two molecules of
5-aminolaevulinate

Porphobilinogen

Four molecules of porphobilinogen then condense "head to tail" to form a **linear tetrapyrrole** in a reaction catalysed by ***porphobilinogen deaminase***. The product cyclizes to form the **tetrapyrrole ring** of uroporphyrinogen.

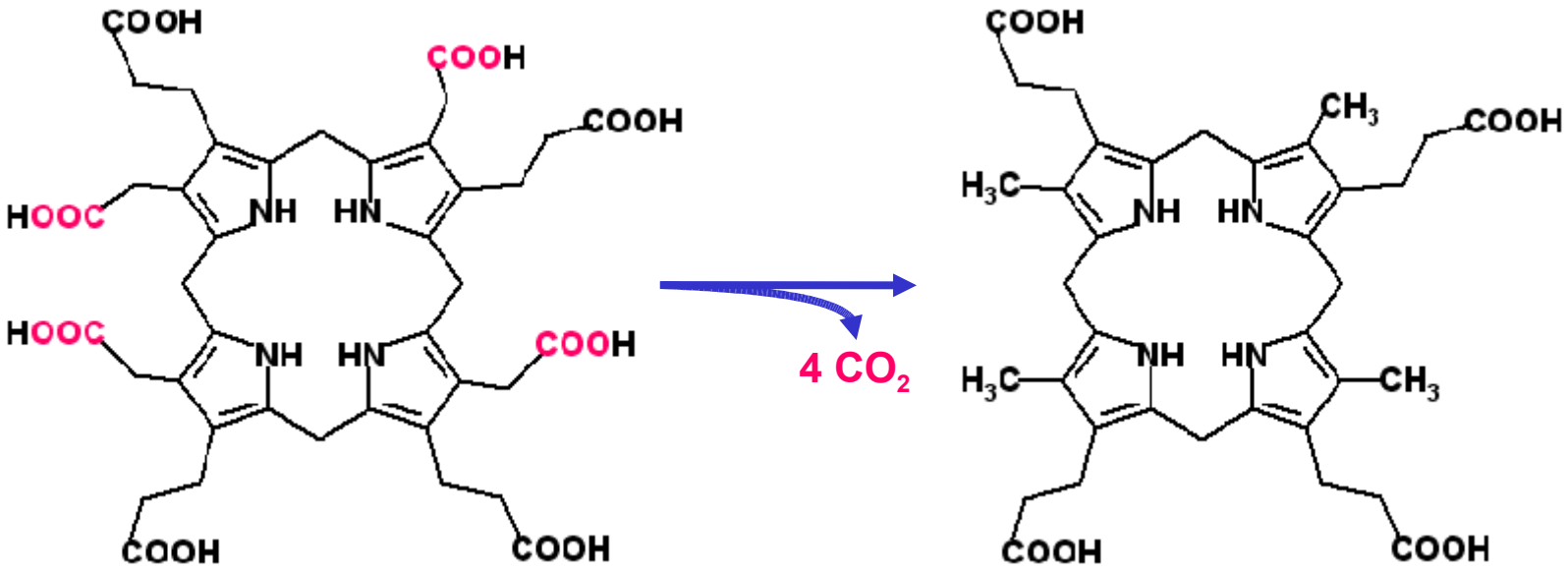
Under physiological circumstances due to the presence of a protein modifier called ***co-synthase***, **uroporphyrinogen III** with an asymmetrical arrangement of side chains of the ring D is formed. Only traces of symmetrical uroporphyrinogen I are produced.



Four molecules of **porphobilinogen**

Uroporphyrinogen III

In subsequent reactions, the side chains and the degree of saturation of the porphyrin ring are modified:

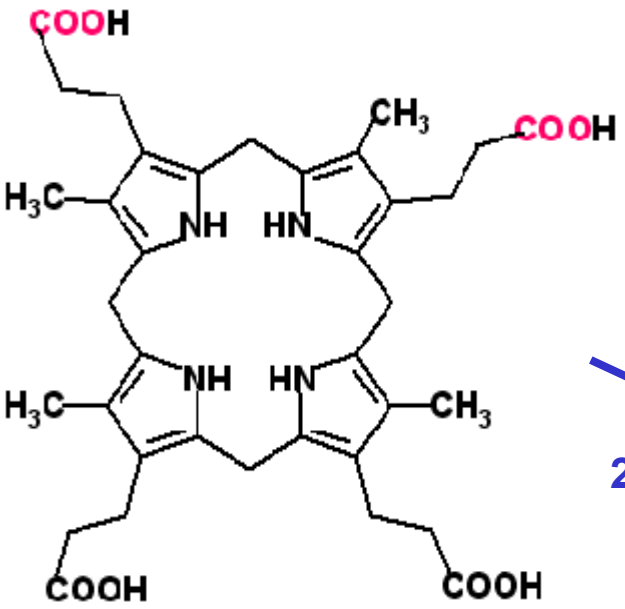


Uroporphyrinogen III
(eight carboxylic groups of four acetates and four propionates)

Coproporphyrinogen III
(four carboxylic groups of propionates)

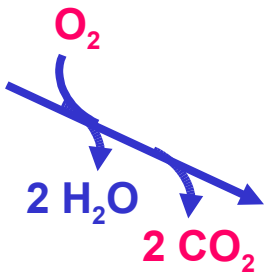
This reaction takes place in the **cytosol**. Coproporphyrinogen is then **transported into the mitochondria**, where the biosynthesis of haem is completed by two oxidative steps and a ferrous cation is built in.

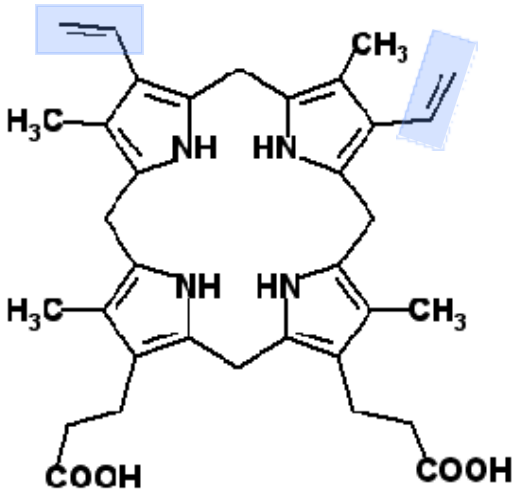
The conversion of two of the propionate side chains into vinyl groups yields **protoporphyrinogen IX**. then follows the desaturation of the porphyrin ring.

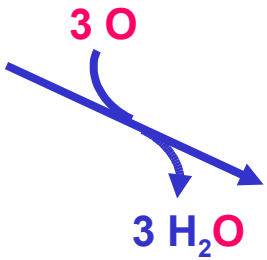


Coproporphyrinogen III

Protoporphyrinogen IX
(colourless)

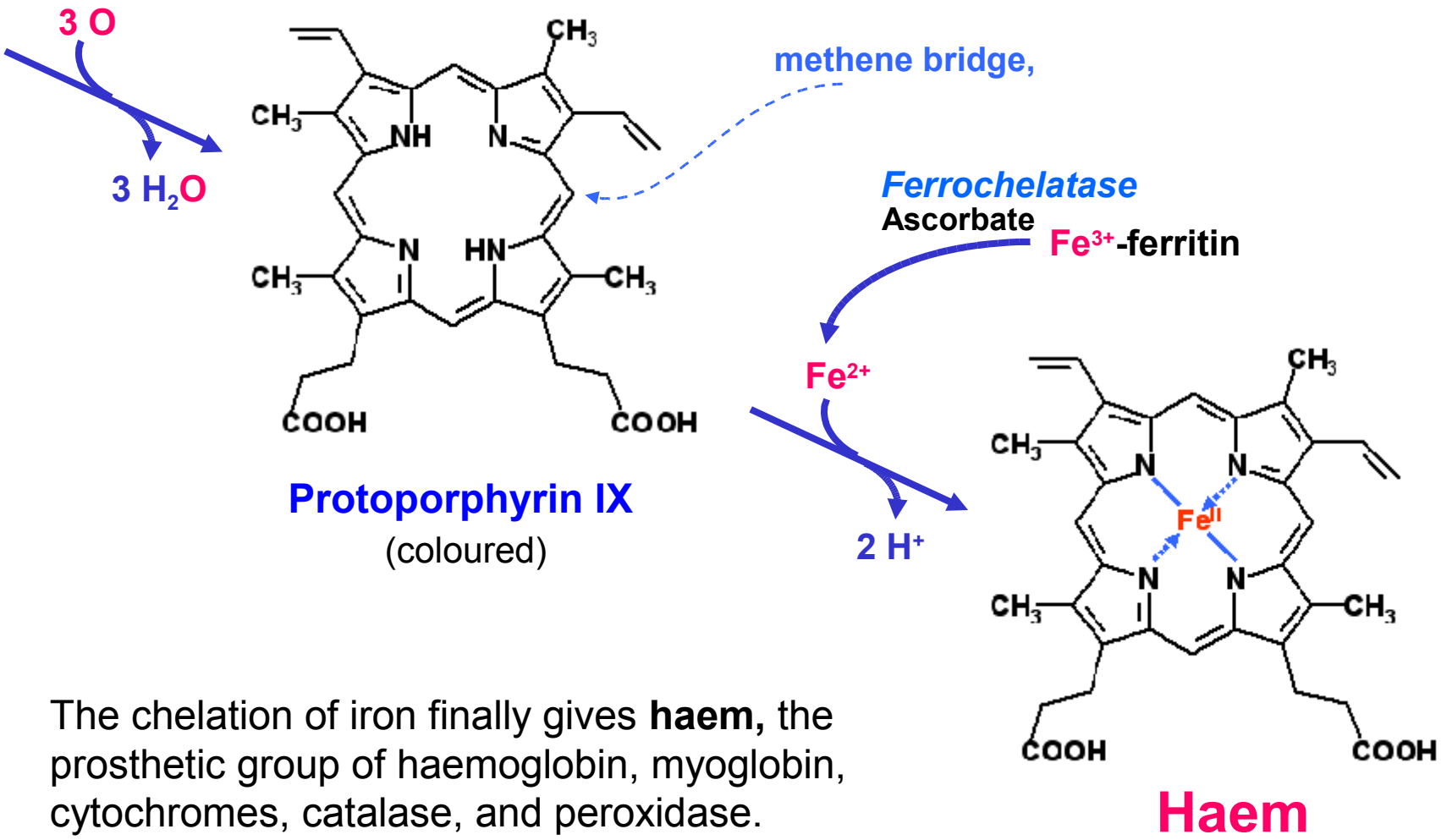




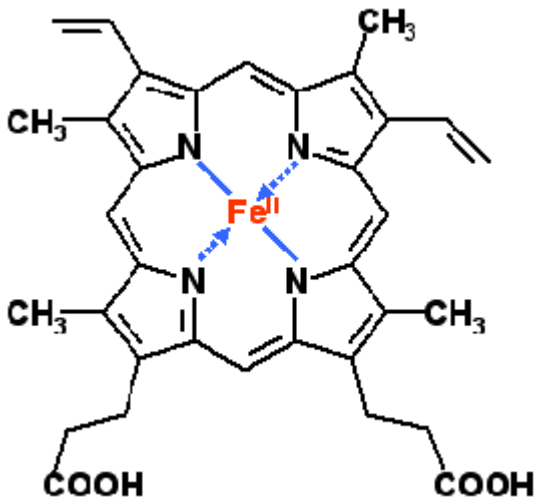


The <u>isomer numbers</u>: Uro- and coproporphyrinogen has theoretically only four isomers that differ in the position of acetates and propionates, type III and I are natural products. Protoporphyrin has three different types of substituents, we may imagine 15 isomers. Product of the biosynthesis that originates from coproporphyrin III, is called protoporphyrin IX.

The desaturation of the ring (methylene bridges are converted into methene bridges) forms a **fully conjugated system of double bonds** – the product is of intensive colour.



The chelation of iron finally gives **haem,** the prosthetic group of haemoglobin, myoglobin, cytochromes, catalase, and peroxidase.



Haem

Approx. **0.4 mmol** synthesized per day

Proteosynthesis of globin α- and β-chains

HAEMOGLOBIN monomers (1 Fe)

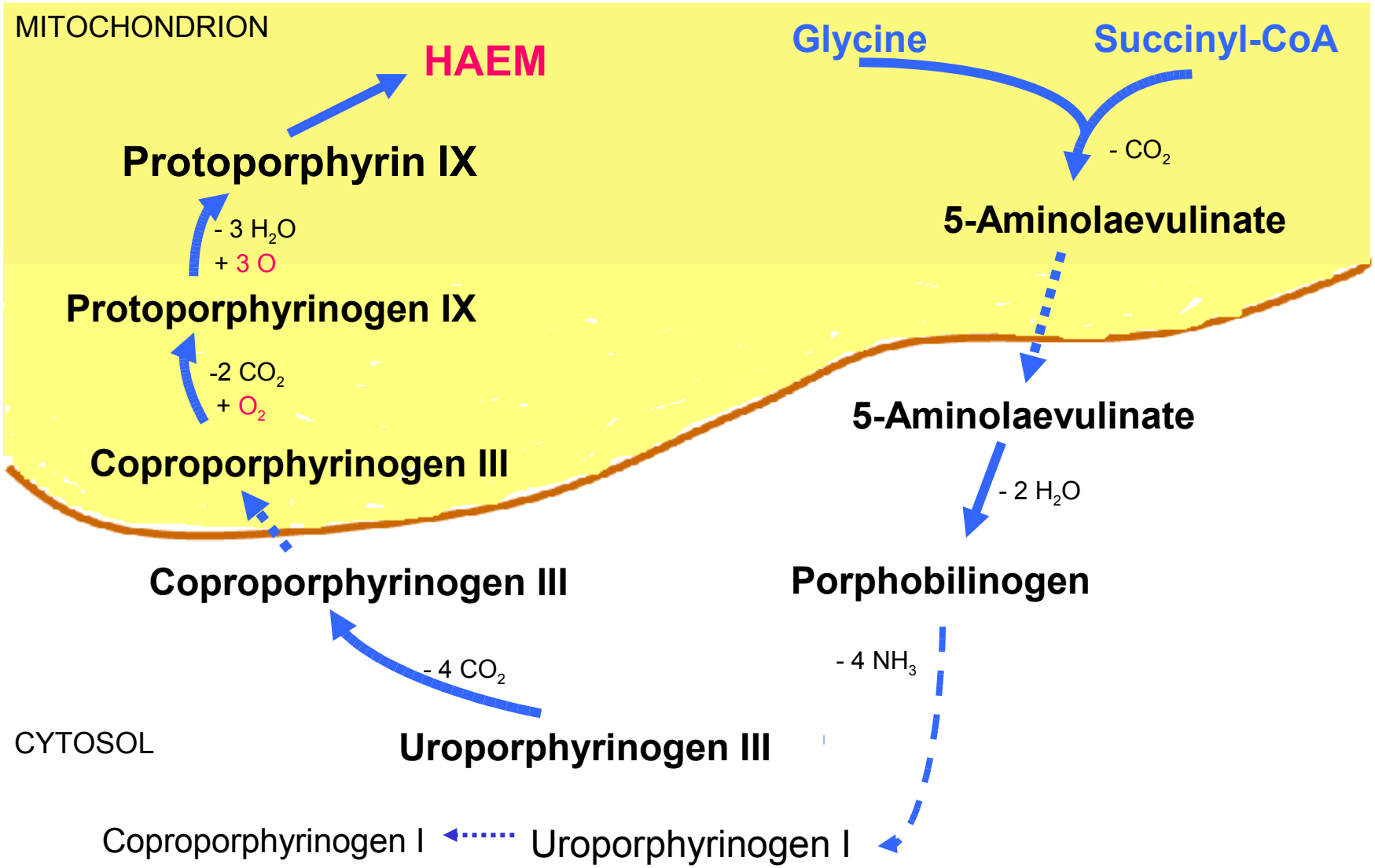
HAEMOGLOBIN $\alpha_2\beta_2$ (4 Fe)

Myoglobin
Cytochromes
Catalase, peroxidase

The rate-limiting step of the overall biosynthesis is synthesis of 5-ALA. In the liver, where only 15 % of haem are synthesized; the feedback control (inhibition) depends of the availability of **haem** or its Fe^{III} oxidation product **haemin.**

85 % of the body's haem groups are synthesized **in the erythroid cells**.

Recapitulation:



Porphyrias

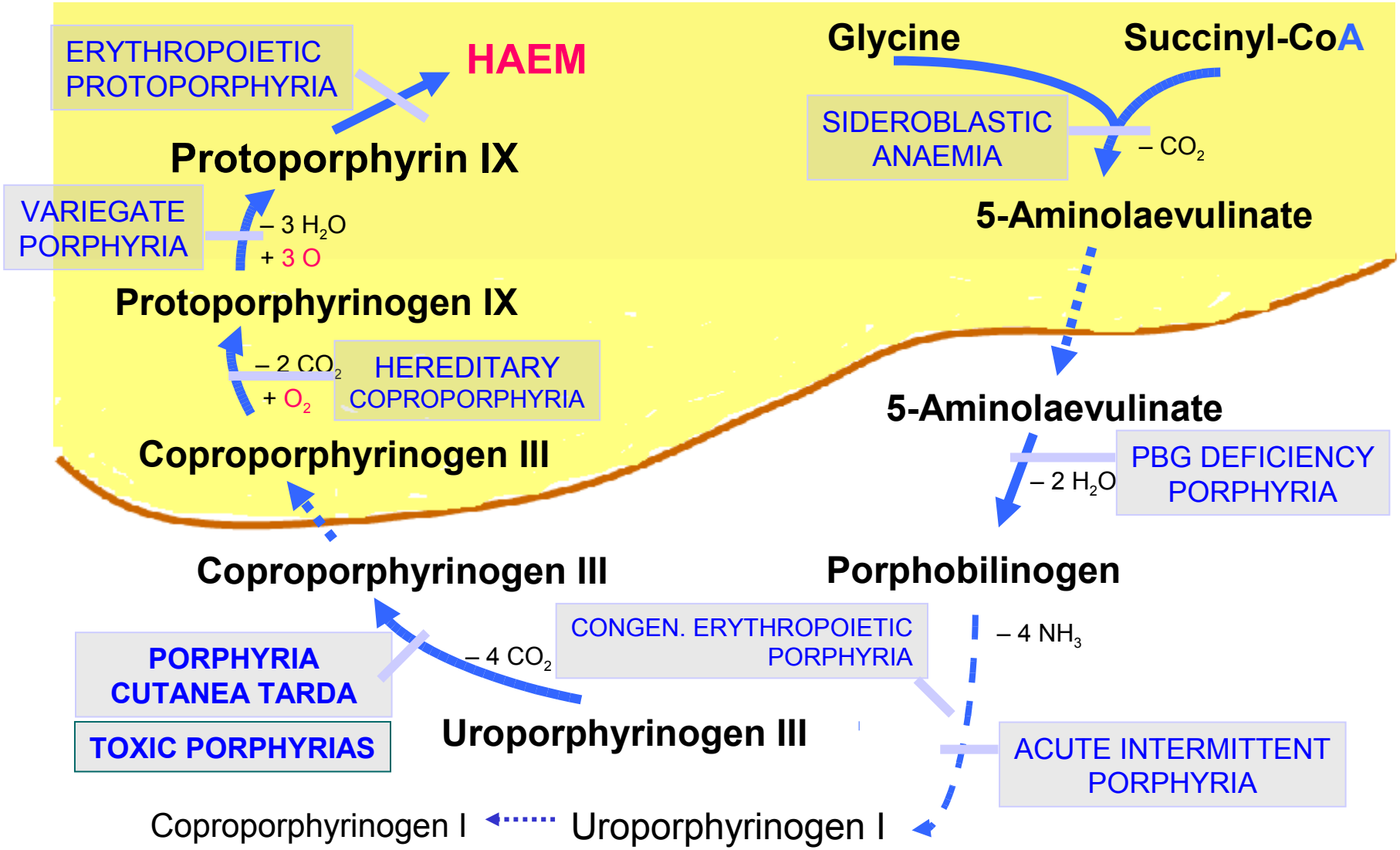
The porphyrias are mostly genetic diseases characterized by defects in haem synthesis.

In spite of haem synthesis may provide enough haem for the body due to reduced feedback inhibition, there is often the **overproduction of porphyrins or their precursors** – increased excretion of porphyrins in the urine and faeces.

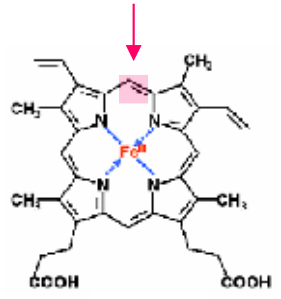
The various symptoms depend on the type of enzyme defect.
Some of them are

- skin lesions on exposure to sunlight
  (porphyrins are photosensitizing agents) – erythemas, scarring,
- disturbances of erythropoiesis,
- disturbances of the liver functions,
- neuropsychiatric disturbances.

Porphyrias


ERYTHROPOIETIC PROTOPORPHYRIA
HAEM
Protoporphyrin IX
VARIEGATE PORPHYRIA
– 3 H2O
+ 3 O
Protoporphyrinogen IX
– 2 CO2
+ O2
HEREDITARY COPROPORPHYRIA
Coproporphyrinogen III
Glycine
Succinyl-CoA
SIDEROBLASTIC ANAEMIA
– CO2
5-Aminolaevulinate
5-Aminolaevulinate
– 2 H2O
PBG DEFICIENCY PORPHYRIA
Coproporphyrinogen III
Porphobilinogen
PORPHYRIA CUTANEA TARDA
TOXIC PORPHYRIAS
– 4 CO2
CONGEN. ERYTHROPOIETIC PORPHYRIA
– 4 NH3
Uroporphyrinogen III
ACUTE INTERMITTENT PORPHYRIA
Coproporphyrinogen I
Uroporphyrinogen I

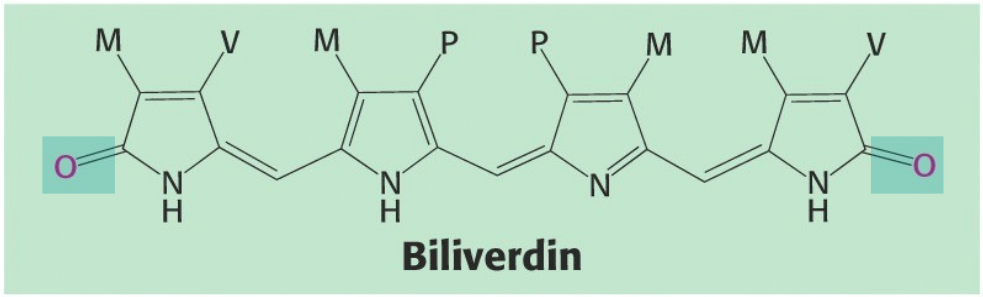
Degradation of haemoglobin in the reticuloendothelial system:



Haem

$2\ O_2 + NADPH$ → $CO + H_2O + NADP^+$ + Fe^{3+}

Haemoxygenase (cyt P450)



Biliverdin

$NADPH + H^+$ → $NADP^+$

Biliverdin reductase

Bilirubin