Citric acid cycle

Synthesis of heme. Hemoproteins

Three phases of nutrient catabolism

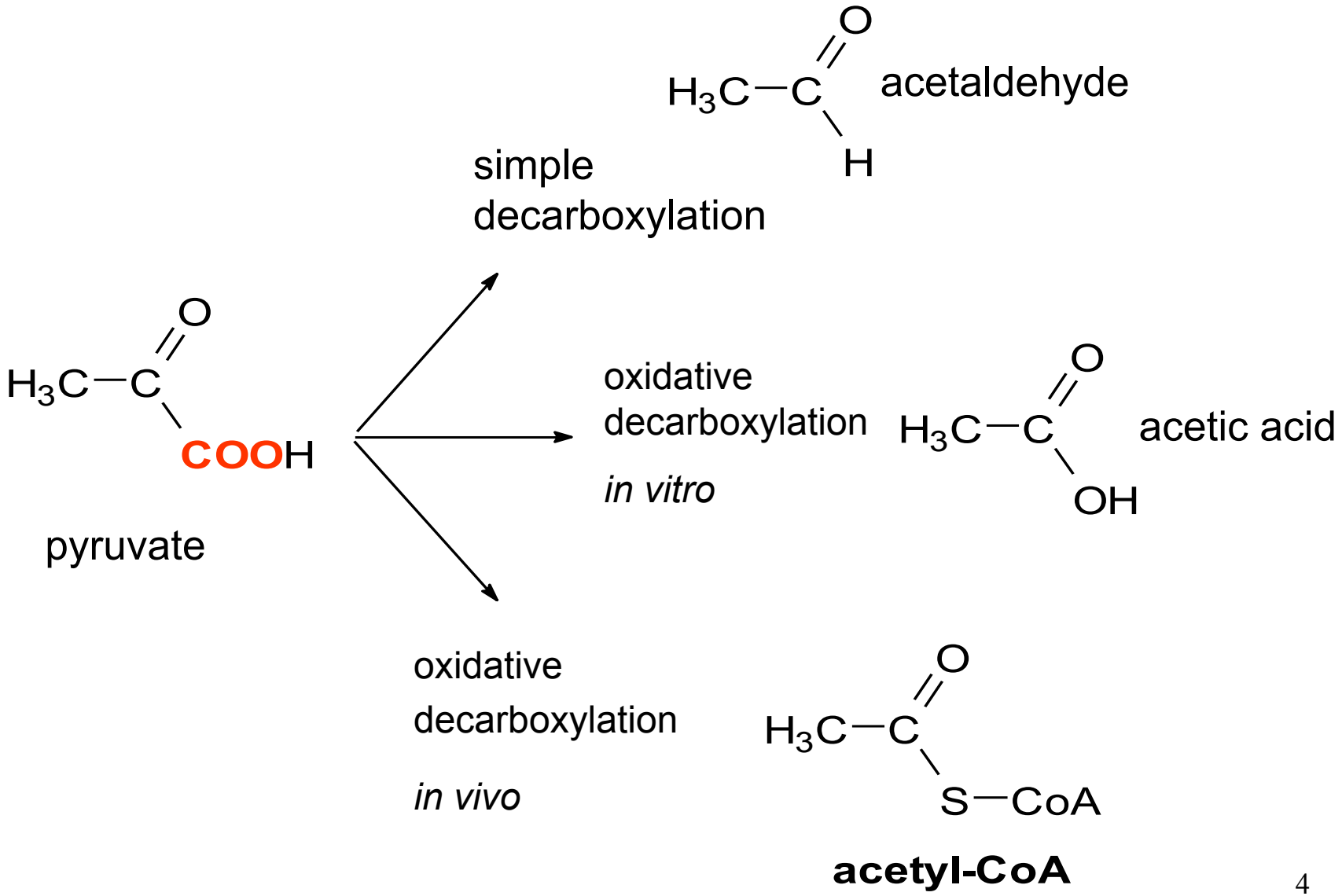
Phase	The conversions of nutrients	ATP yield
I.	Hydrolytic cleavage of nutrients during digestion: Starch → maltose → glucose Proteins → peptides → amino acids Lipids → fatty acids	none
II.	Intracellular catabolism of nutrients: Glc, FA, AA → → → pyruvate → acetyl-CoA Production of ATP in glycolysis (2 ATP/Glc) Production of reduced cofactors ($NADH+H^+$, $FADH_2$)	small
III.	Citrate cycle: acetyl-CoA → 2 CO_2 + red. cofactors + ATP Respiratory chain: oxidation of reduced cofactors Aerobic phosphorylation: synthesis of ATP from ADP + P_i	the biggest

Sources of acetyl-CoA

- oxidative decarboxylation of pyruvate (from glucose and 6 AA)
- β-oxidation of fatty acids
- catabolism of some amino acids (Thr, Trp, Lys, Leu, Ile)
- ketone bodies utilization in extrahepatal tissues:

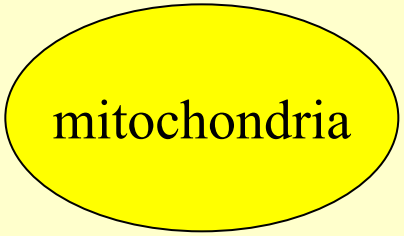
  acetoacetate → acetoacetyl-CoA → 2 acetyl-CoA
- catabolism of ethanol → acetaldehyde → acetate → acetyl-CoA

Compare different ways of pyruvate decarboxylation

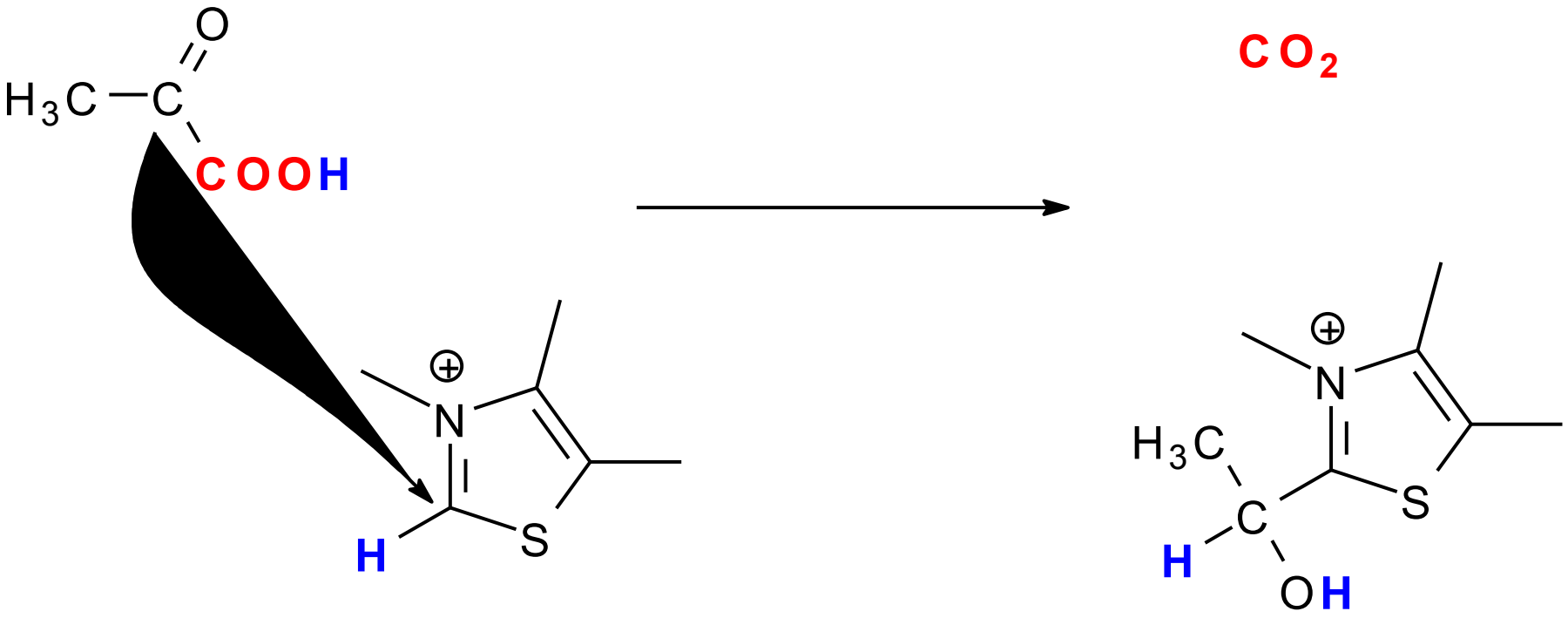


Oxidative decarboxylation of pyruvate is catalyzed by pyruvate dehydrogenase complex: three enzymes and five cofactors

1. thiamin diphosphate (TDP)
2. lipoate
3. coenzyme A
4. FAD
5. NAD^+



(1) Decarboxylation of pyruvate

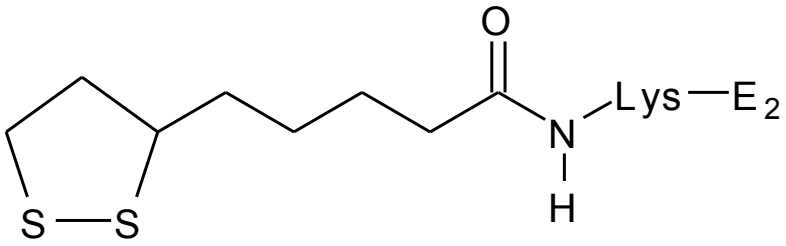


TDP

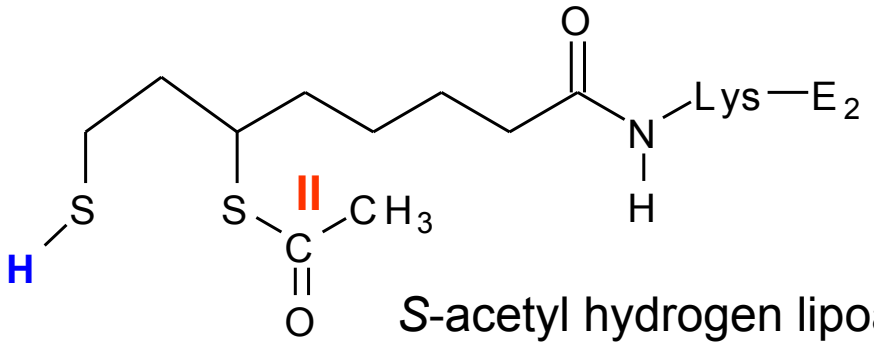
thiazolium ring

hydroxyethyl-TDP

(2) Transfer of acetyl to lipoate is redox reaction

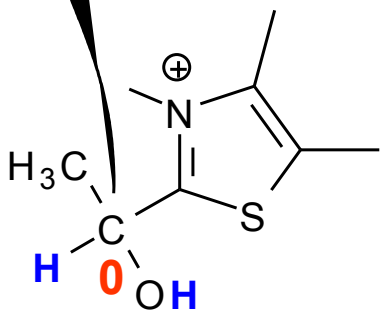


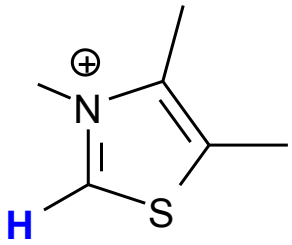
lipoate attached to enzyme



S-acetyl hydrogen lipoate

(thioester)

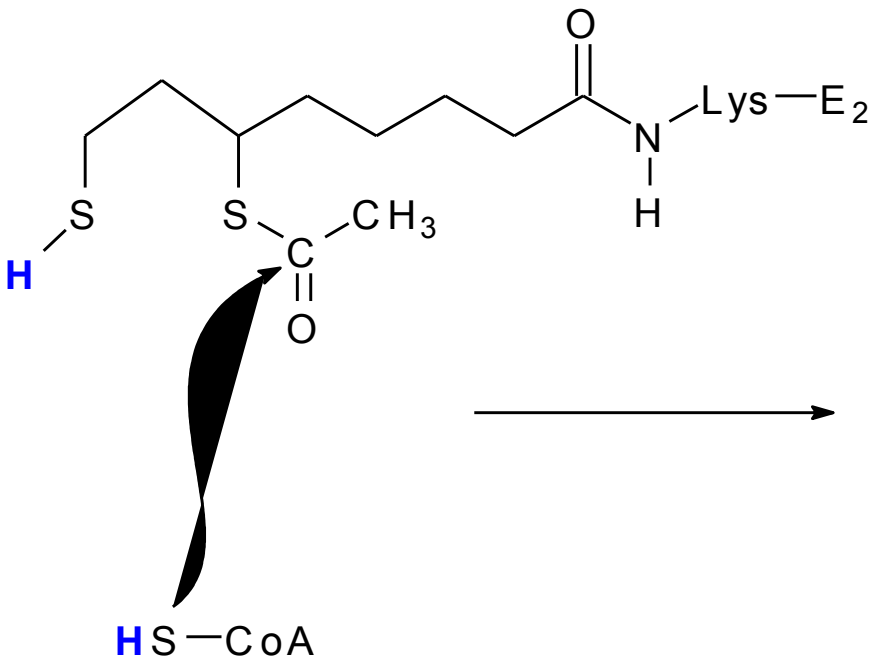


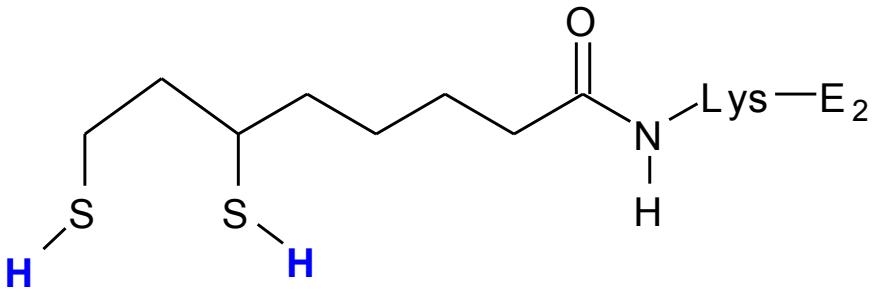


TDP

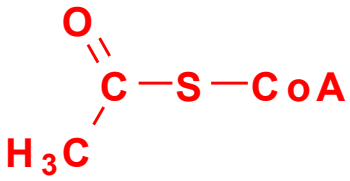
- hydroxyethyl group is dehydrogenated to thioester during transfer
- one H atom reduces sulfur atom of lipoate to –SH group
- the second H atom goes back to TDP

(3) Transfer of acetyl to coenzyme A



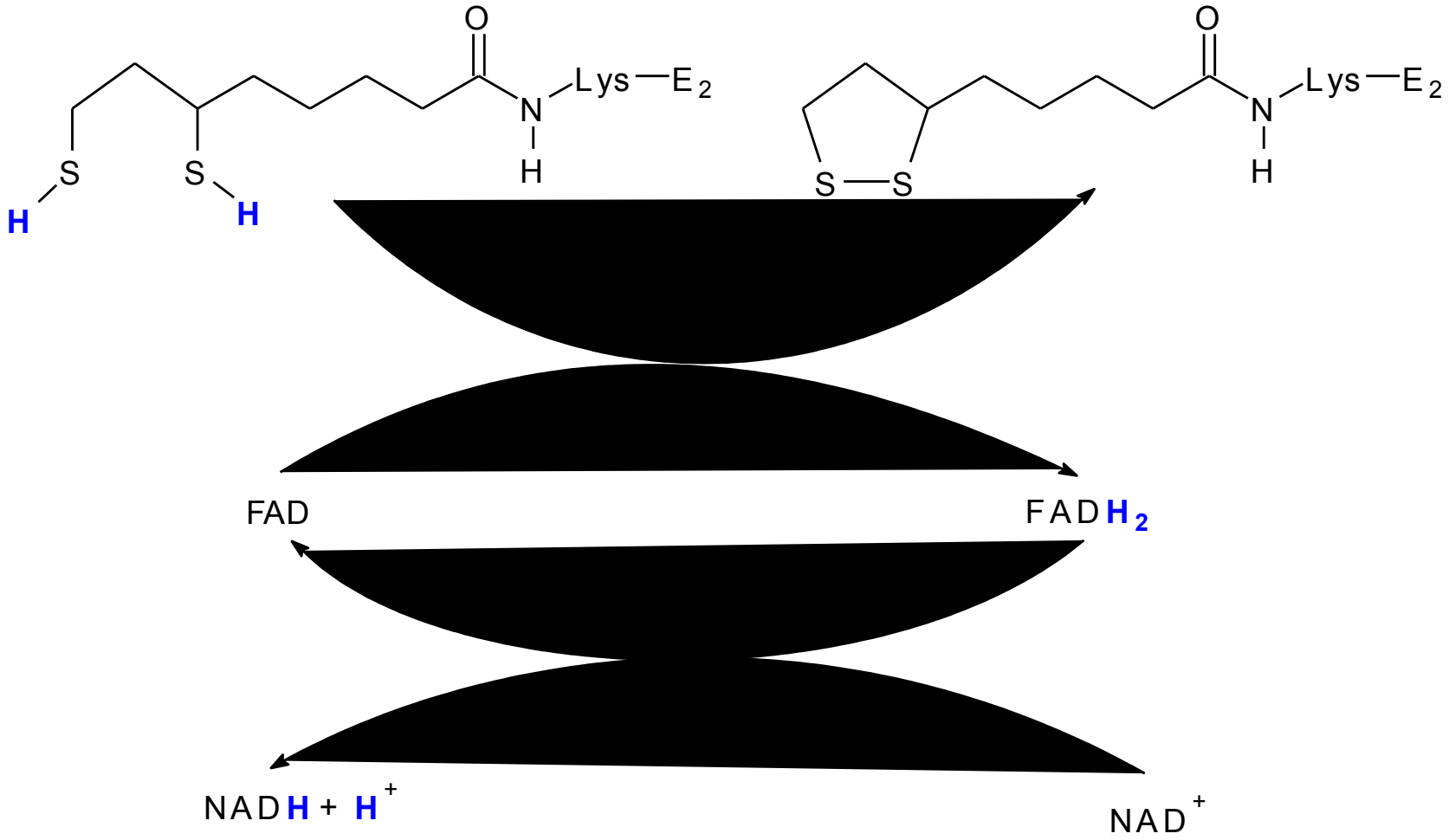


dihydrogen lipoate



acetyl-CoA

(4) Transfer of 2H to NAD^+ *via* FAD



Balance reaction

$$CH_3\text{-}CO\text{-}COOH + CoA\text{-}SH + NAD^+ \rightarrow CO_2 + CH_3\text{-}CO\text{-}S\text{-}CoA + NADH{+}H^+$$

Pyruvate dehydrogenase is allosterically inhibited

by end products: acetyl-CoA + NADH

Citric acid cycle (CAC)

Krebs cycle, tricarboxylic acid cycle (TCA)

- final common pathway for oxidation of all major nutrients
- located in mitochondria, active in all cells that possess mitochondria
- **acetyl-CoA** from metabolism of nutrients is **oxidized to two molecules of CO_2**

(CH_3-CO-S-CoA + 3 H_2O → 2 CO_2 + 8 H + CoA-SH)

- CAC products:

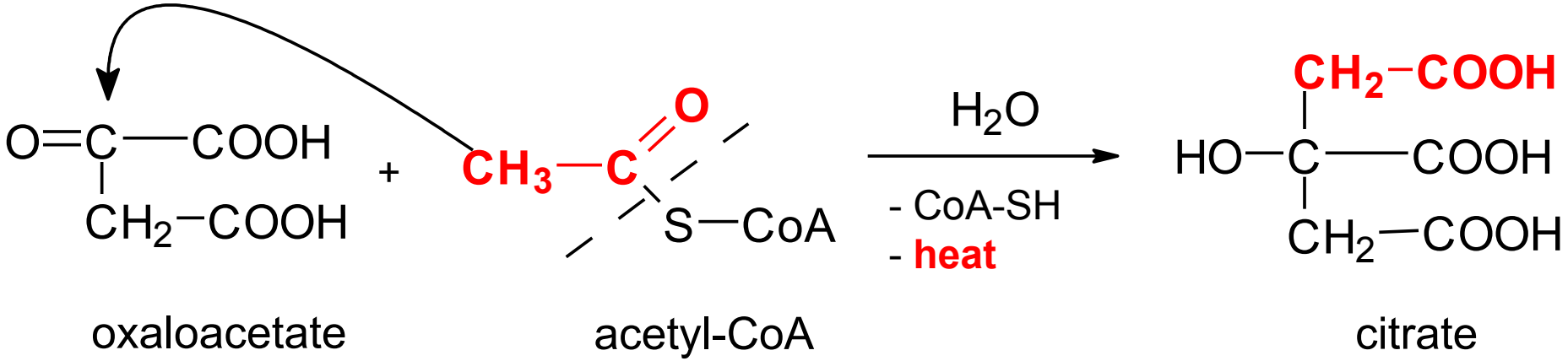
  CO_2 → expired by lungs

  four oxidative steps → reduced cofactors → respiratory chain

  GTP → ATP

- most reactions are reversible, only **three** reactions are irreversible

(1) Oxaloacetate + Acetyl-CoA



Reaction type: condensation

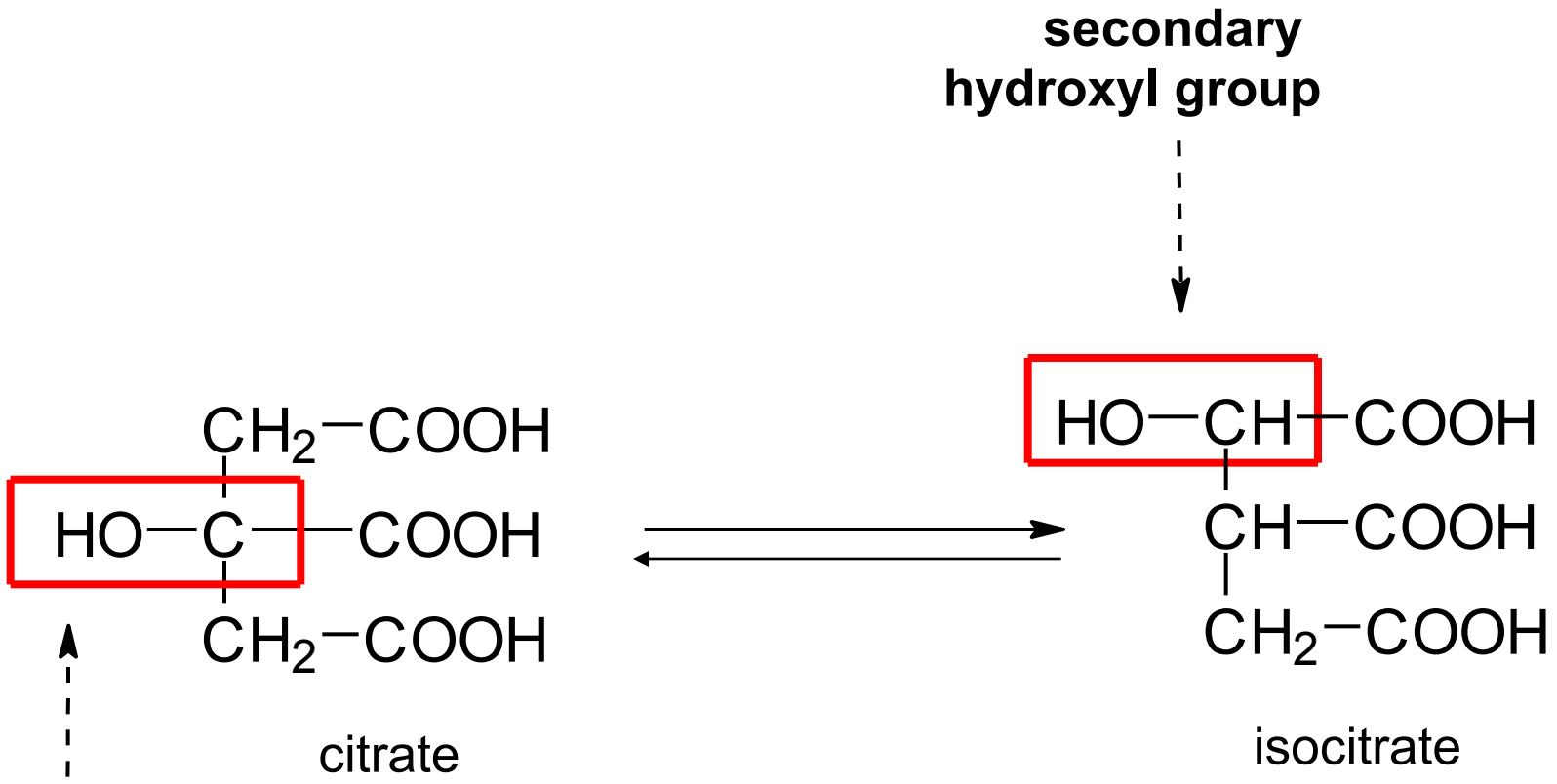
Enzyme: citrate synthase

Cofactor: coenzyme A

Note: **exothermic + irreversible**

low-energy compound ⇒
for backward reaction
in cytosol ATP needed

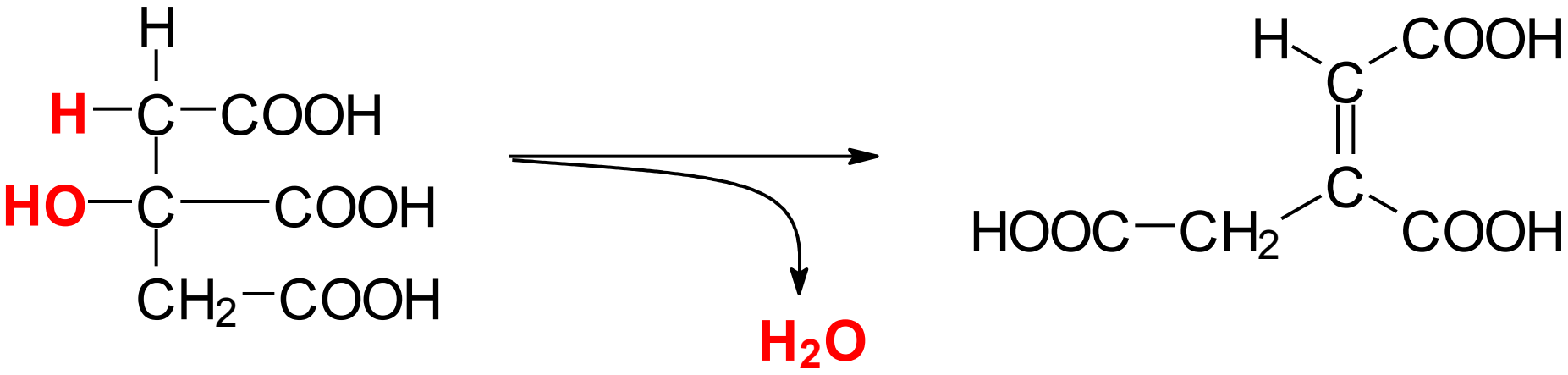
(2) Citrate → Isocitrate



Reaction type: isomeration Enzyme: aconitase Cofactor: Fe-S

Note: two-step-reaction, intermediate is *cis*-aconitate

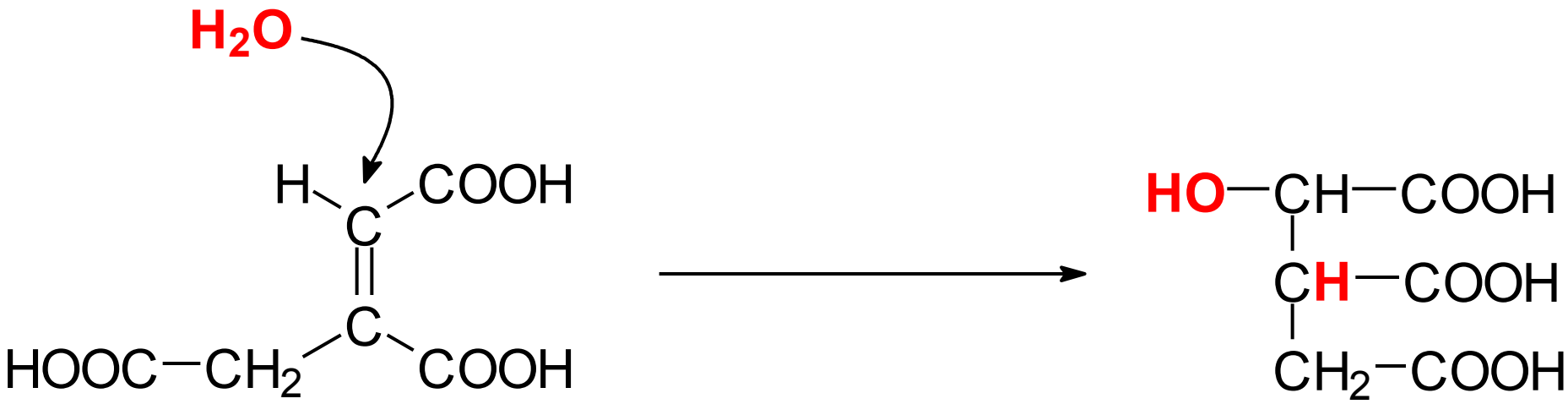
(2a) Dehydration of citrate



citrate

cis-aconitate

(2b) Hydratation of cis-aconitate



cis-aconitate

isocitrate

stereospecific reaction

Aconitase is inhibited by fluoroacetate

FCH_2COOH

reacts with oxaloacetate
to give fluorocitrate

CAC is stopped

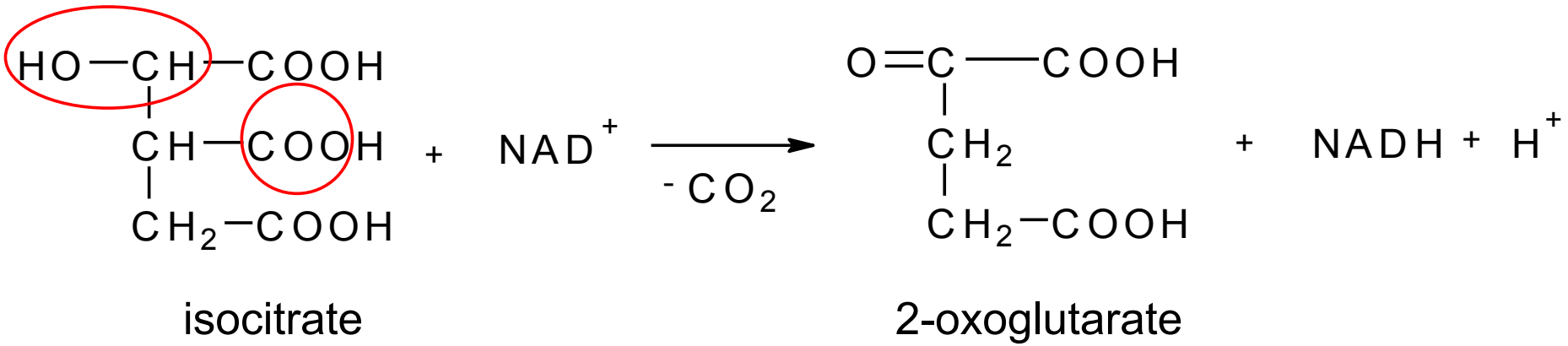
LD_{50} for human is 1 mg/kg

rat poison

Dichapetalum cymosum

(see also Med. Chem. II, p. 65)

(3) Isocitrate → 2-oxoglutarate

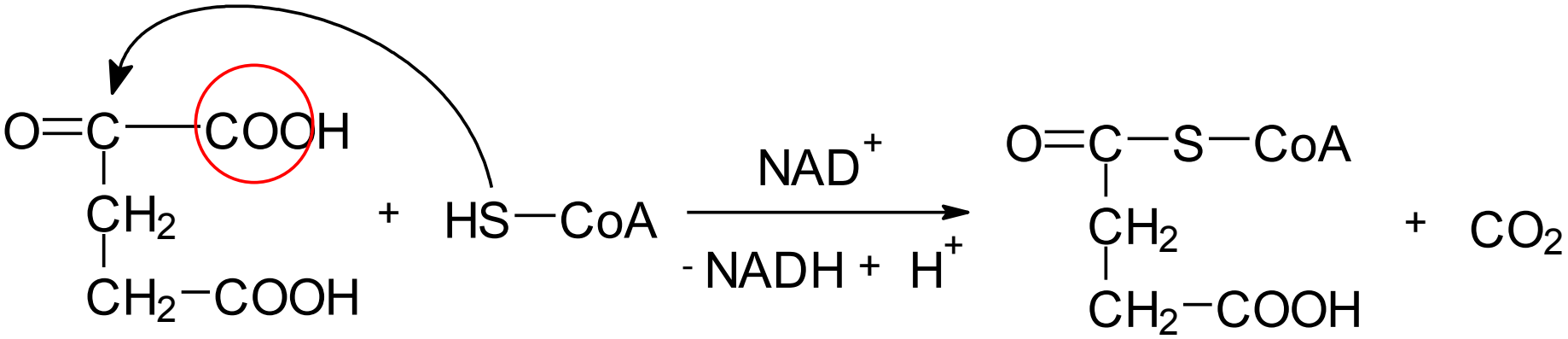


Reaction type: dehydrogenation + decarboxylation

Enzyme: isocitrate dehydrogenase

Cofactor: NAD^+ Note: **irreversible**

(4) 2-Oxoglutarate → succinyl-CoA



2-oxoglutarate

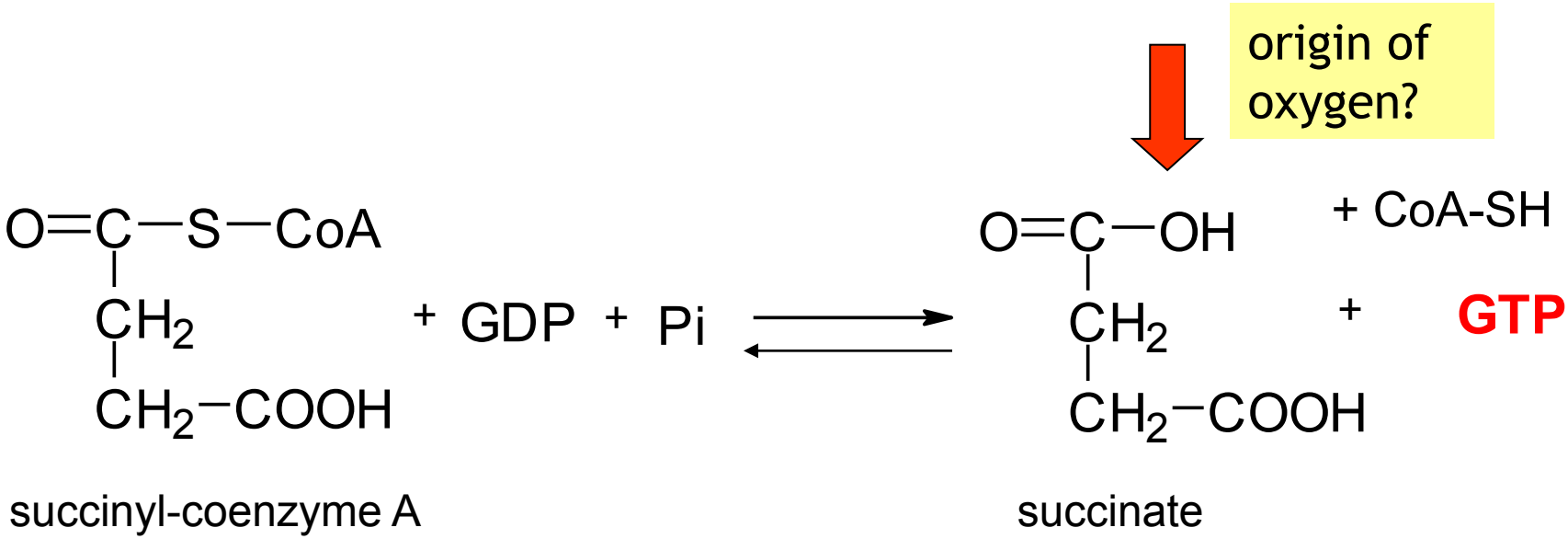
succinyl-coenzyme A thioester macroergic intermediate

Reaction type: oxidative decarboxylation Enzyme: 2-oxoglutarate dehydrogenase complex

Cofactors: TDP, lipoate, CoA-SH, FAD, NAD^+

Note: **irreversible**, similar to pyruvate dehydrogenase reaction (five coenzymes)

(5) Succinyl-CoA + GDP + P_i



Reaction type: substrate phosphorylation

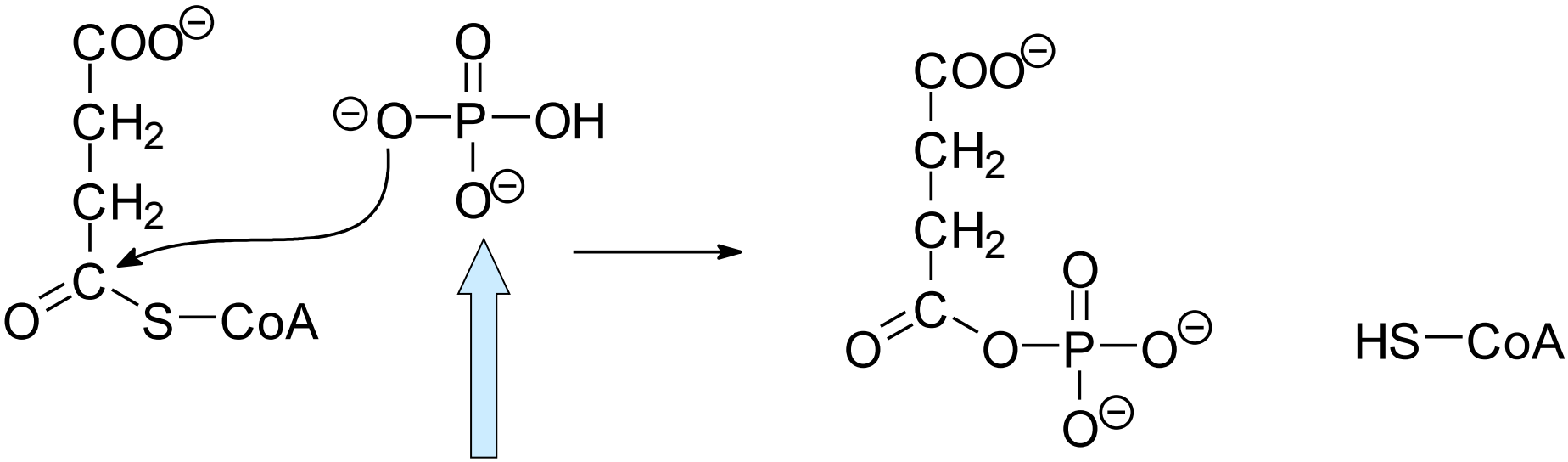
Enzyme: succinyl-CoA synthetase (succinate thiokinase)

Cofactor: coenzyme A

GTP is formed in three-step reaction

Chemical energy of macroergic succinyl-CoA is gradually transformed into two macroergic intermediates and finally to macroergic GTP (Passing a hot potato)

(5a) Addition of phosphate to succinyl-CoA



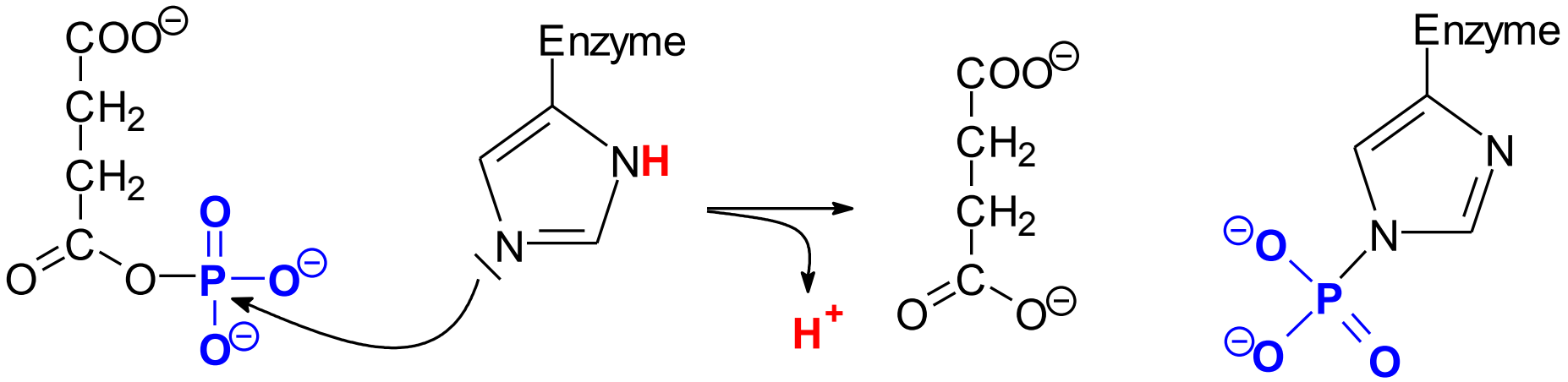
succinyl-CoA

four oxygen atoms in phosphate

succinylphosphate

mixed anhydride

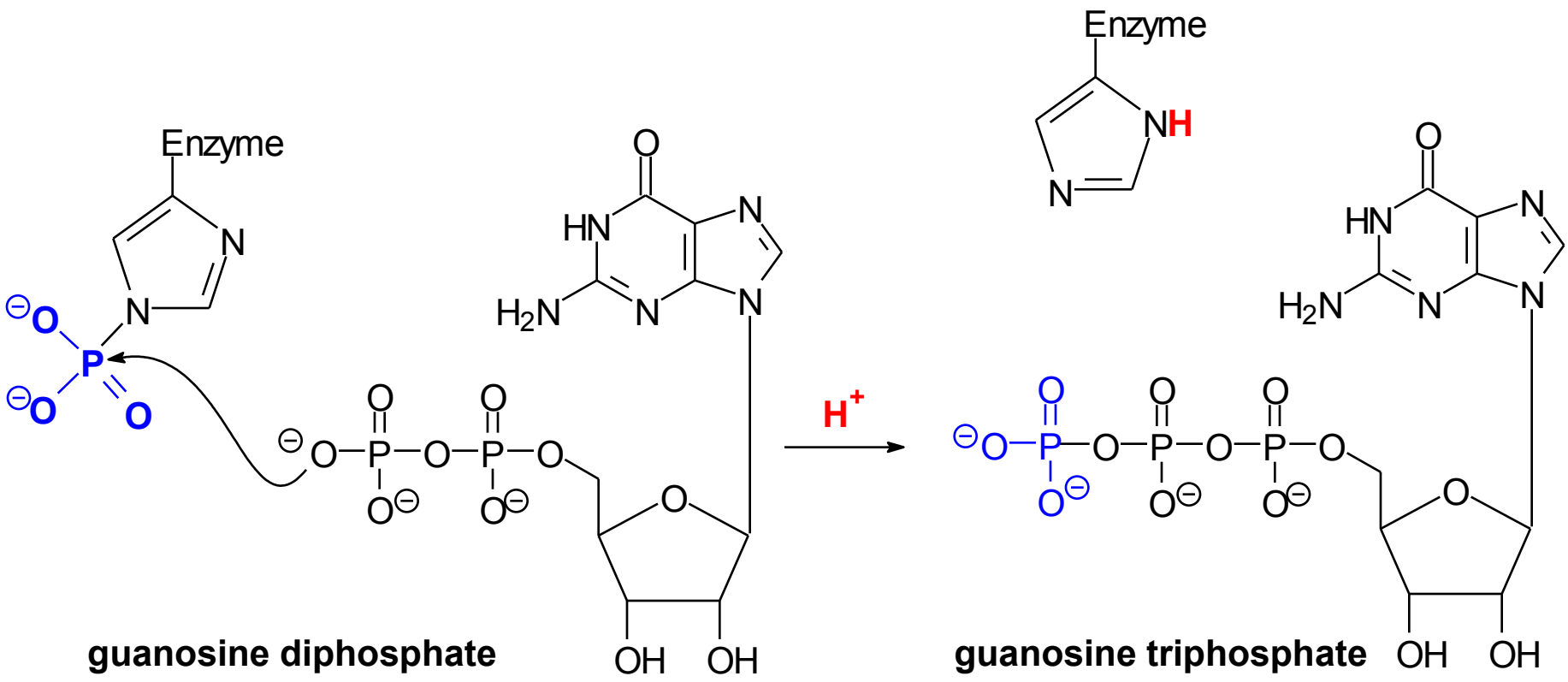
(5b) Phosforylation of His in the active site of enzyme



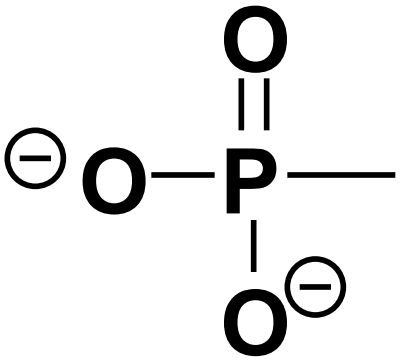
succinylphosphate succinate phospho-His

substituted phosphoamide

(5c) Phosforylation of GDP



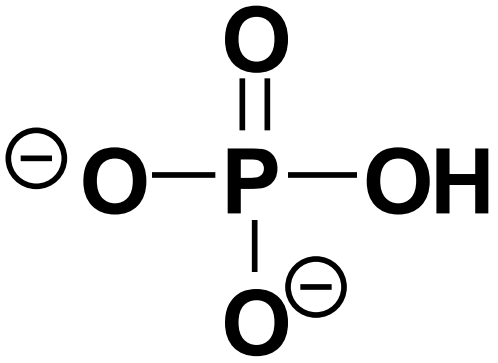
Distinguish



phosphoryl

$-PO_3^{2-}$

virtual group



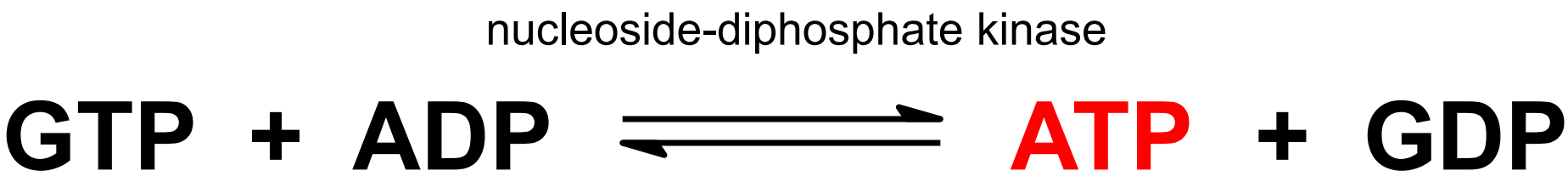
phosphate

HPO_4^{2-} (P_i)

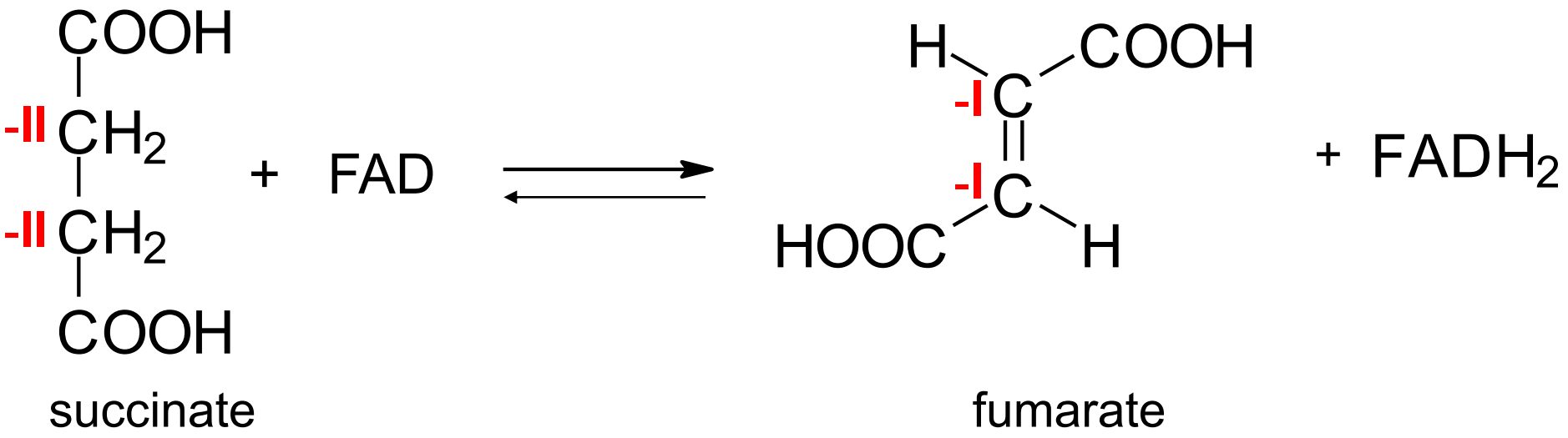
phosphate inorganic

real compound

GTP is quickly converted to ATP



(6) Succinate → fumarate

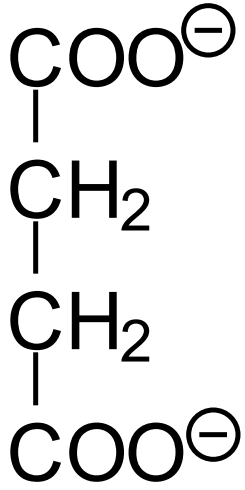


Reaction type: dehydrogenation (-CH_2-CH_2- bond)

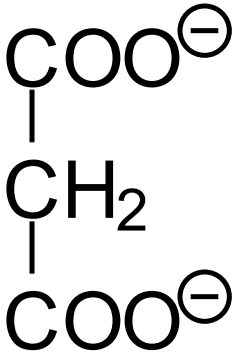
Enzyme: succinate dehydrogenase

Cofactor: FAD

Malonate is competitive inhibitor of succinate dehydrogenase



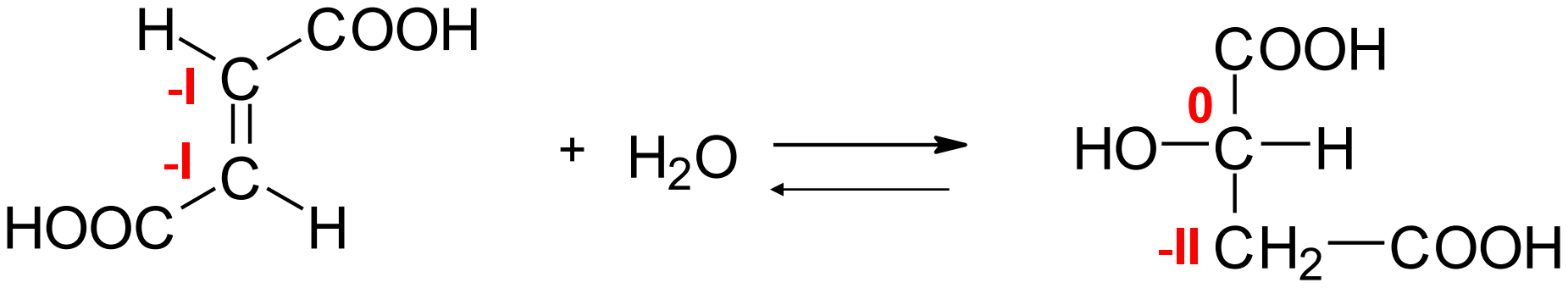
succinate



malonate

Do not confuse:
malonate malate

(7) Fumarate → L-malate



fumarate

L-malate

Σ = -II

Σ = -II

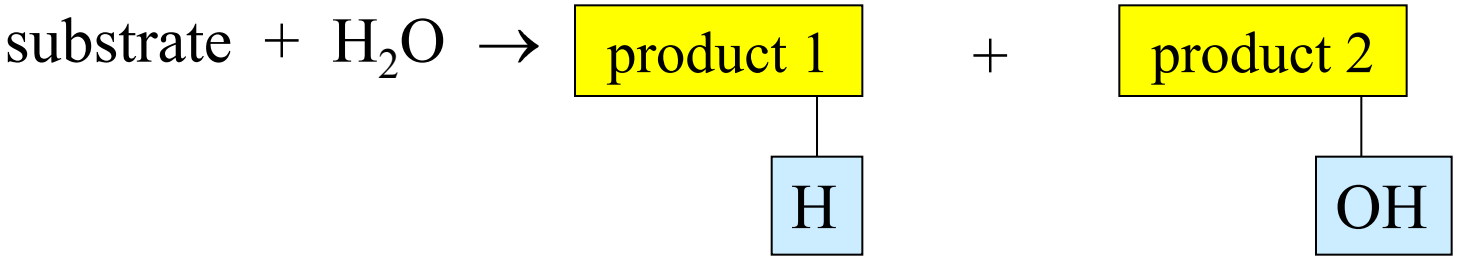
Reaction type: hydration Enzyme: fumarase Cofactor: none

Notes: 1) addition of water on double bond is **stereospecific**

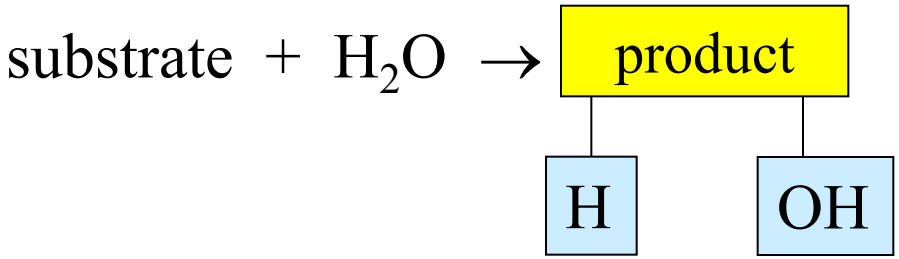
2) hydration is not redox reaction

Distinguish: hydrolysis hydration

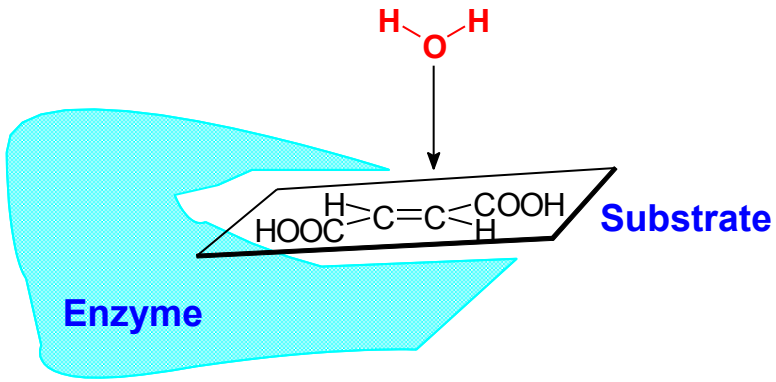
Hydrolysis = decomposition of substrate by the action of water
(typical in esters, amides, peptides, glycosides, anhydrides)



Hydration = addition of water (to unsaturated substrates)

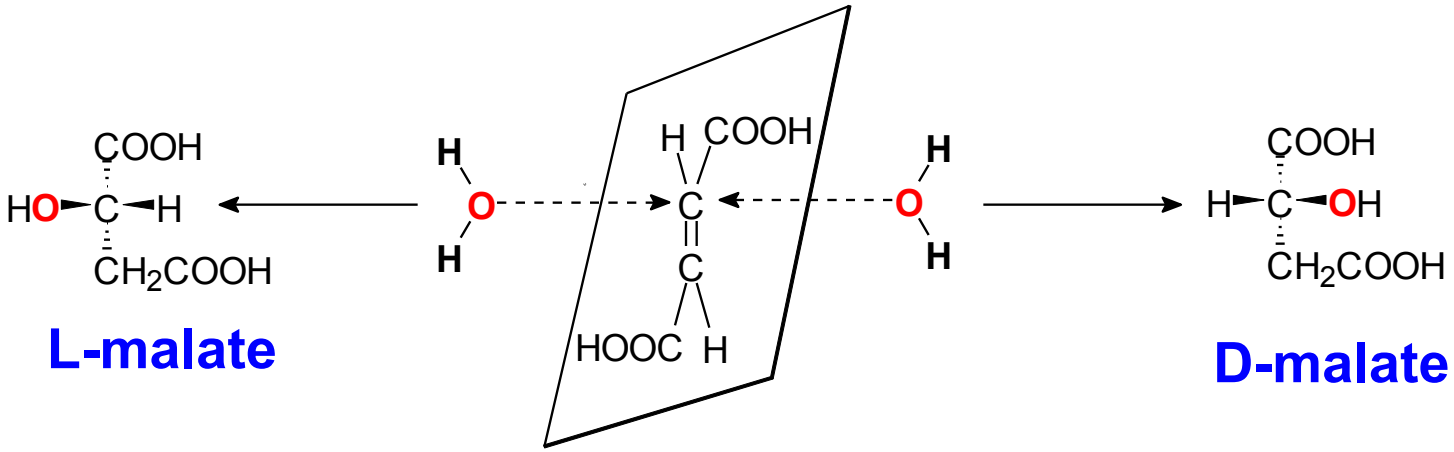


Compare: Hydration of fumarate *in vivo* and *in vitro*



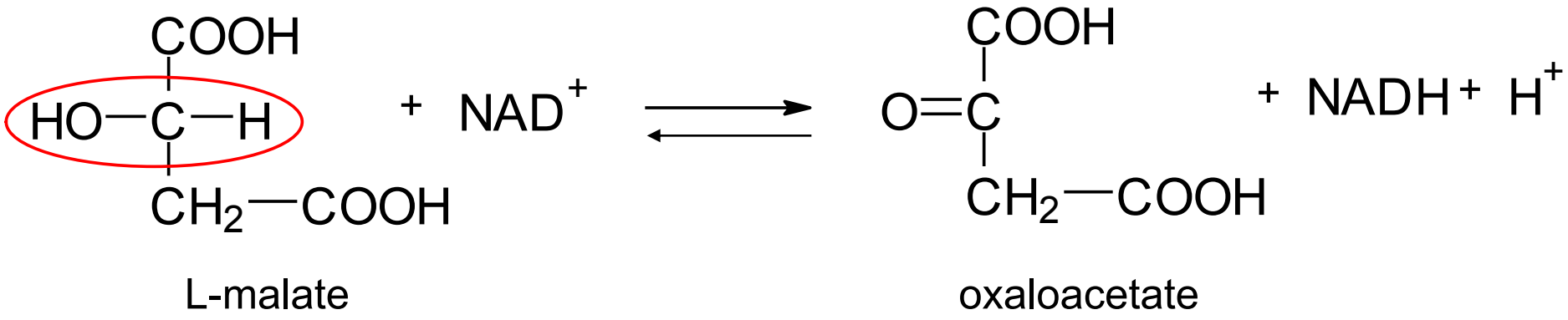
in vivo: (enzymatic reaction):

only one enantiomer is formed (L-malate)



in vitro: formation of racemate

(8) L-malate → oxalacetate

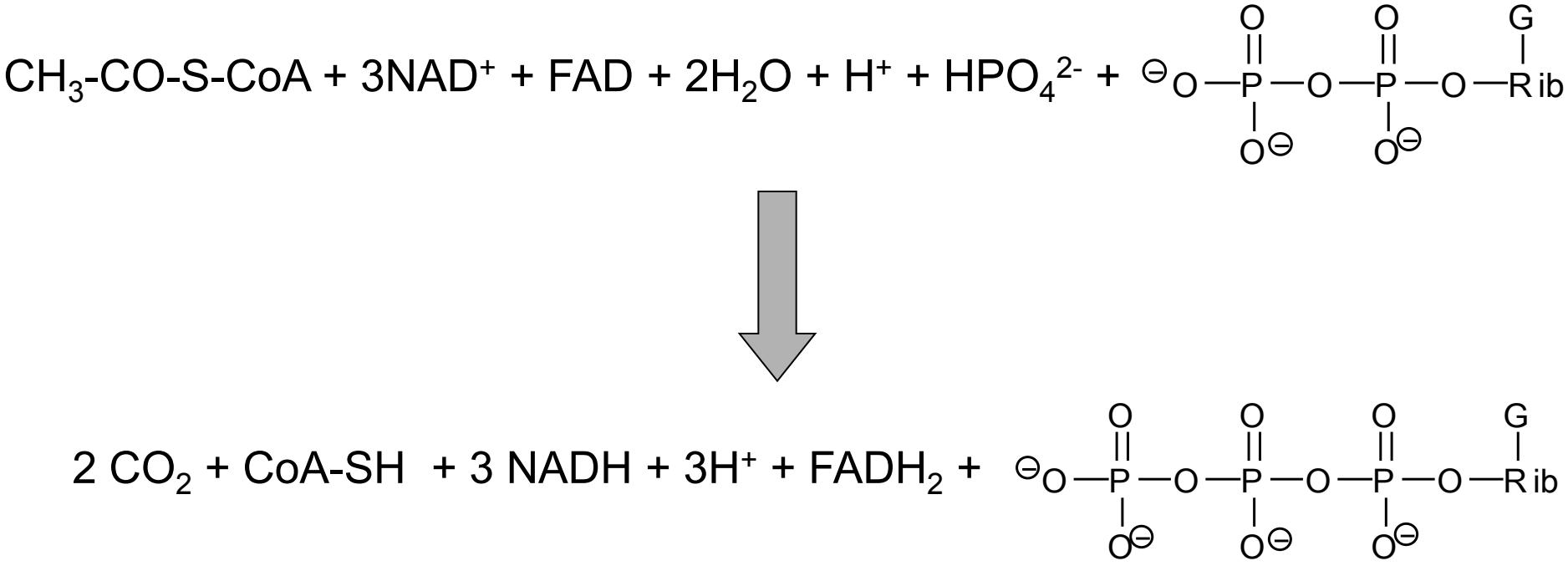


L-malate oxaloacetate

Reaction type: dehydrogenation

Enzyme: malate dehydrogenase Cofactor: NAD^+

The net equation of citrate cycle



- two C atoms are completely oxidized to 2 CO_2
- 8 H atoms are released in the form of reduced cofactors

  (3 $NADH+H^+$, 1 $FADH_2$)

The energetic yield

Products of CAC	Equivalent of ATP (Resp. chain)
1 × GTP	1
3 × $NADH + H^+$	9
1 × $FADH_2$	2
	Total: 12 ATP*

* new calculations: 10 ATP

Factors affecting CAC

- Energy charge of the cell:
- ATP/ADP ratio and $NADH/NAD^+$ ratio
- Allosteric inhibition
- Inhibition by products
- Supply of oxygen - CAC can proceed only at aerobic conditions (reduced cofactors must be reoxidized in respiratory chain)

Key enzymes for regulation of citrate cycle: irreversible reactions

Enzyme	ATP[a]	NADH[a]	Other effect
Pyruvate dehydrogenase	⊖	⊖	⊖ acetyl-CoA[b]
Citrate synthase	⊖		⊖ citrate[b]
Isocitrate dehydrogenase	⊖	⊖	⊕ ADP[c]
2-OG dehydrogenase		⊖	⊖ succinyl-CoA[b]

[a] allosteric inhibitor – signal of high energy status of cell

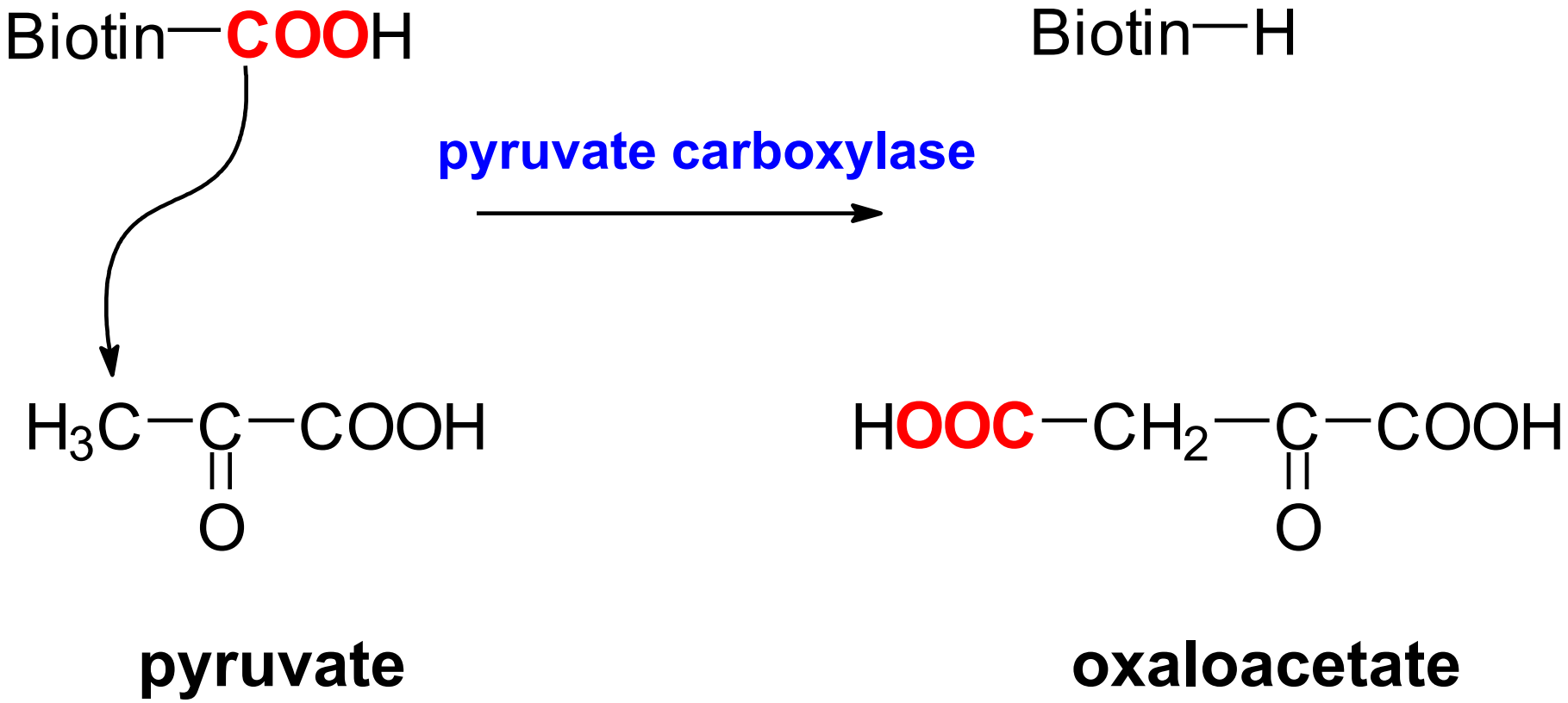
[b] feed-back inhibitor (inhibition by a product)

[c] allosteric activator

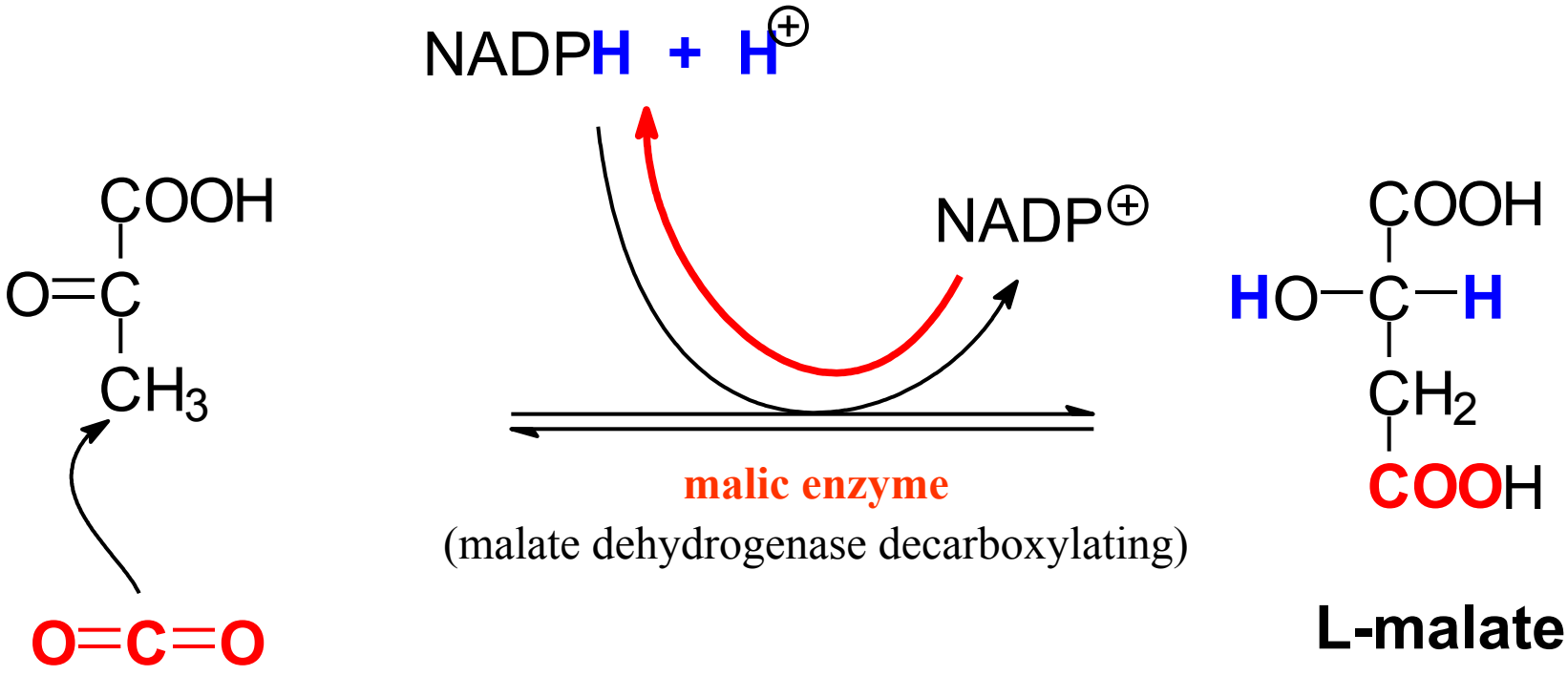
Anaplerotic reactions of CAC

- Reactions that supply the intermediates of citrate cycle
- **Carboxylation of pyruvate → oxalacetate**
- (Reductive carboxylation of pyruvate → malate)
- Transamination of aspartate → oxaloacetate
- Catabolism of Phe + Tyr → fumarate
- Aspartate in the synthesis of urea/purines → fumarate
- Catabolism of Val, Ile, Met → succinyl-CoA
- Transamination of glutamate → 2-oxoglutarate

Carboxylation of pyruvate (biotin)



Reductive carboxylation of pyruvate



Reaction is more important for production of NADPH for reductive synthesis (FA, cholesterol)

Amphibolic character of CAC

Final **catabolic** pathway:

oxidation of acetyl-CoA to 2 CO_2

Also other compounds,

which are metabolized to CAC

intermediates, can serve as substrates

of the cycle

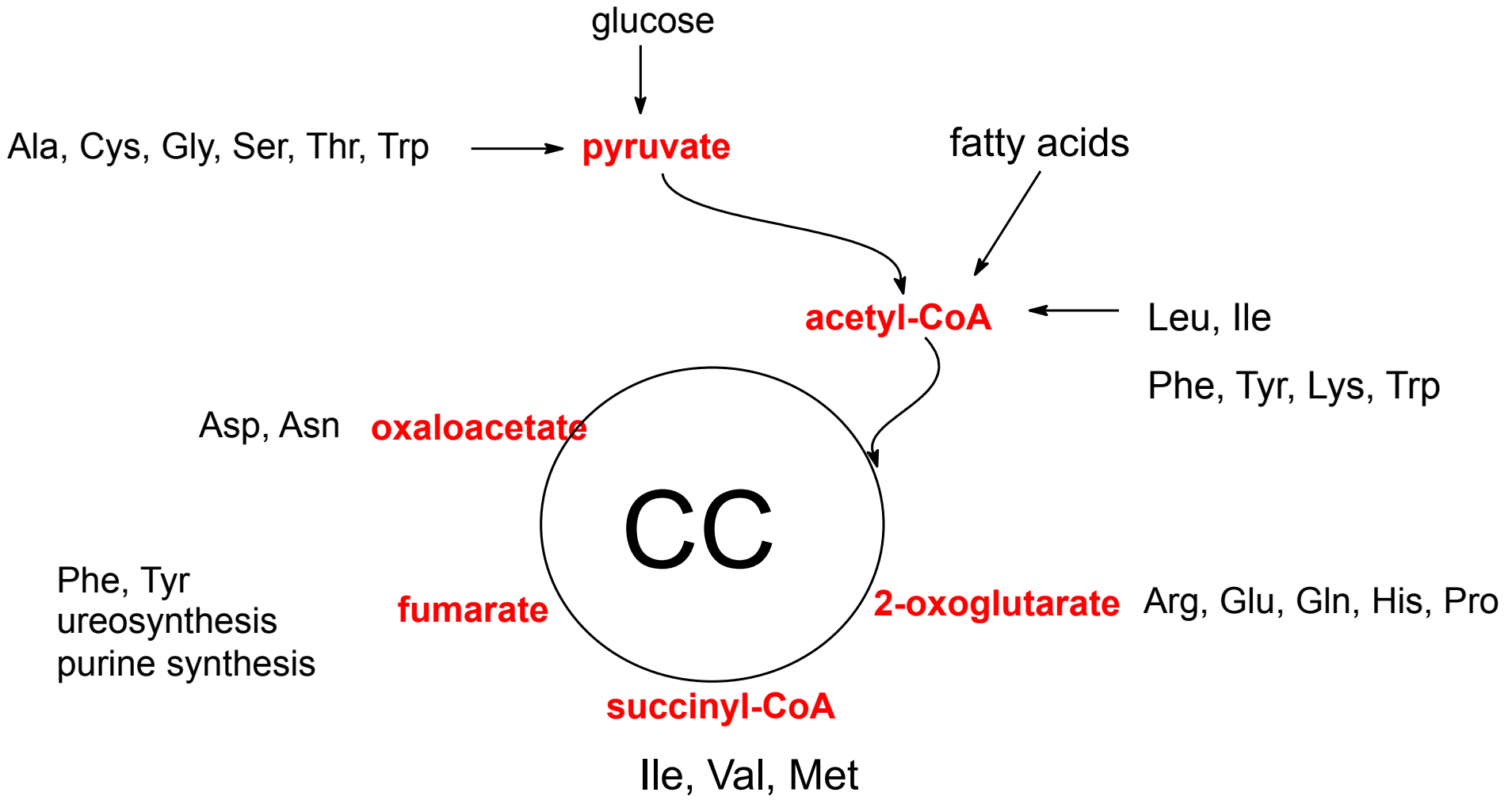
CAC provides important

metabolic intermediates for

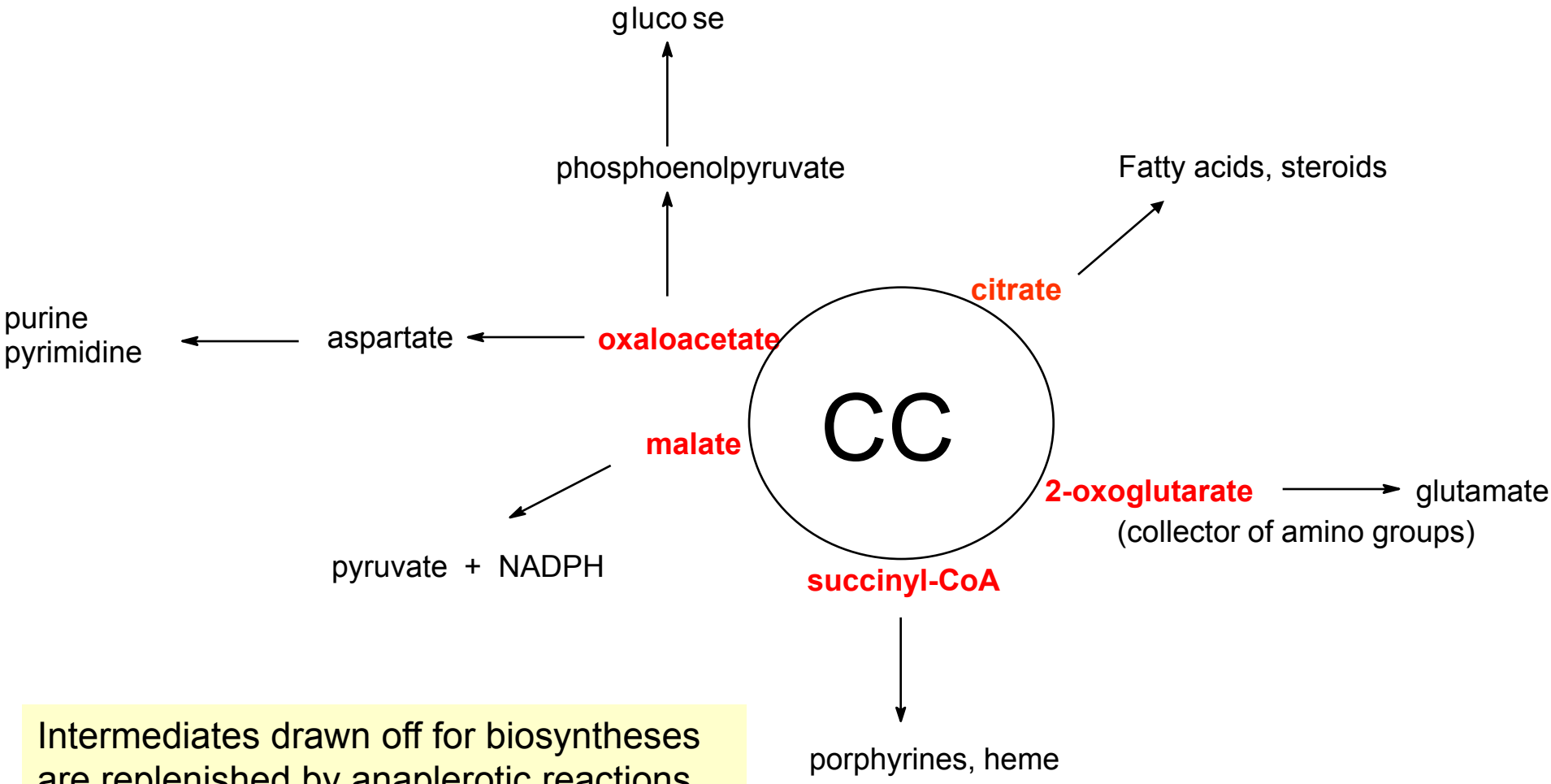
anabolic processes:

gluconeogenesis, transamination

Catabolic processes - entries into the cycle

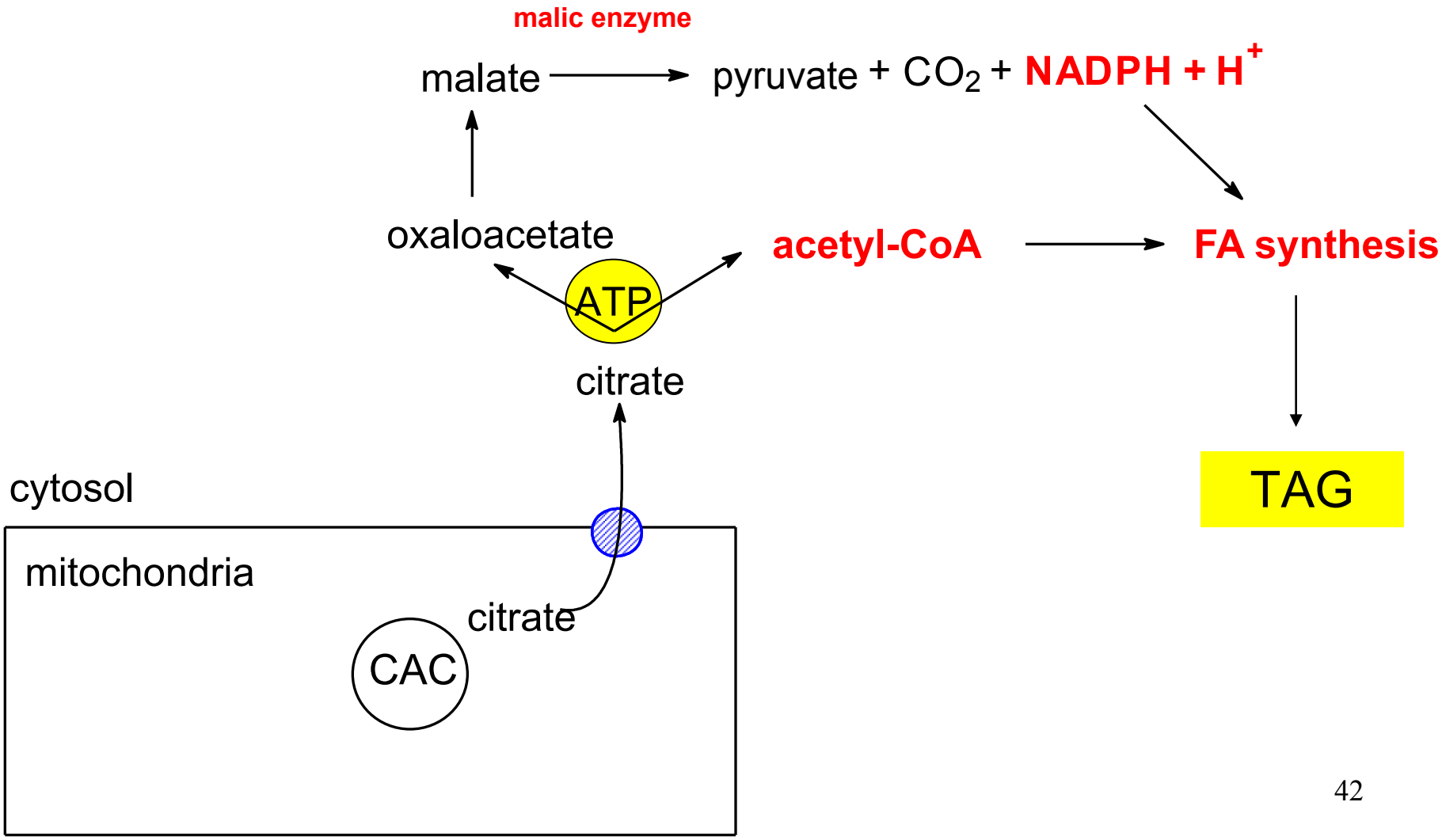


Anabolic processes – intermediates for syntheses

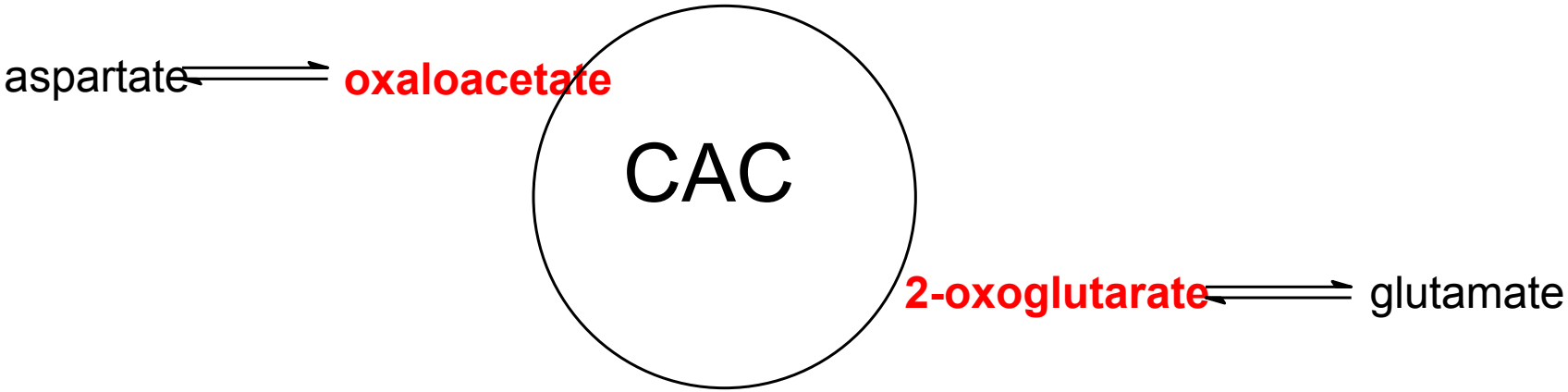


Intermediates drawn off for biosyntheses are replenished by anaplerotic reactions

CAC and the synthesis of lipids



CAC and transamination

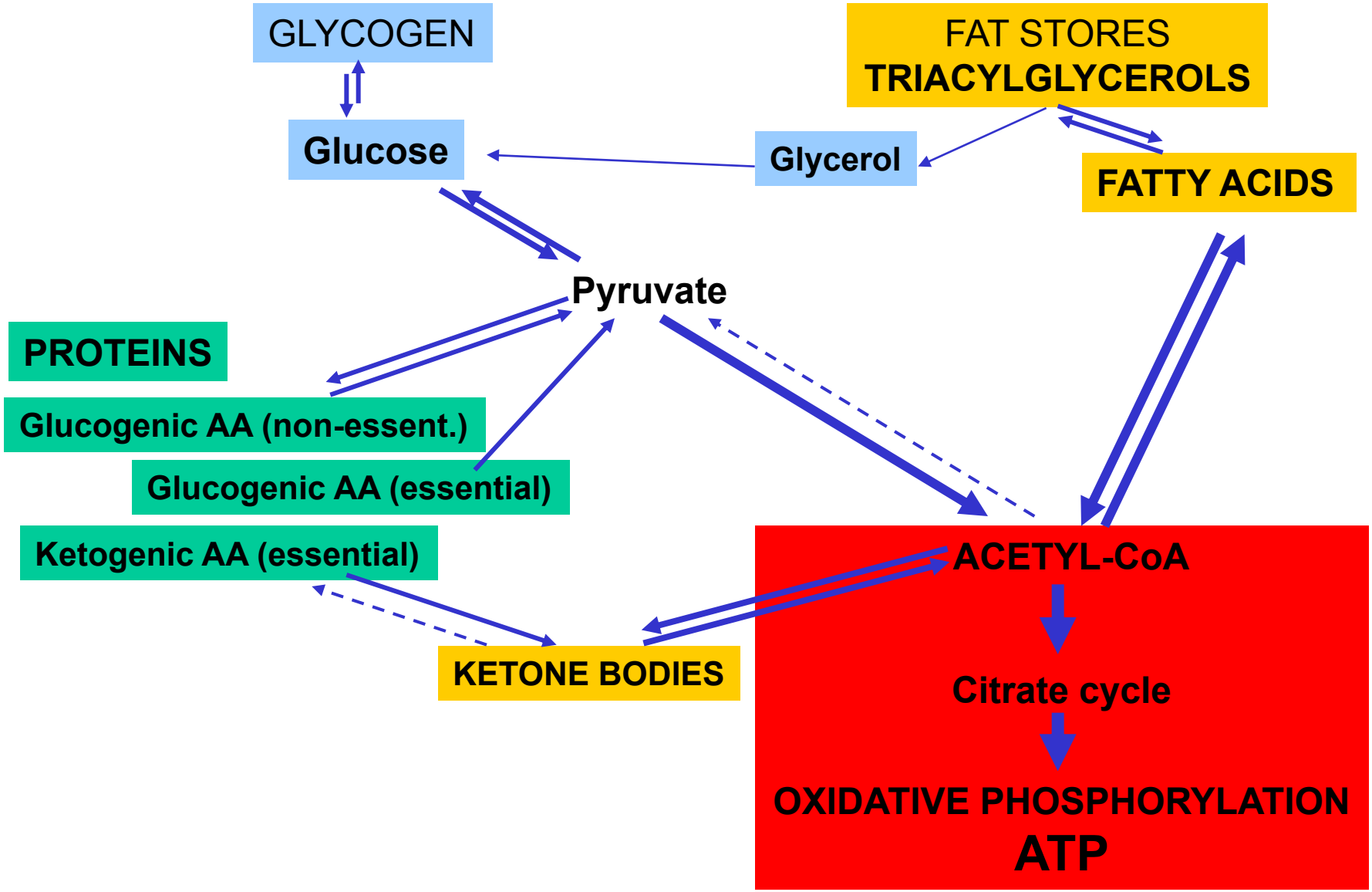


CAC and vitamins

Complete

Vitamin	Reaction in citrate cycle
Riboflavin	
Niacin	
Thiamin	
Pantothenic acid	

Relationships in major metabolic pathways



Interconversions between nutrients

Interconversion	Commentary
Sugars → lipids	very easy and quickly
Lipids ~~→~~ glucose	not possible, pyruvate dehydrogenase reaction is irreversible
Amino acids → glucose	most AA are glucogenic
Glucose intermediates → AA	pyruvate and CAC intermediate provide arbon skeleton for some amino acids
Amino acids → lipids	in excess of proteins
Lipids ~~→~~ amino acids	pyruvate dehydrogenase reaction is irreversible ketogenic AA and most mixed AA are essential

Saccharides are the most **universal nutrients –**
the overdose is transformed in the fat stores,
carbon skelet of non-essential amino acids can 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 and metabolic pathways

Pathway	Liver	CNS	Kidneys	Muscles	Adipocyte	Ery
CAC	+	+	+	+	+	-
FA β-oxidation	++	-	+	++	-	-
FA synthesis	+++				+++	-
Ketogenesis	+	-	-	-	-	-
KB oxidation*	-	+	+	+++	+	-
Glycolysis	+	+++	+	+++	+	+++
Gluconeogenesis	+++	-	+	-	-	-

* KB = ketone bodies

Cellular compartmentation of major metabolic pathways

Nucleus	DNA replication, RNA synthesis (= DNA transcription)
Mitochondria	oxidative decarboxylation of pyruvate, CAC, RCh, FA β-oxidation, synthesis of KB / urea / heme / Gln, AST reaction
Rough ER	proteosynthesis on ribosomes (translation of mRNA)
Smooth ER	synthesis of TAG / chol., FA desaturation, hydroxylations of xenobiotics
Lysosomes	non-specific hydrolysis of various substrates
Cell membrane	transport of molecules/ions/information = transporters/channels/receptors
Golgi apparat.	glycosylation of proteins, sorting and export of proteins
Peroxisomes	formation and decomposition of H_2O_2 and peroxides
Cytosol	glycolysis, gluconeogenesis, glycogen metabolism, pentose cycle, transamination, synthesis of FA / urea / urate / heme; ethanol → acetaldehyde

Metabolic effects of insulin

Liver	↑ Glucose phosphorylation
	↑ Glycolysis
	↓ Gluconeogenesis
	↑ Synthesis of glycogen
	↓ Glycogenolysis
	↑ Synthesis of fatty acids
	↑ Pentose phosphate cycle

Adipose tissue	↑ Glucose uptake (GLUT 4)
	↑ Glycolysis
	↑ Pentose phosphate pathway
	↑ Ox. decarboxylation of pyruvate
	↑ Hydrolysis of TG in lipoproteins
	↑ Synthesis of TG
	↓ Lipolysis

Muscle	↑ Glucose uptake (GLUT 4)
	↑ Glycolysis
	↑ Synthesis of glycogen
	↓ Glycogenolysis
	↑ Synthesis of proteins

Metabolic effects of glucagon [not on muscles]

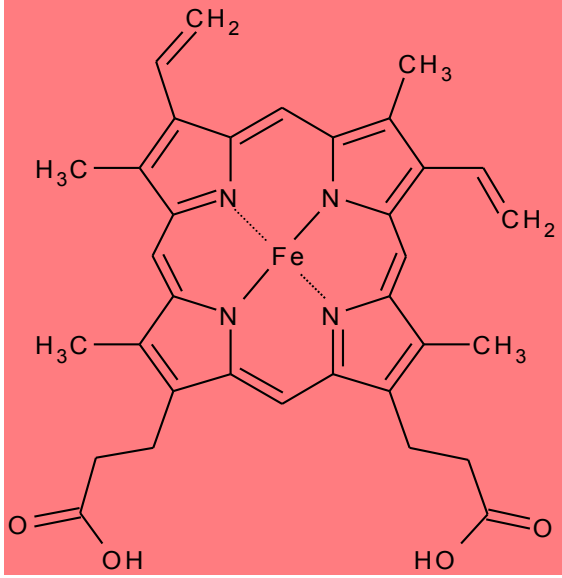
Liver	↓ Glycolysis
	↑ Gluconeogenesis
	↓ Synthesis of glycogen
	↑ Glycogenolysis
	↓ Synthesis of fatty acids
	↑ Oxidation of fatty acids
Adipocytes	↑ Lipolysis (HSL, hormone sensitive lipase)

Biosynthesis of heme

~

Hemoproteins

Heme



Prostetic group of many proteins

(hemoglobin, myoglobin, cytochromes)

Synthesis in the body:

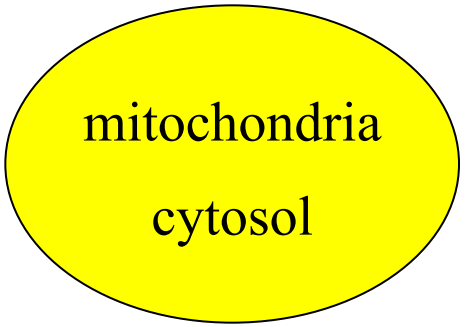
70-80 % in erythroid cells in bone marow - hemoglobin

15 % liver – cytochroms P450 and other hemoproteins

Heme consists of porphyrin ring coordinated with iron cation

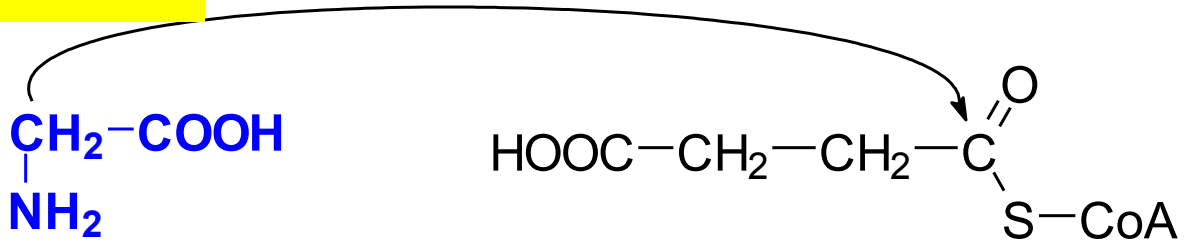
Biosynthesis of heme

- initial compound for synthesis is succinyl-CoA (intermediate of CAC)
- source of nitrogen is glycine
- reactions are located in mitochondria and cytosol
- regulation: ALA-synthase



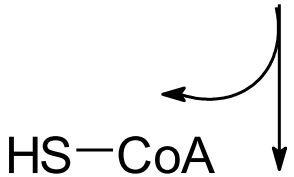
Synthesis of δ-aminolevulinate (ALA)

mitochondria



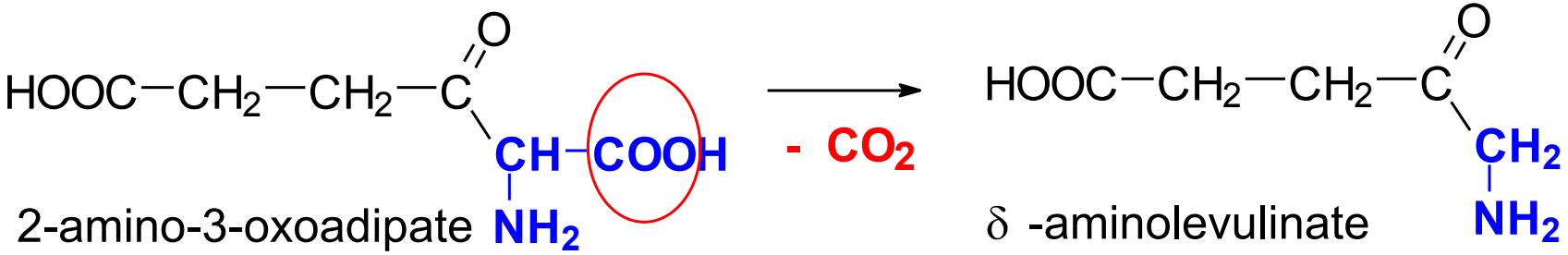
glycin

succinyl-CoA



ALA-synthase

pyridoxalphosphate is cofactor



2-amino-3-oxoadipate

δ -aminolevulinate

(5-amino-4-oxobutanoic acid)

ALA-synthase is the rate-controlling enzyme of porphyrine biosynthesis

Half-life about 1 hour

ALA-synthase

- is inhibited by heme (allosteric inhibition)

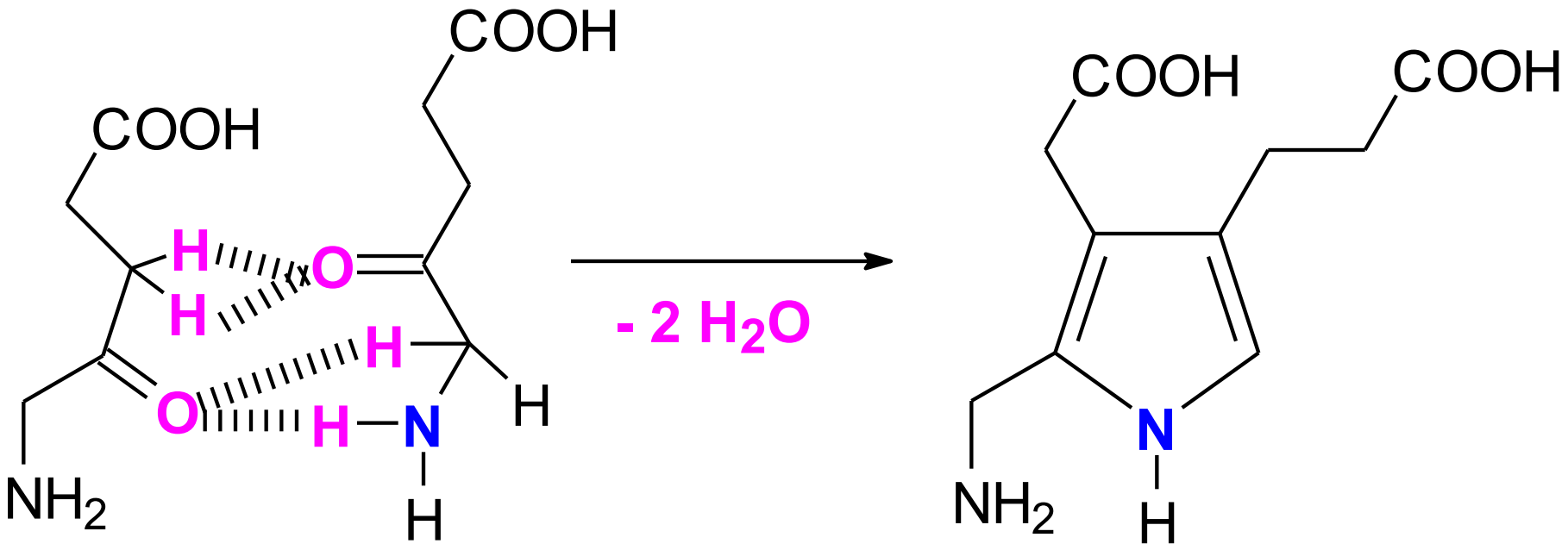
- synthesis of enzyme is repressed by heme

- is induced by some drugs (barbiturates, phenytoin, griseofulvin)

- cytochrome P-450 is needed for biotransformation of drugs/xenobiotics

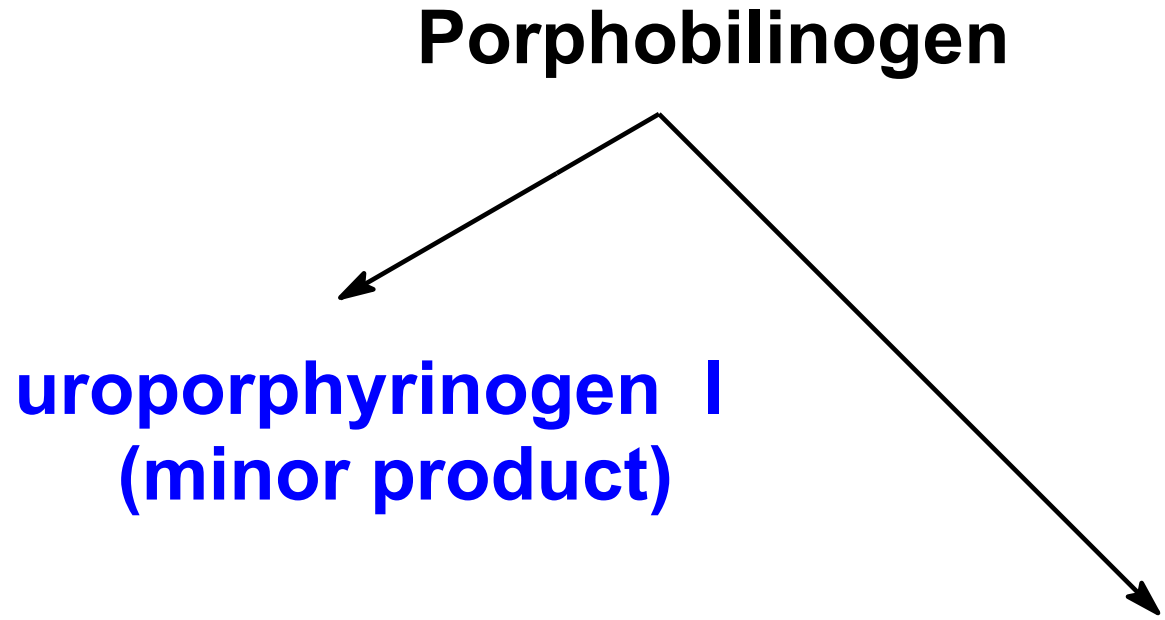
Condensation to substituted pyrrole

cytosol



δ-aminolevulinate **porphobilinogen**

Condensation of porphobilinogen



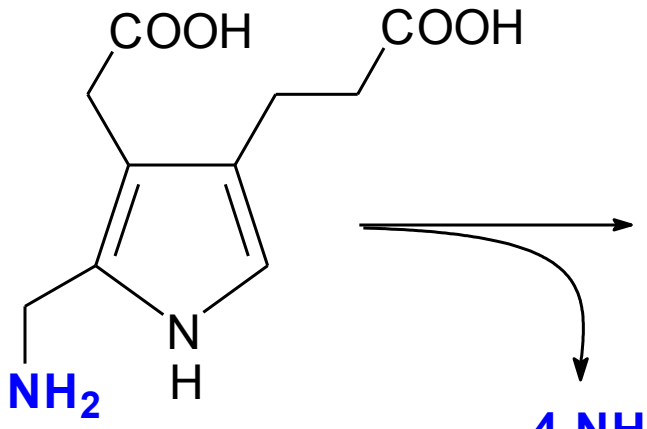
Under physiological circumstances, due to the presence of a protein modifier called co-synthase, uroporphyrinogen III with an **asymmetrical** arrangement of side chains of the ring D is formed. Only traces of symmetrical uroporphyrinogen I are produced

uroporphyrinogen III
(main product)

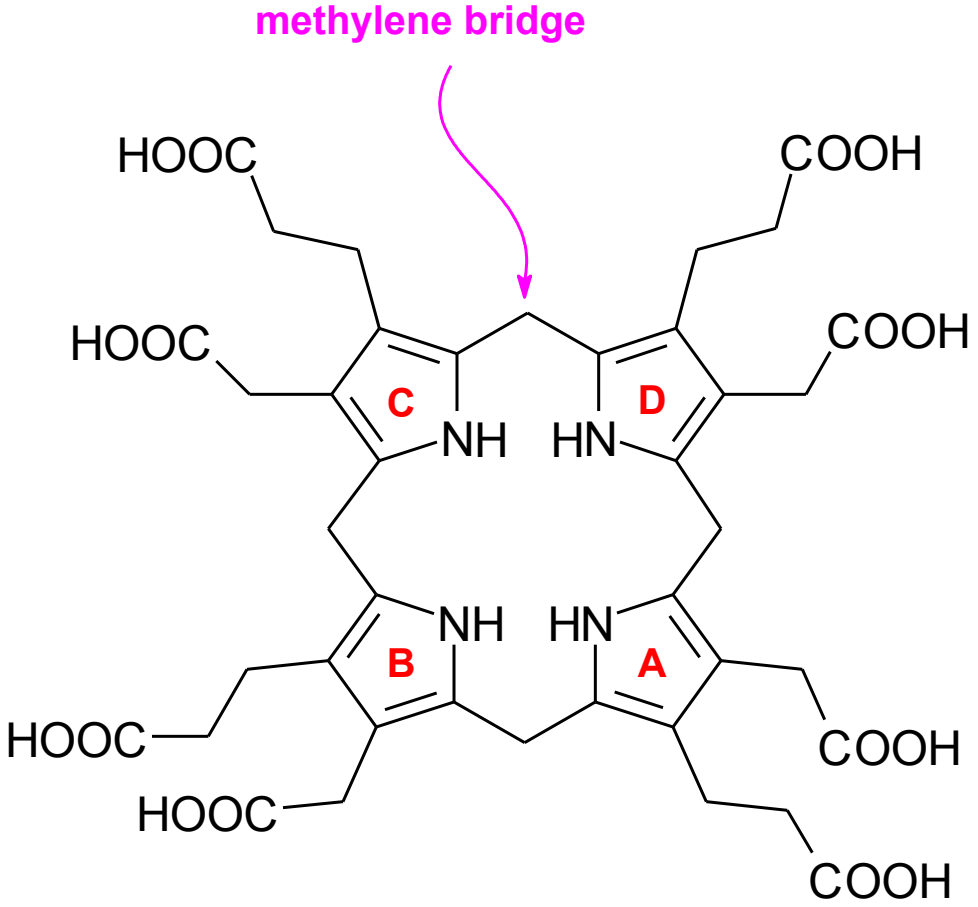
Condensation of porphobilinogen

cytosol

4



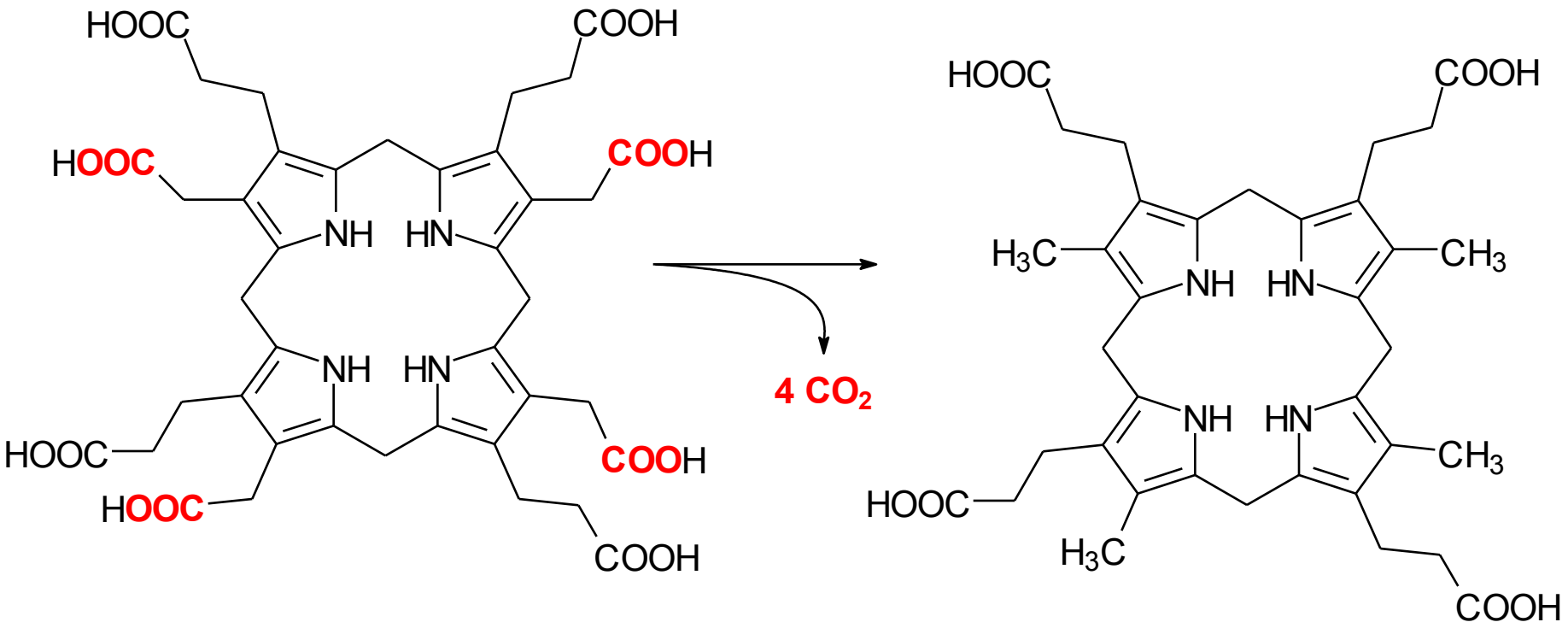
porphobilinogen



uroporphyrinogen III

Decarboxylation of four acetates – formation of methyl groups

cytosol

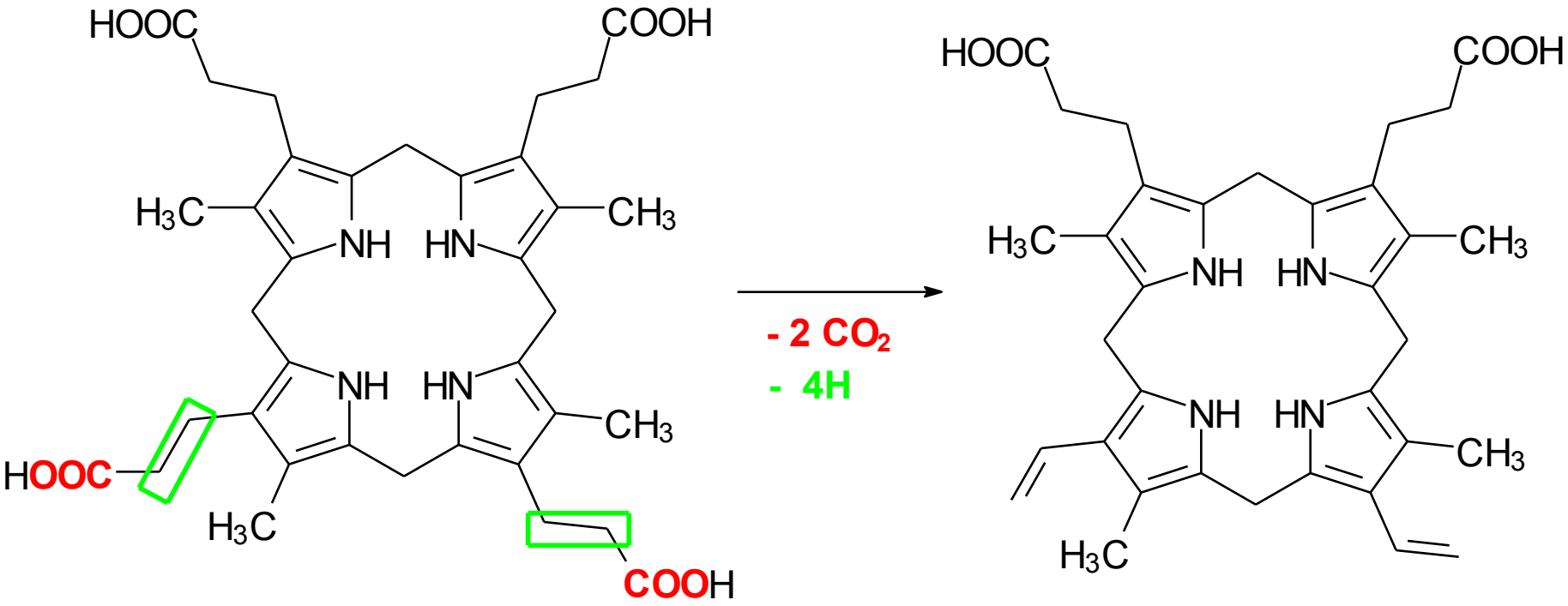


uroporphyrinogen III

coproporphyrinogen III

Formation of vinyl groups from two propionates

mitochondria

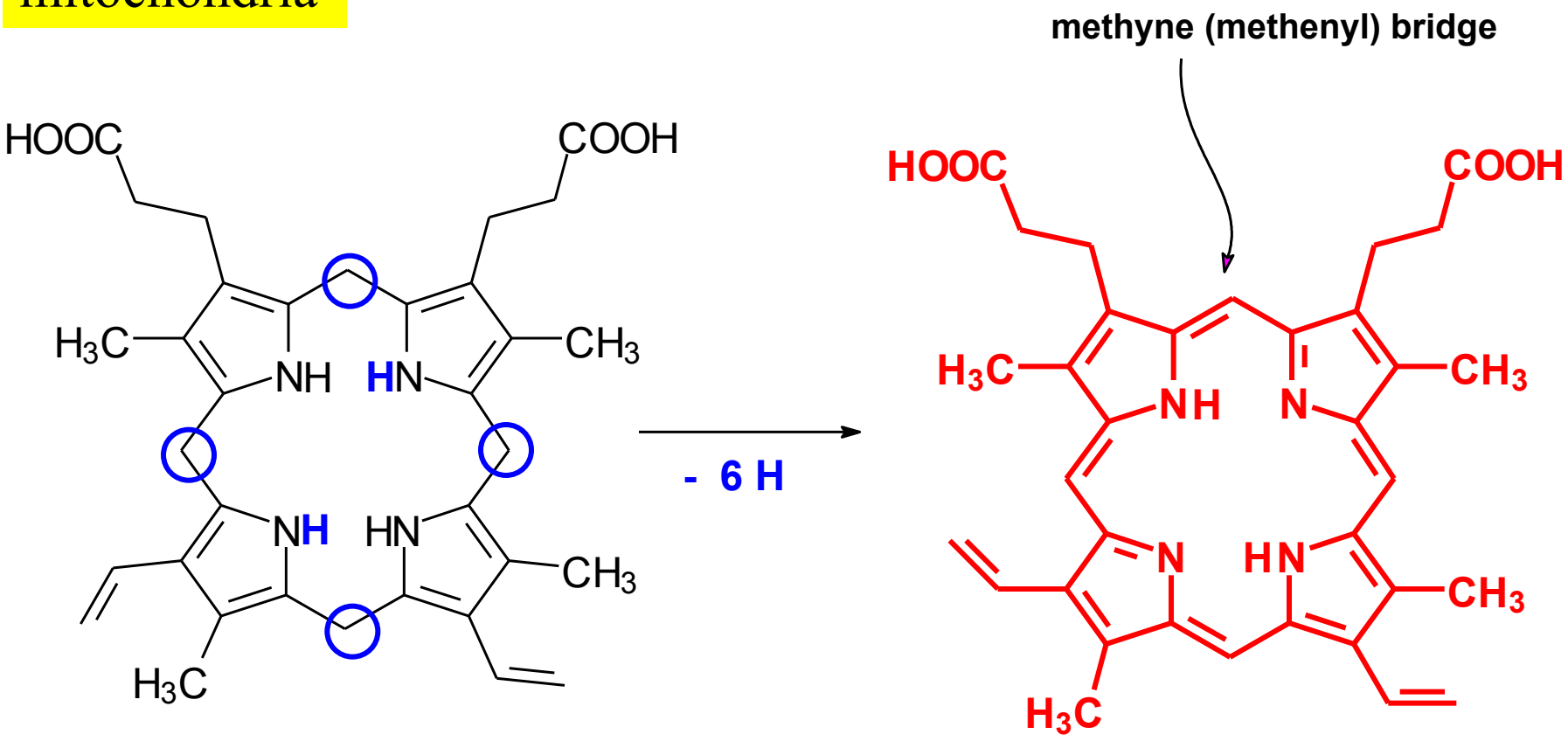


coproporphyrinogen III

protoporphyrinogen IX

Formation of conjugated system

mitochondria



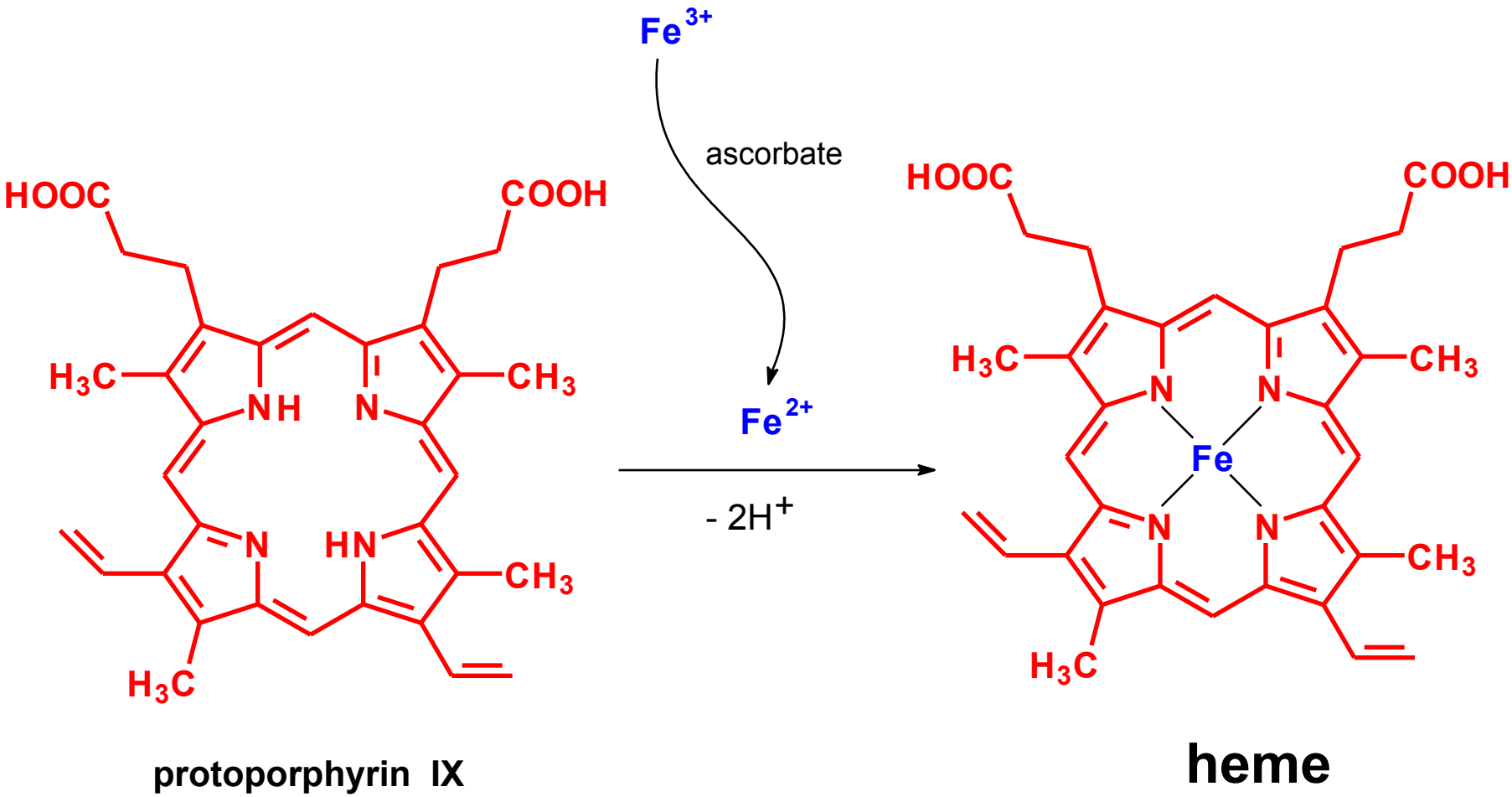
protoporphyrinogen IX

colourless

protoporphyrin IX

red

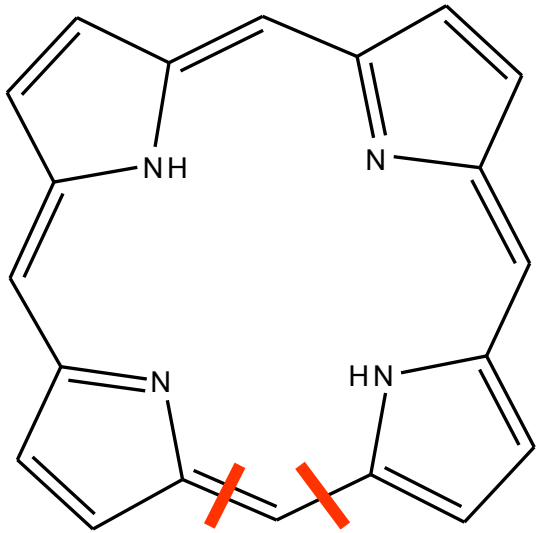
Heme is coloured chelate with Fe^{2+}



protoporphyrin IX

heme

CO and bilirubin are formed by the degradation of heme



3 O_2
3 NADPH+H^+ | oxidative cleavage (heme oxygenase)

CO + biliverdin

carbonyl-hemoglobin

bilirubin

more details
in the 4th semester

Porhyrias are caused by partial deficiency of one of the heme synthesizing enzymes

Primary (genetic)

- Defective enzyme of heme biosynthesis
- Overproduction and accumulation of intermediates (ALA, PBG)
- Porphyrinogens in skin - photosensitivity

Secondary

- Inactivation of enzymes as a consequence of disease or poisoning
- Similar symptoms

Hemoproteins

Protein	Redox state of Fe	Function
Hemoglobin	Fe^{2+}	Transport of O_2 in blood
Myoglobin	Fe^{2+}	Deposit of O_2 in muscles
Catalase	$Fe^{2+} \leftrightarrows Fe^{3+}$	Decomposition of H_2O_2
Peroxidase	$Fe^{2+} \leftrightarrows Fe^{3+}$	Decomposition of peroxides
Cytochroms	$Fe^{2+} \leftrightarrows Fe^{3+}$	Components of resp. chain
Cytochrom P-450	$Fe^{2+} \leftrightarrows Fe^{3+}$	Hydroxylation
Desaturases of FA	$Fe^{2+} \leftrightarrows Fe^{3+}$	Desaturation of FA

Oxidation number of Fe in various hemes

Does not change

- Fe^{2+}
- prosthetic group for O_2 transport
- hemoglobin, myoglobin
- heme is hidden in hydrophobic pocket of globin
- oxidation of Fe^{2+} means the loss of function

Does change

- $Fe^{2+} \leftrightarrows Fe^{3+}$
- cofactor of oxidoreductases
- cytochromes, heme enzymes
- heme is relatively exposed
- reversible redox change is the primary function = the transfer of one electron

Hemoglobin and myoglobin bind O_2

Hemoglobin

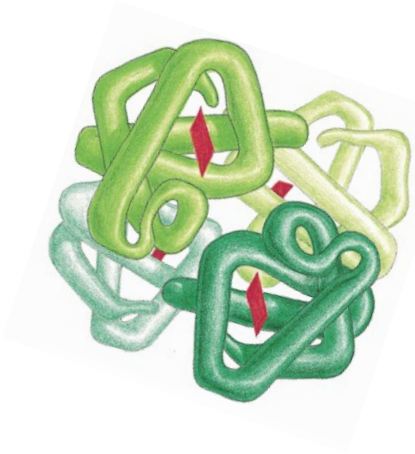
- transports O_2 from lungs to tissues
- from tissues to lungs, it transports some H^+ and CO_2 (carbamino-Hb)
- tetramer $\Rightarrow$ sigmoidal saturation curve
- two conformations: T-deoxyHb(2,3-BPG), R-oxyHb
- binding O_2 in lungs $\rightarrow$ release of H^+
- binding H^+ in tissues $\rightarrow$ release of O_2
- buffer system in erythrocytes (His)

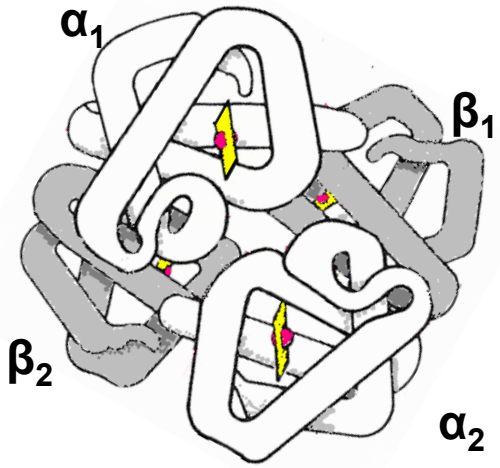
Bohr effect !

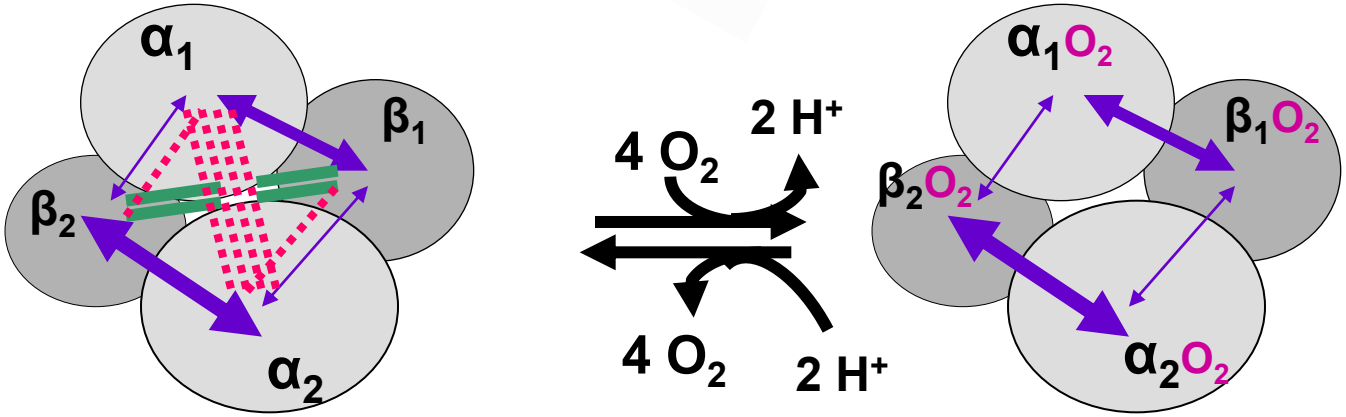
Myoglobin

- muscle hemoprotein – deposition (reserve) of oxygen
- monomer $\Rightarrow$ saturation curve hyperbolic = stronger binding O_2

Quaternary structure of hemoglobin







deoxygenated hemoglobin
(2,3-bisphosphoglycerate)
T-conformation

oxyhemoglobin
R-conformation

Derivatives of hemoglobin

Carbonylhemoglobin

- CO has great affinity to Fe^{2+} in heme
- physiological level: 1 - 15 % from total Hb (environment, smokers etc.)

Glycated hemoglobin

- non-enzymatic reaction with free glucose, $-NH_2$ group of Hb (N-terminus, Lys) and aldehyde group of glucose
- physiological level: 2.8 – 4.0 % (from total Hb)

Methemoglobin (hemiglobin)

- oxidation of heme iron, $Fe^{2+} \rightarrow Fe^{3+}$, physiol. level: 0.5 - 1.5 %
- oxidation agents: nitrites, alkyl nitrites, aromatic amines, nitro compounds
- Hb mutation: hemoglobin M (HbM), the replacement of $F8^{His \rightarrow Tyr}$
- deficit of methemoglobin reductase

Language note: Methemoglobin

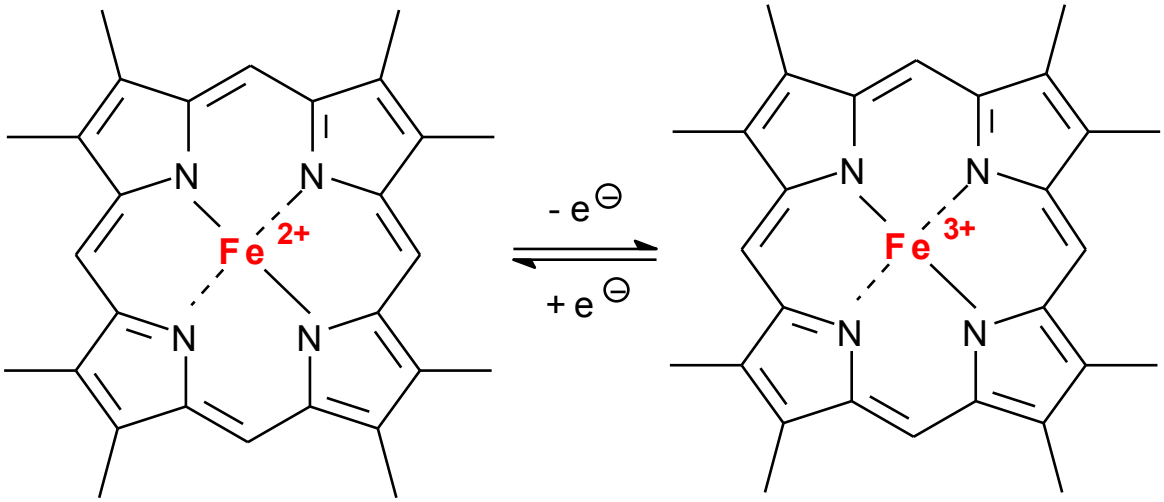
- **it has nothing to do with methyl group !!!**
- abbreviated from *metahemoglobin*
- the prefix *meta-* (from Greek) indicates change, transformation, alteration
- other examples with the prefix *meta*:
  metabolic (= catabolic + anabolic)
  metamorphosis, metazoan ...

Linguistic note:

How to express two redox states of iron

	Fe^{2+}	Fe^{3+}
Infix	**-o**	**-i**
Biochemical names	hem**o**globin, ferr**o**portin, ferr**o**xidase	hem**i**globin = methemoglobin, ferr**i**tin, transferr**i**n, lactoferr**i**n, gastroferr**i**n, ferr**i**c reductase
Chemical names		
Latin	ferr**o**si chloridum	ferr**i** chloridum
Old English	ferr**o**us chloride	ferr**i**c chloride
New English	iron(II) chloride	iron(III) chloride

Heme as cofactor of oxidoreductases transfers one electron



Examples of heme enzymes

Catalase: $H_2O_2 \rightarrow \frac{1}{2} O_2 + H_2O$

Myeloperoxidase: $H_2O_2 + Cl^- + H^+ \rightarrow HClO + H_2O$

Cytochrome P450 (CYP)

- superfamily of **heme** enzymes (many isoforms)
- catalyze mainly **hydroxylation** of various substrates
- exhibits wide substrate specifity (advantage for the body)
- can be induced and inhibited by many compounds
- occurs in most tissues (except of muscles and erythrocytes)
- the highest activity in the liver (ER)

Abbreviation: P = pigment, 450 = wave length (nm) of a absorption peak after binding CO

Hydroxylation by CYP 450 occurs in endogenous and exogenous substrates

- Endoplasmic reticulum:

  squalene, cholesterol, bile acids, calciol,

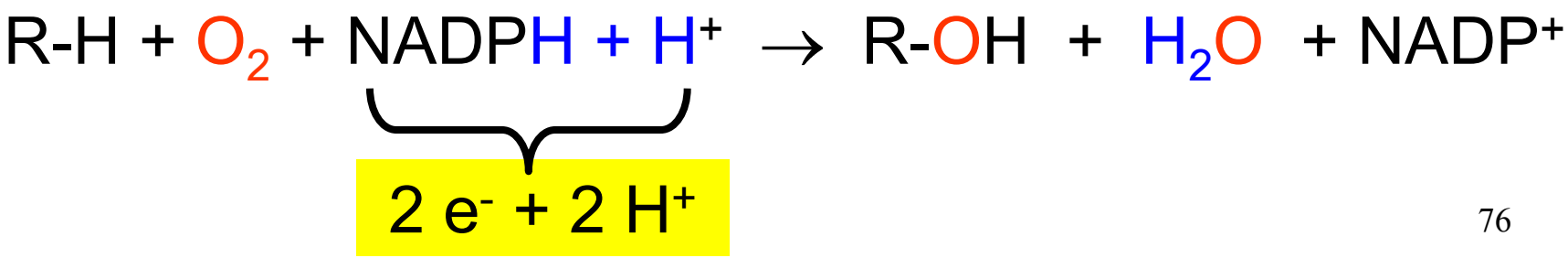
  FA desaturation, prostaglandins, xenobiotics

- Mitochondria:

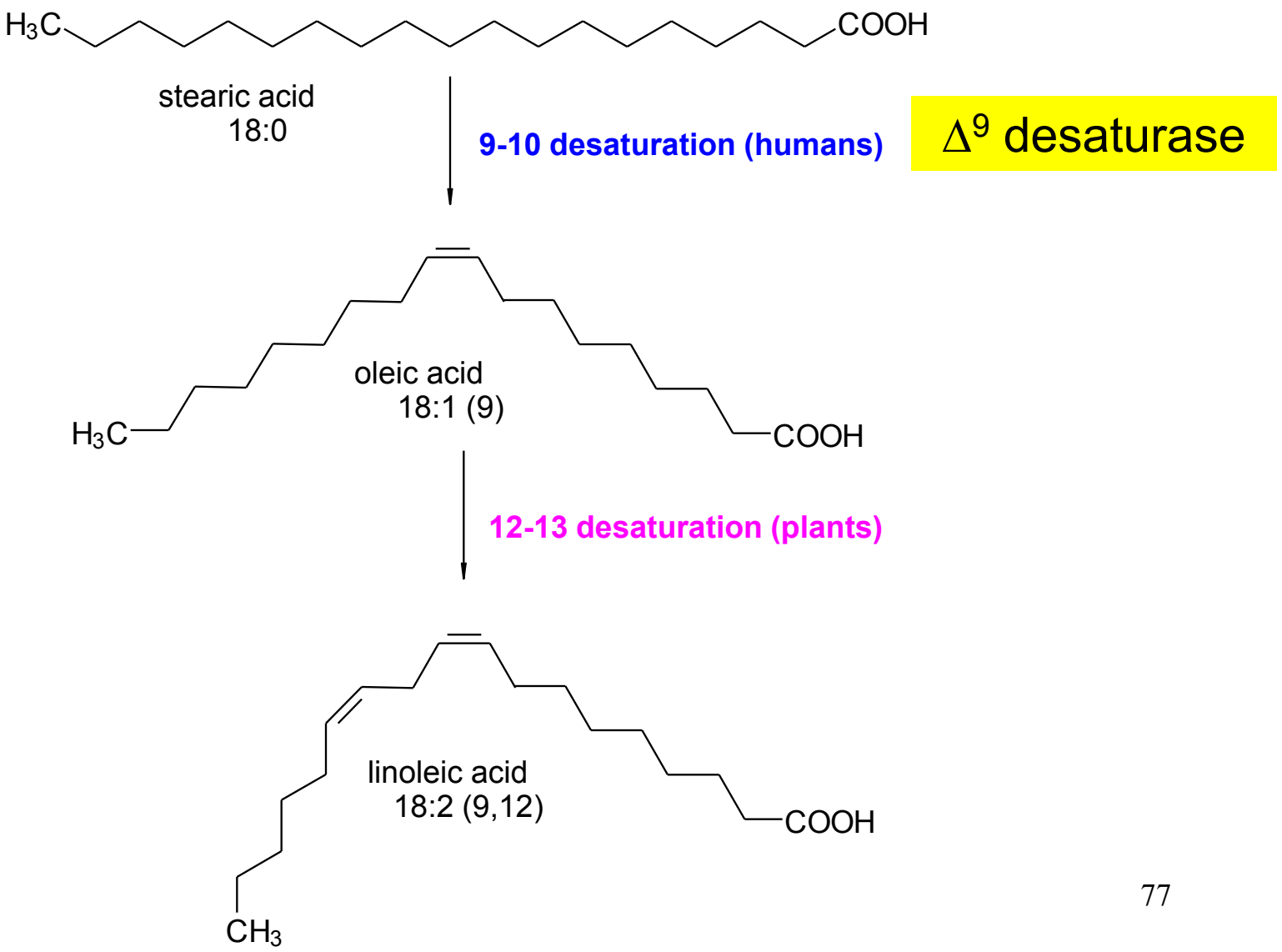
  steroidal hormones

Mechanism of hydroxylation

- the formation of hydroxyl group
- **mono**oxygenase: **one** O atom from O_2 molecule is incorporated into substrate between C and H (R-H $\rightarrow$ R-OH)
- the second O atom and 2H from NADPH+H^+ produce water



Desaturation of fatty acids



Desaturation of FA requires cytochrome b_5