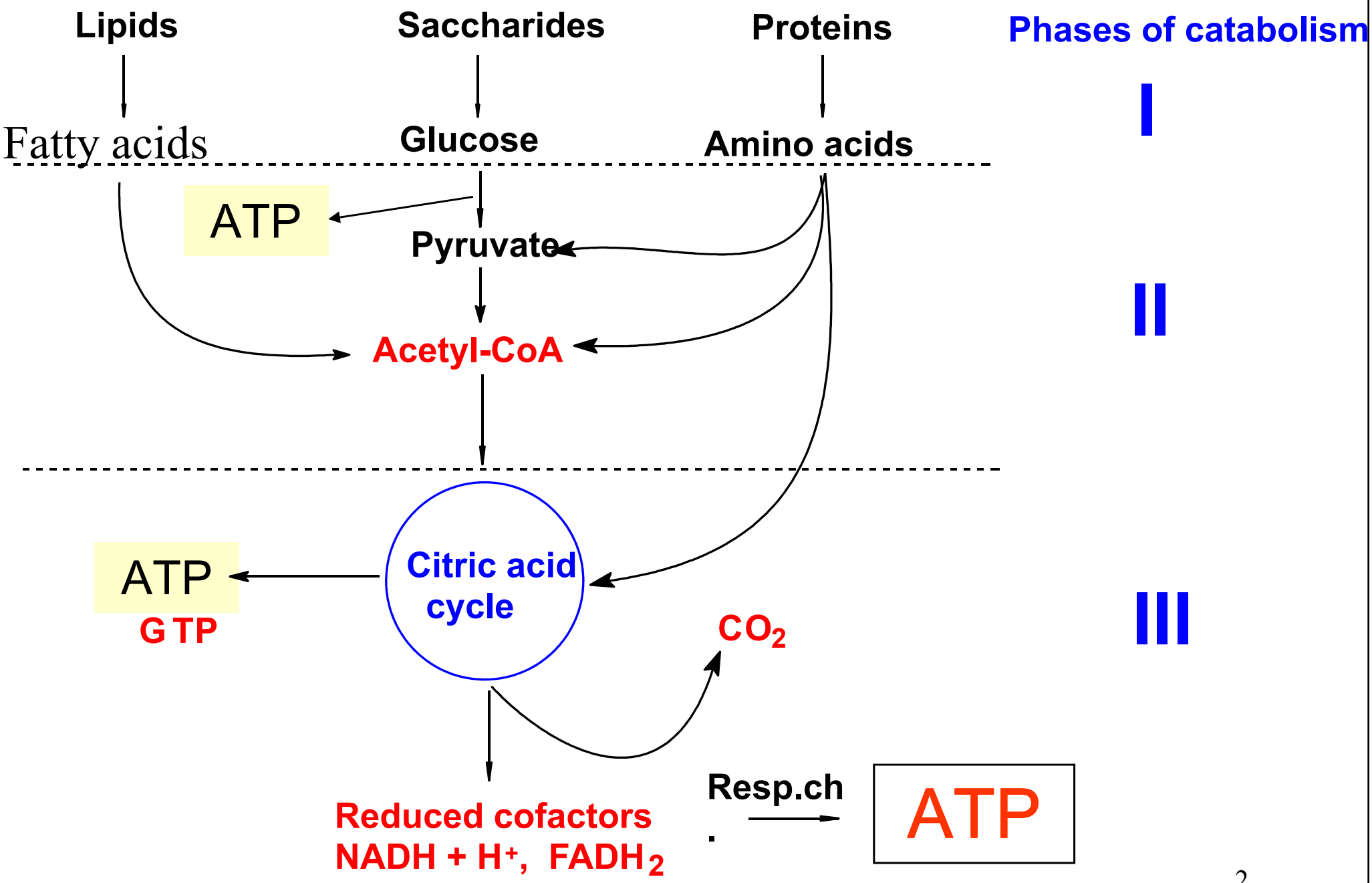
Citric acid cycle


Lipids
Saccharides
Proteins
Phases of catabolism
Fatty acids
Glucose
Amino acids
ATP
Pyruvate
Acetyl-CoA
ATP
GTP
Citric acid cycle
CO_2
Reduced cofactors
$NADH + H^+$, $FADH_2$
Resp.ch
.
ATP
I
II
III

Three phases of nutrient catabolism

- I.Hydrolysis of biopolymers to smaller units in digestion tract – **no yield of energy**

II. Metabolism of glucose → acetylCoA – **small amount of ATP + reduced cofactors,**

metabolism of amino acids → pyruvate, acetylCoA or some intermediates of TCA – **some reduced cofactors**

beta oxidation of FA – acetyl-CoA + **reduced cofactors**

III. Oxidation of acetyl-CoA in citric acid cycle – **GTP + reduced cofactors**

oxidation of reduced cofactors in respiratory chain – **ATP (highest yield of energy)**

Citric acid cycle

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 glucose, fatty acids, some aminoacids, keton bodies, is **oxidized to 2 molecules of CO_2**

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

Formation of acetyl-CoA

- oxidative decarboxylation of pyruvate
- β-oxidation of fatty acids
- catabolism of some amino acids
- Keton bodies → acetoacetylCoA → acetylCoA (in extrahepatal tissues)
- metabolism of ethanol

Citric acid cycle

- Products of TCA:

  CO_2 → is expired

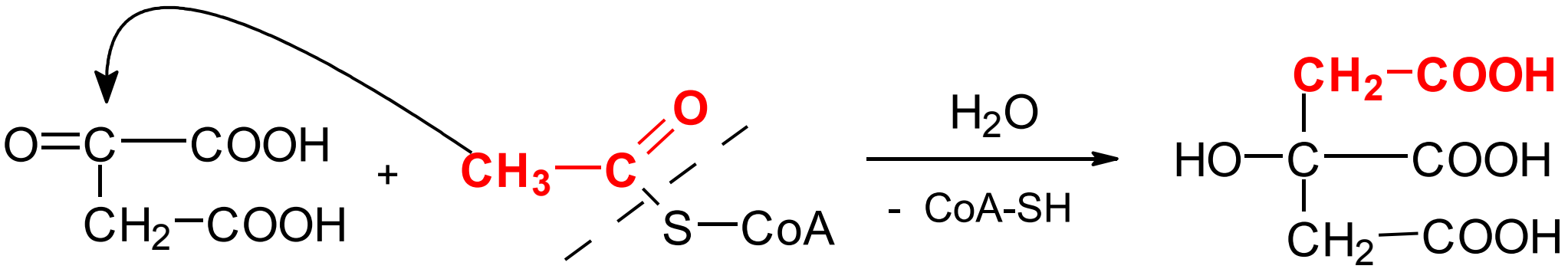
  four oxidative steps → reduced cofactors → respiratory chain

  GTP → ATP

Most of reactions are reversible, only 3 reactions are irreversible

http://www.wiley.com/college/pratt/0471393878/student/animations/citric_acid_cycle/index.html

(1) Oxalacetate + Acetyl-CoA



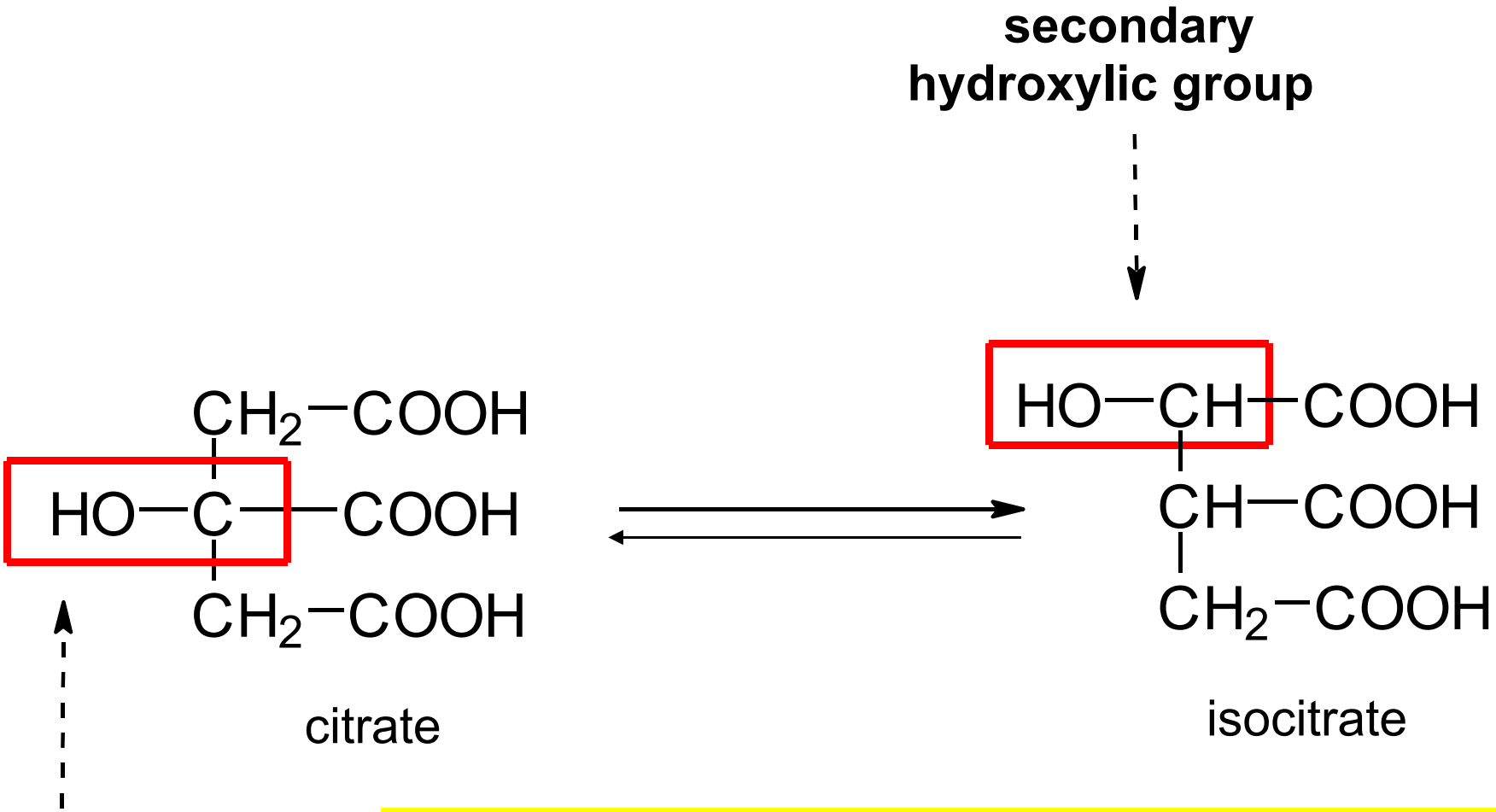
oxalacetate **acetyl-koenzym A** **citrate**

Reaction type: condensation

Enzyme: citrate synthase

Cofaktor: coenzym A Note: **irreversible**

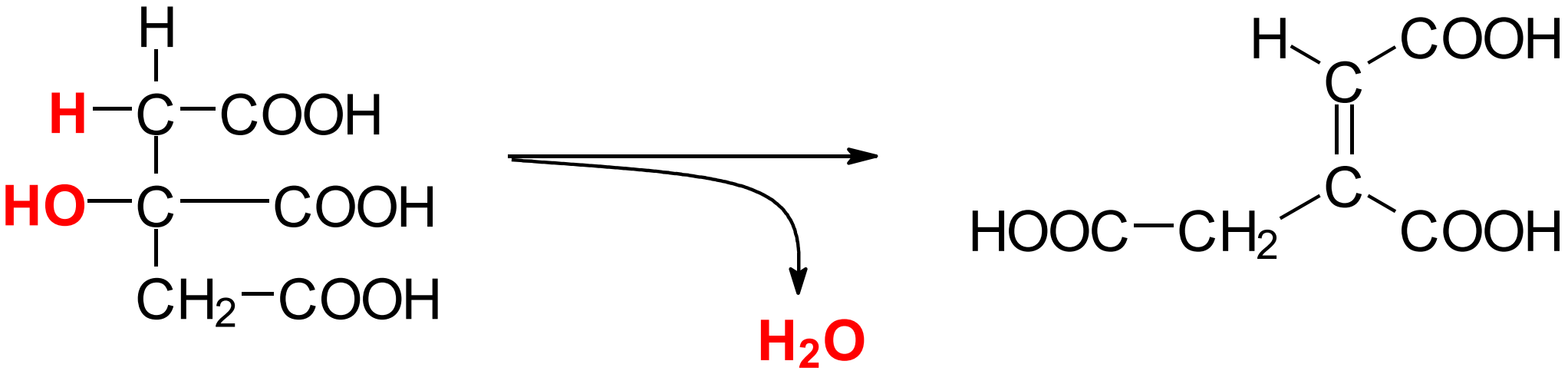
(2) Citrate → Isocitrate



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

Note: in fact two steps reaction, intermediate is cis-aconitate

(2a) Dehydratation of citrate



citrate

cis-aconitate

(2b) Hydratation of cis-aconitate

cis-aconitate

isocitrate

stereospecific reaction

Aconitase is inhibited by fluoracetate

FCH_2COOH

Forms fluorocitrate with OA

TCA is stopped

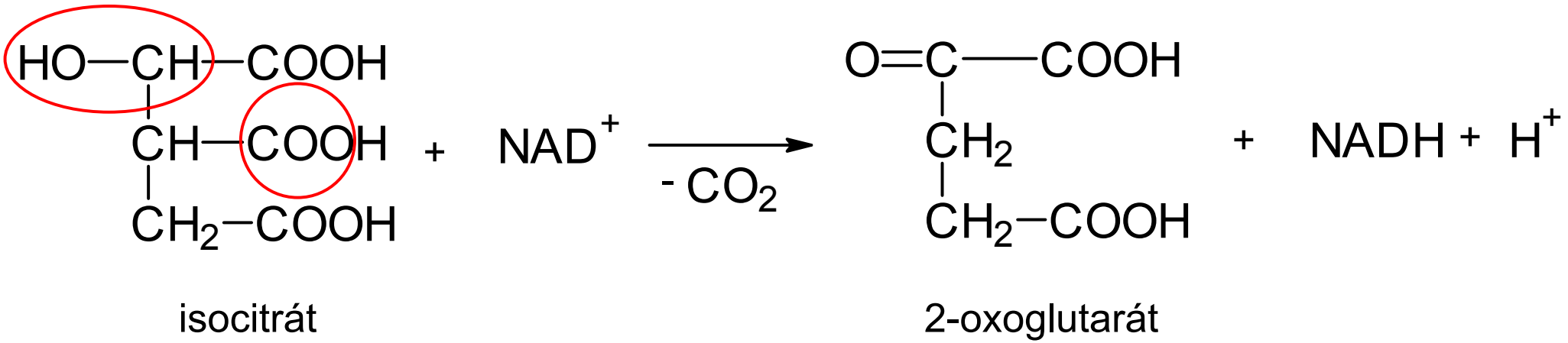
LD_{50} is 1 mg/kg

Rat poison

Dichapetalum cymosum

(see also med.chem II, p.)

(3) Isocitrate → 2-oxoglutarate

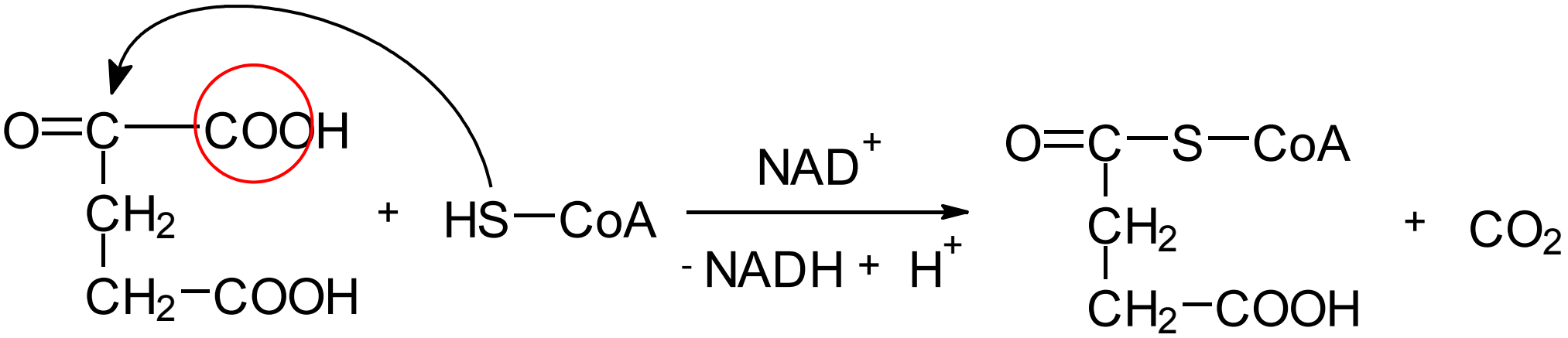


Reaction type: dehydrogenation + decarboxylation

Enzyme: isocitrate dehydrogenase

Cofaktor: NAD^+ Note: **irreversible**

(4) 2-Oxoglutarate → succinyl-CoA



2-oxoglutarate

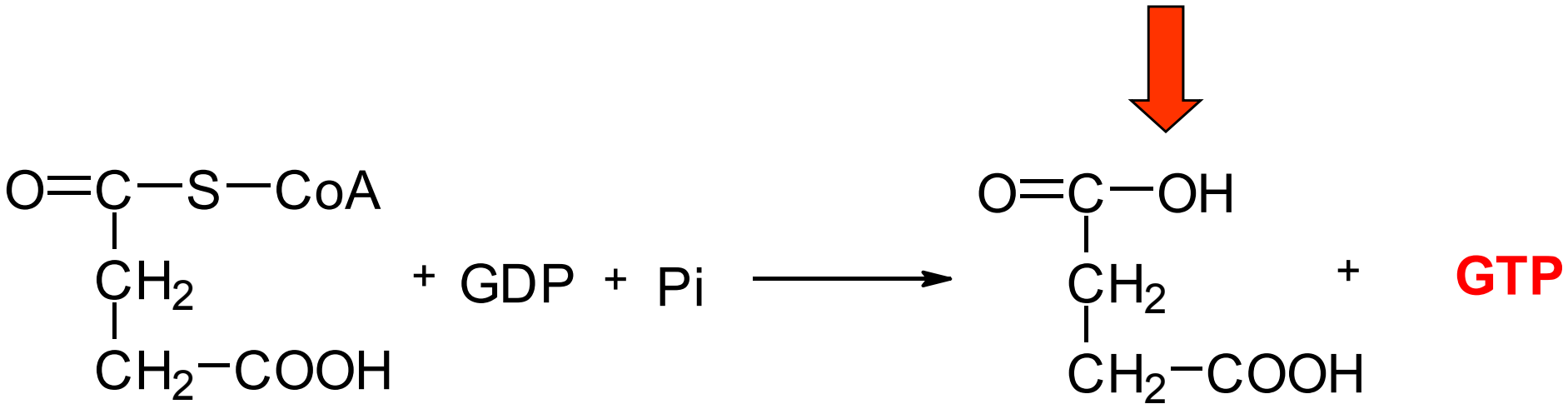
succinyl-coenzyme A thioester macroergic intermediate

Reaction type: oxidative decarboxylation Enzyme: 2-oxoglutarate dehydrogenase

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

Note: **irreversible**, resembles to pyruvate dehydrogenase reaction (identical coenzyme requirements)

(5) Succinyl-CoA + GDP + P_i



succinyl-coenzym A　　succinate

Reaction type: substrate phosphorylation

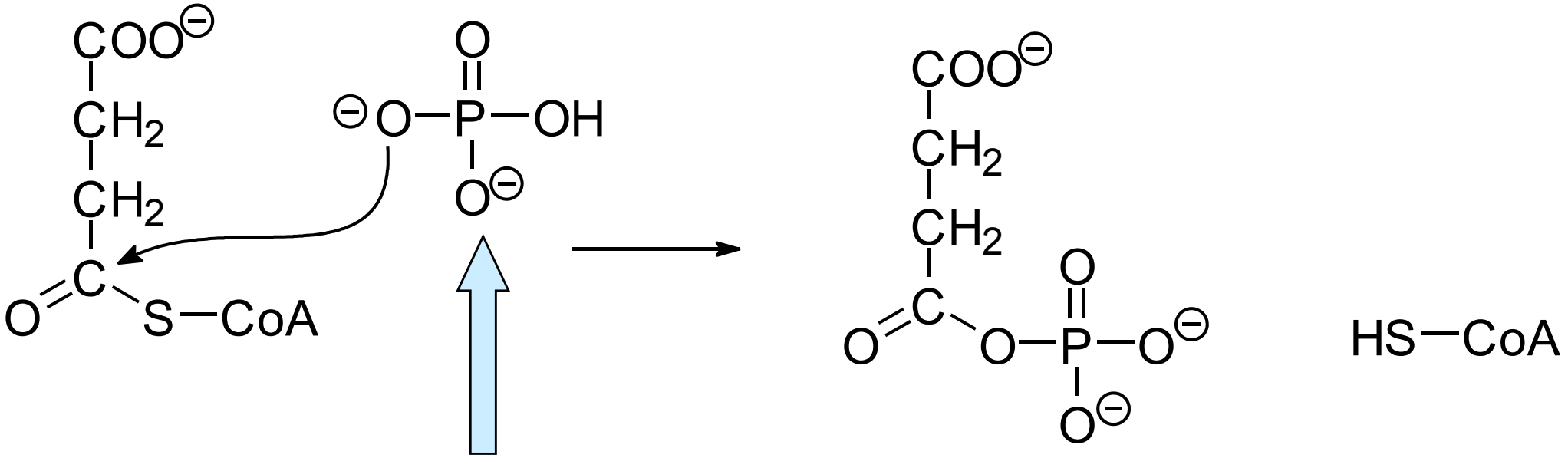
Enzyme: succinyl-CoA syntethase　Cofactor: coenzym A

GTP i formed in three-steps reaction

Chemical energy of macroergic succinyl-CoA is gradually transformed into two macroergic intermediates and in the end to macroergic GTP

(Passing a hot potato)

(5a) Addition of phosphate to succinyl-CoA



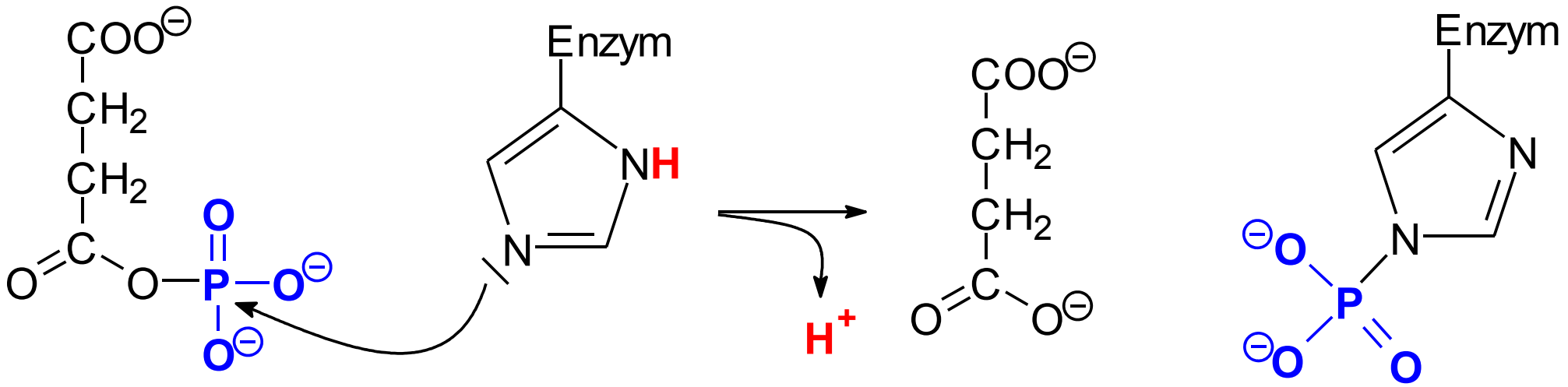
succinyl-CoA

Four oxygens in phosphate

succinylphosphate

mixed anhydride

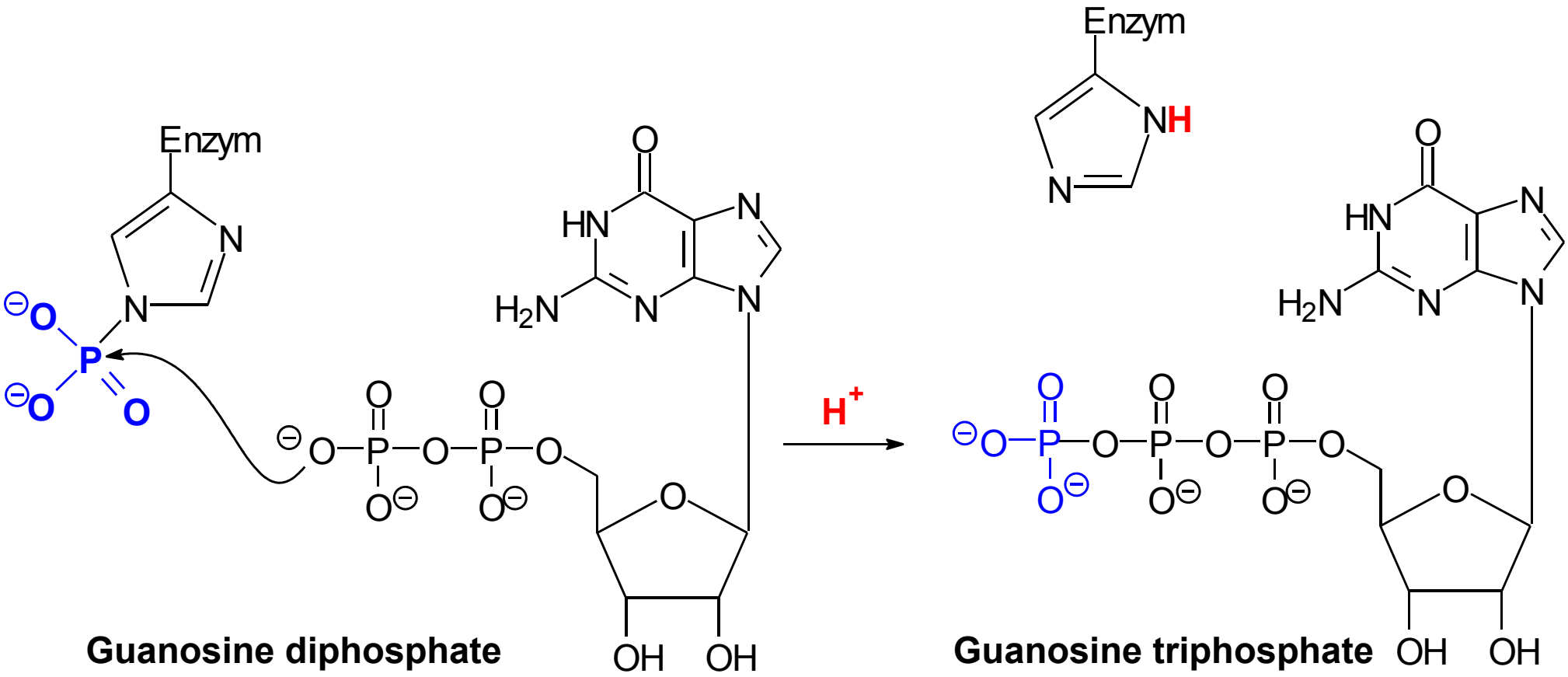
(5b) Phosforylation of His in active center of the enzyme



succinylphosphate succinate fosfo-His

substituted phosphoamide

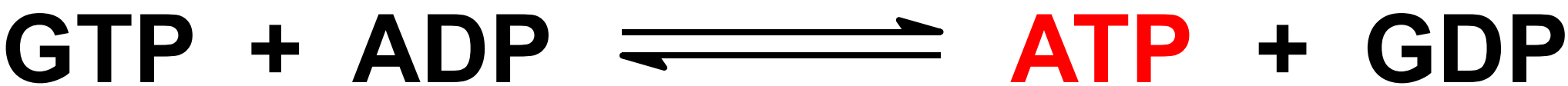
(5c) Phosforylation of GDP



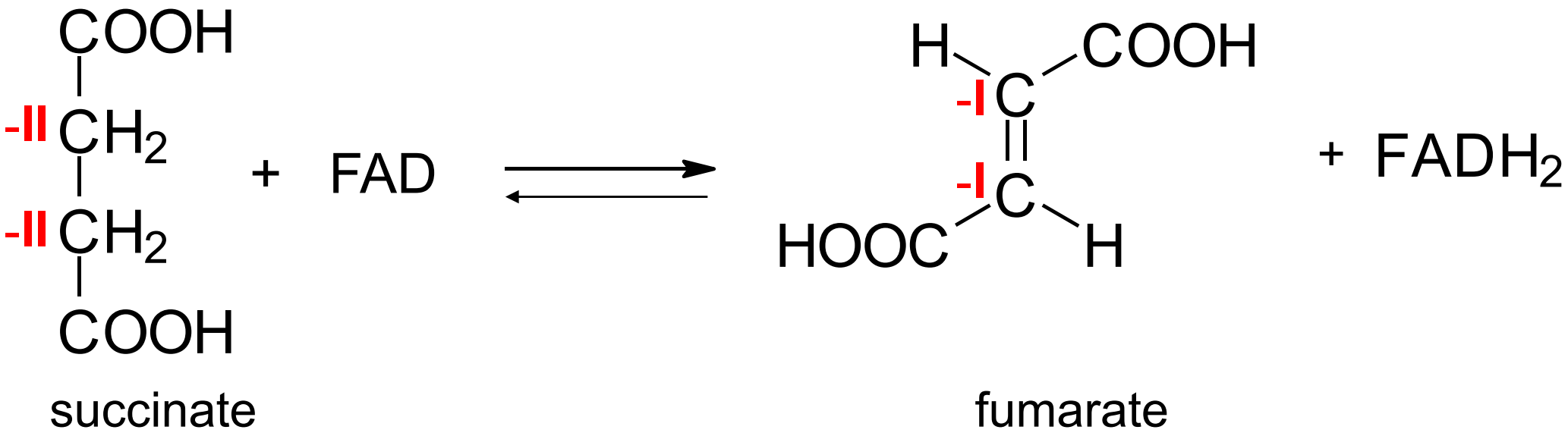
Guanosine diphosphate **Guanosine triphosphate**

GTP is quickly converted to ATP

nucleoside-diphosphate kinase



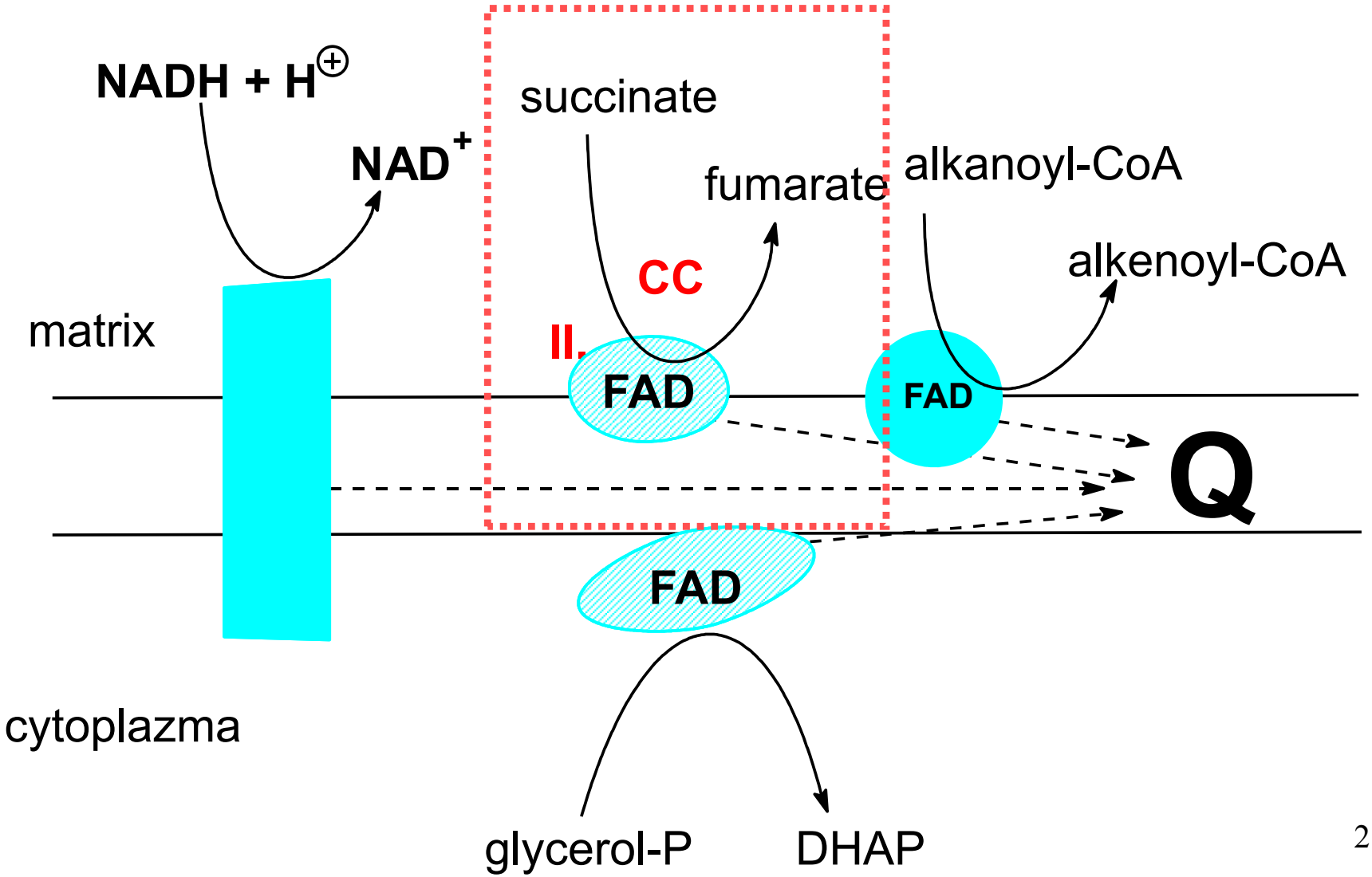
(6) Succinate → fumarate



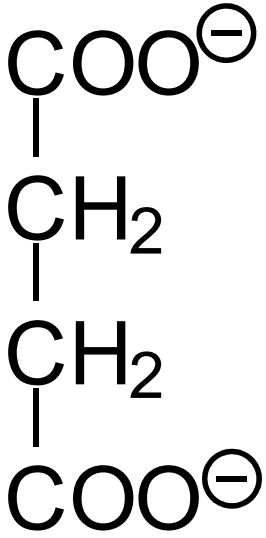
Reaction type: dehydrogenation (-CH_2-CH_2- bond)

Enzyme: succinate dehydrogenase Cofaktor: FAD

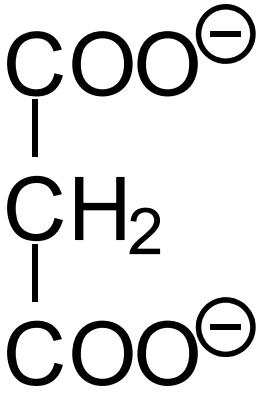
Succinate dehydrogenase is a component of respiratory chain in the inner mitochondrial membrane



Malonate is competitive inhibitor of succinate dehydrogenase



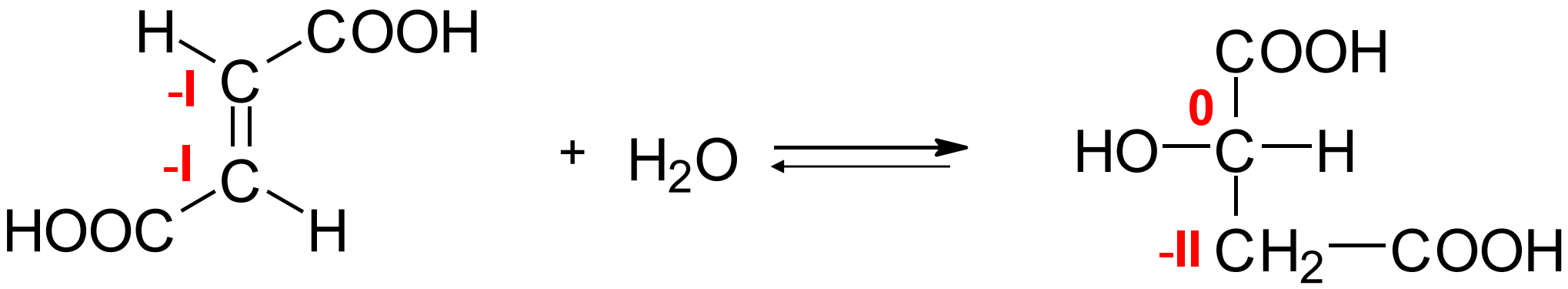
succinate



malonate

Do not confuse:
malonate × malate

(7) Fumarate → L-malate



fumarate

L-malate

Σ = -II

Σ = -II

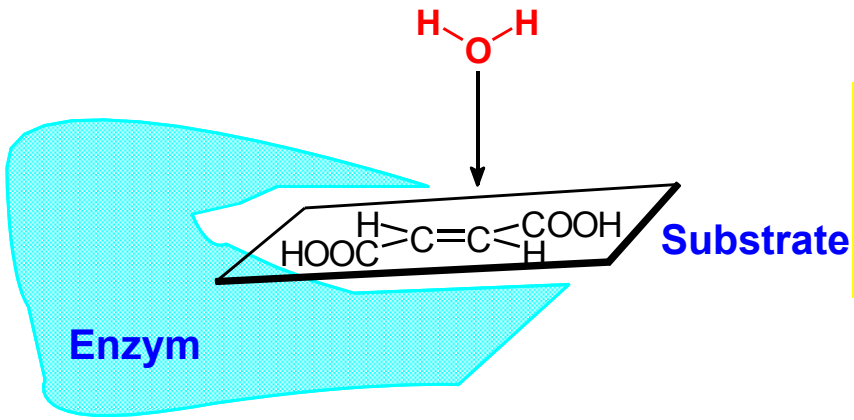
Reaction type: hydration Enzyme: fumarase

Cofactor: no

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

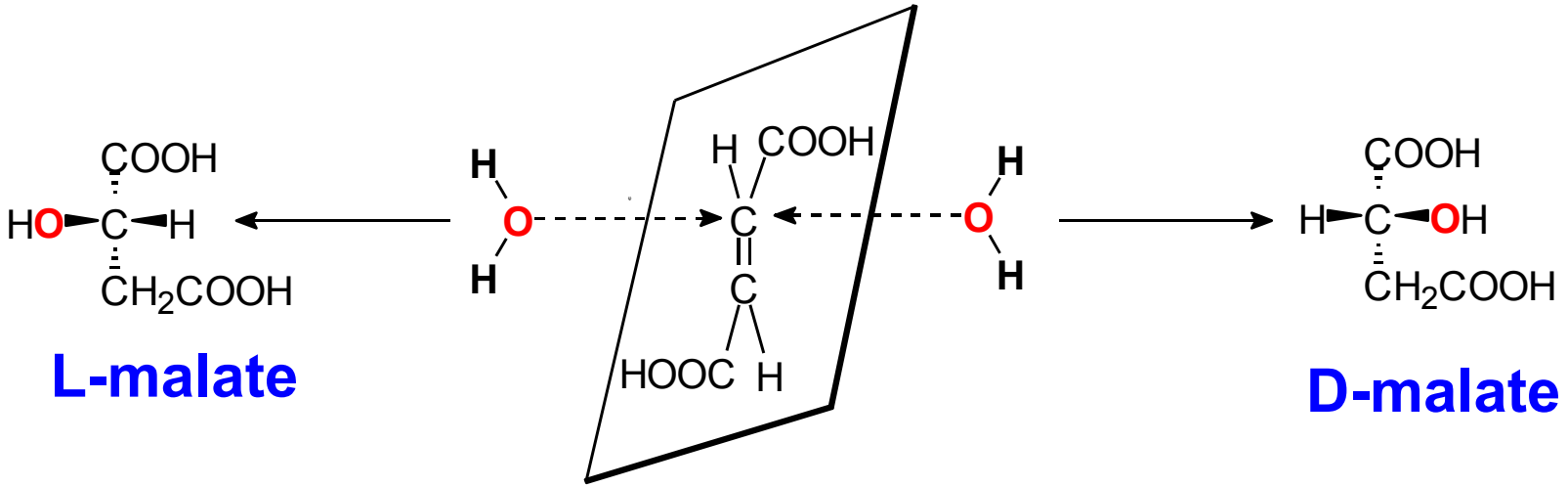
2) hydration is not redox reaction

Compare: Hydration of fumarate



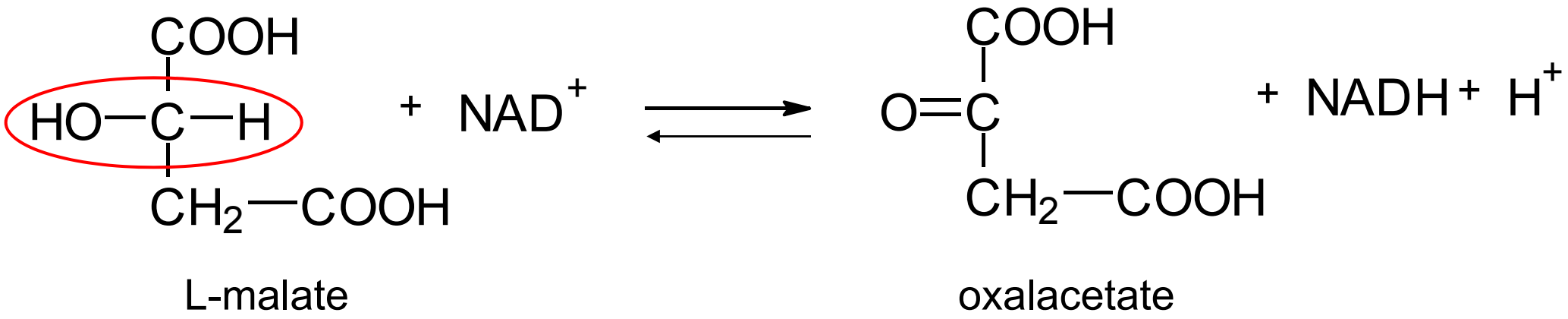
in vivo (by an enzym action):

Only one enantiomer is formed (L-malate)



in vitro: formation of racemate

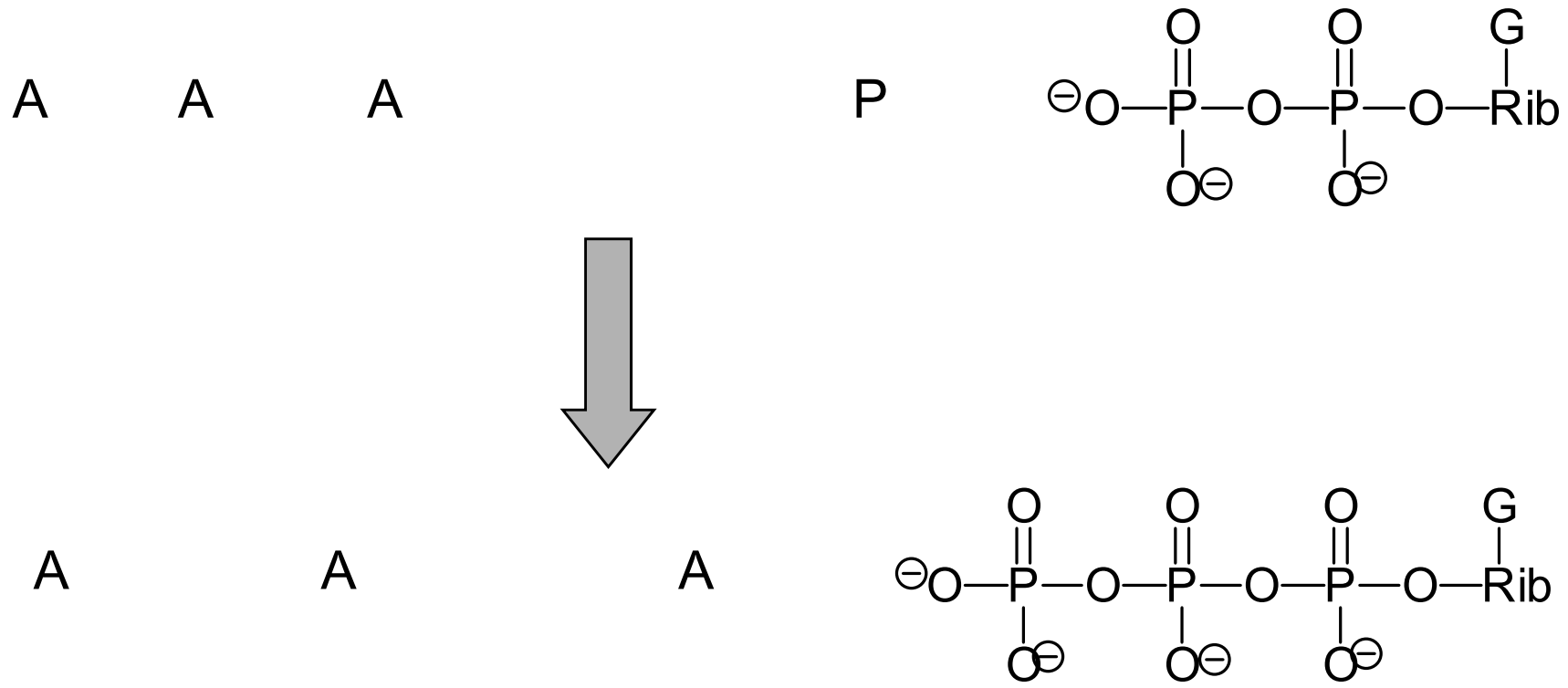
(8) L-malate → oxalacetate



Reaction type: dehydrogenation

Enzyme: malate dehydrogenase Cofactor: NAD^+

The net equation of TCA



- two C-atoms are completely oxidized to 2 CO_2
- 8 H-atoms are released in the form of reduced cofactors ($3 \times NADH+H^+$, $1 \times FADH_2$)

The energetic yield

Products of TCA	Equivalent to ATP (resp.chain)
1 × GTP	1
3 × NADH + H^+	9
1 × $FADH_2$	2

Total: **12 ATP**

Factors affecting TCA

- Energy charge of the cell

$$\text{Energy charge} = \frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$$

- $NADH+H^+/NAD^+$ ratio
- Allosteric inhibition
- Inhibition by products
- Supply of oxygen -TCA can proceed only at aerobic conditions

  (reduced cofactors must be reoxidize in respiratory chain)

Key enzymes for regulation

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

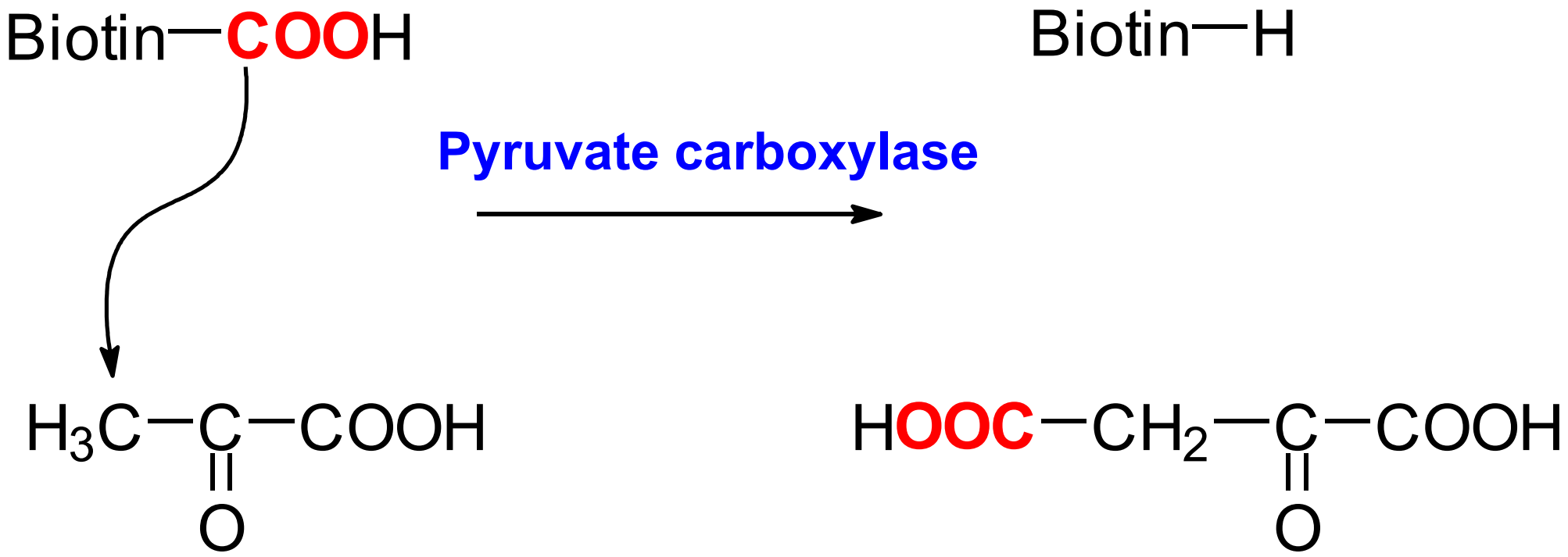
[b] feed-back inhibitor (inhibition by a product)

[c] allosteric activator

Anaplerotic reactions of TCA

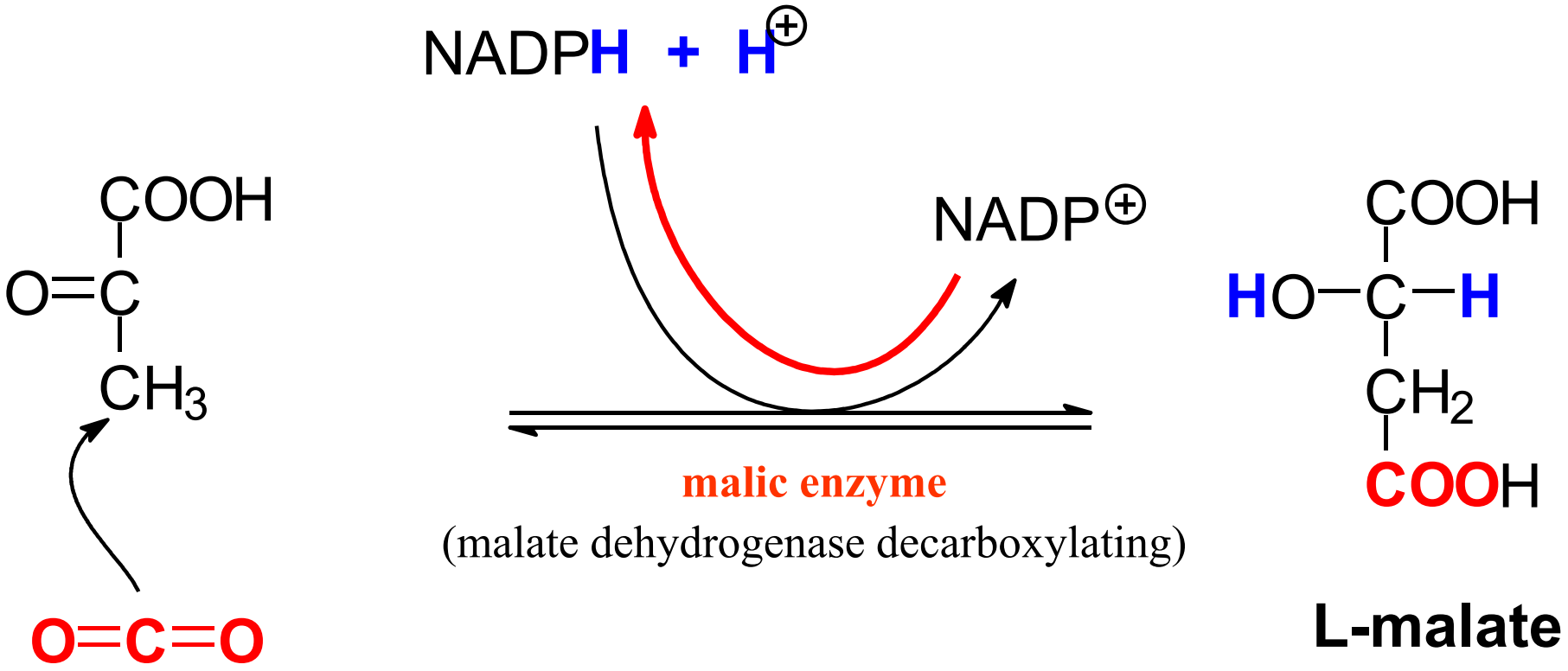
- Reaction that fill up intermediates of TCA:
- **Carboxylation of pyruvate → oxalacetate**
- (reductive carboxylation of pyruvate → malate)
- Transamination of aspartate → oxalacetate
- Catabolismus of Phe, Tyr → fumarate
- Asp (synt. Of urea, purines → fumarate
- catabolisms of Val, Ile, Met → succinyl-CoA
- Transamination of glutamate → 2-oxoglutarate

Carboxylation of pyruvate (biotin)



pyruvate **oxalacetate**

Reductive carboxylation of pyruvate



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

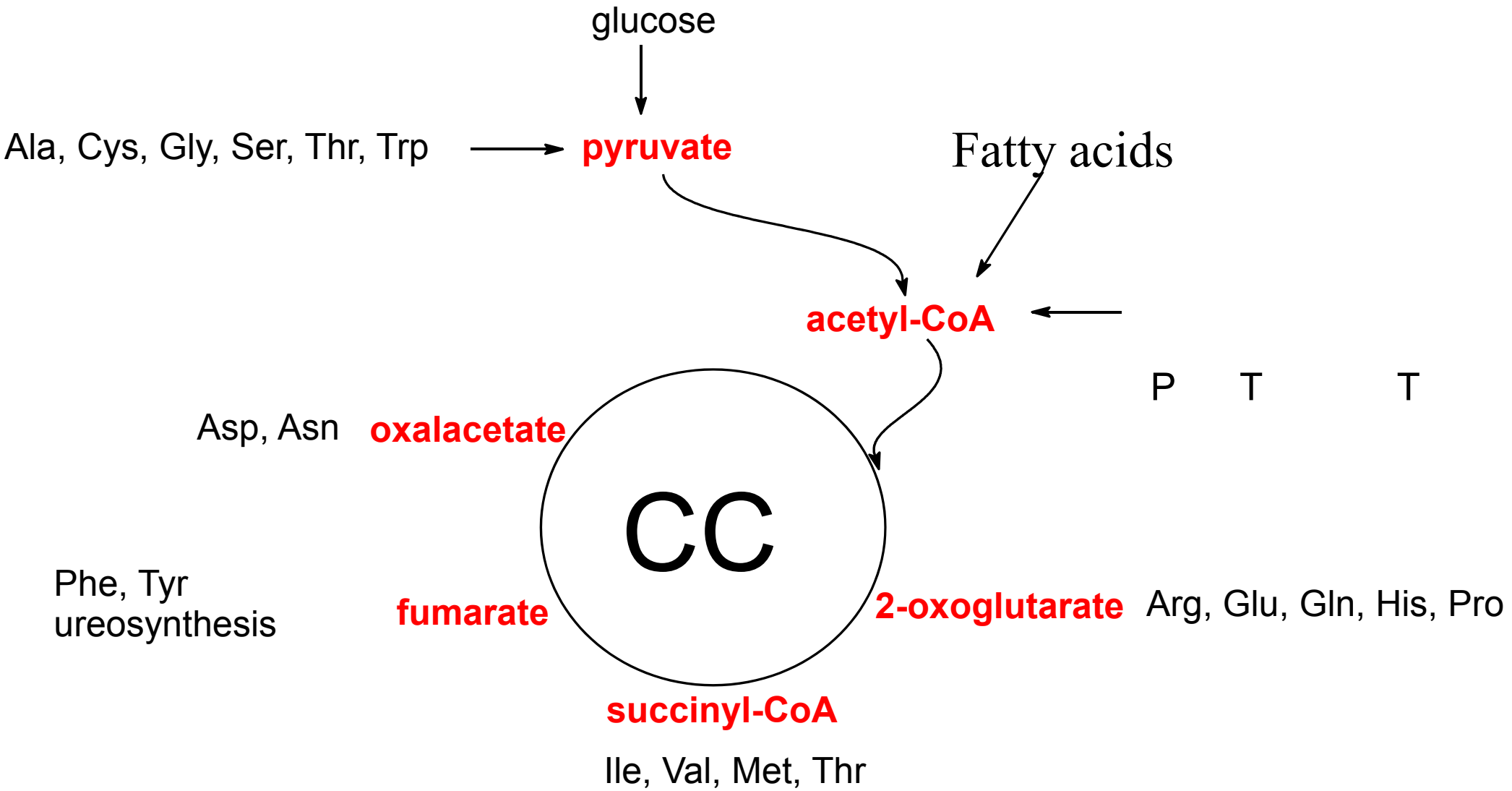
Amphibolic character of TCA

Final catabolic pathway: oxidation of acetyl-CoA to CO_2

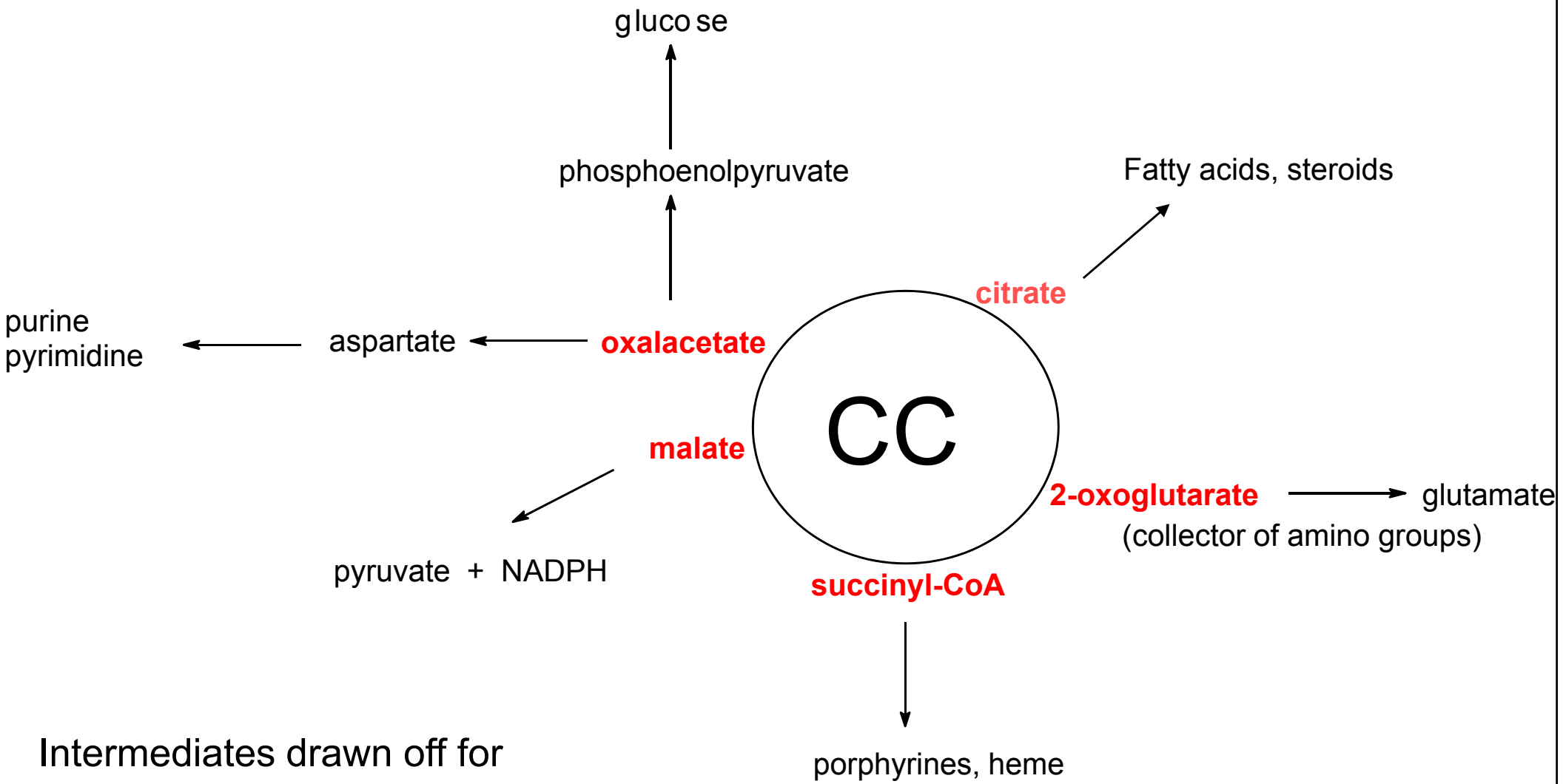
Also other compounds, which are metabolized to the TCA intermediates, **can serve as substrates** of the cycle

TCA provides important **metabolic intermediates** for **anabolic** processes: gluconeogenesis, transamination etc.

Catabolic processes - entries into the cycle:

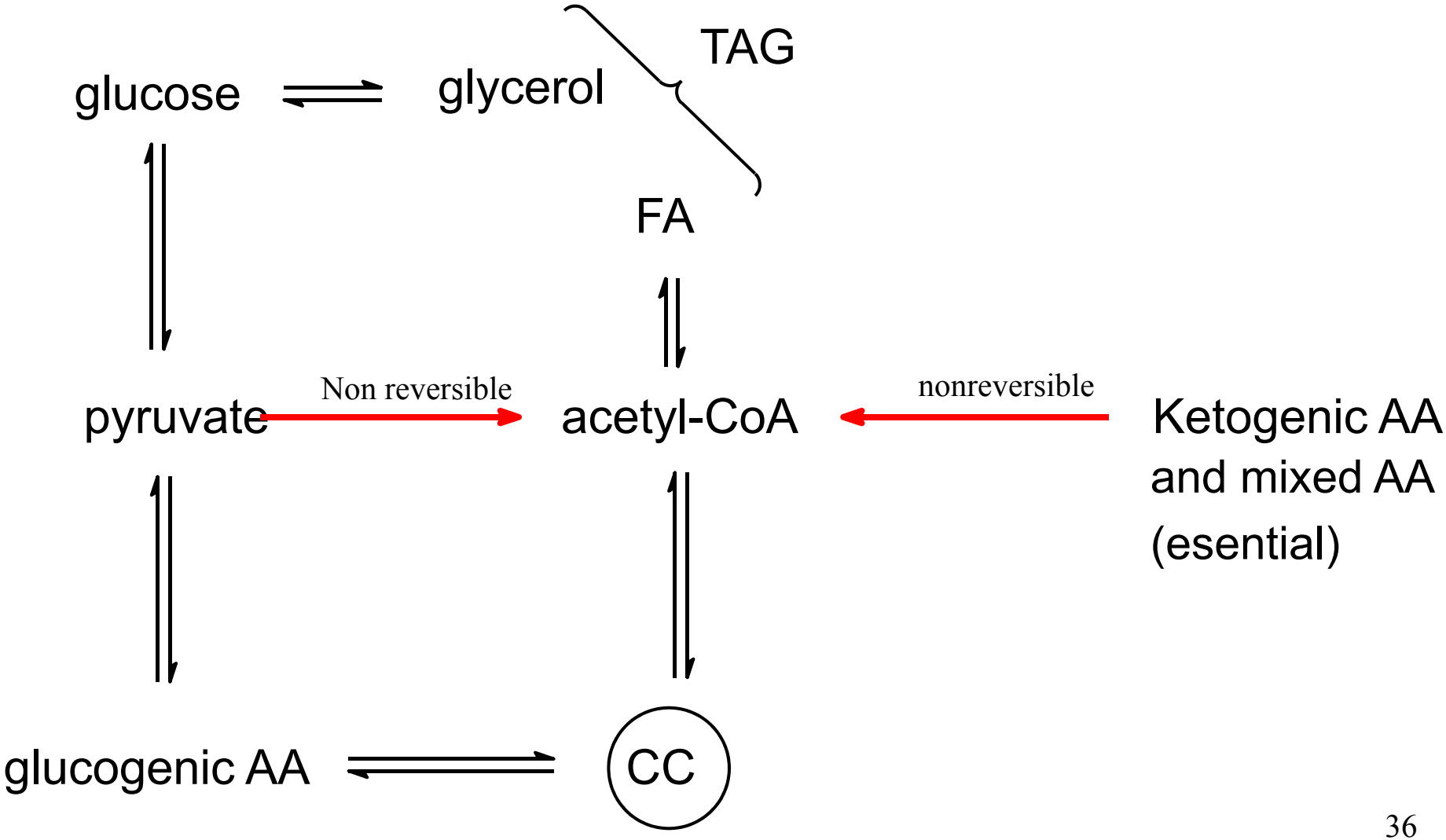


Anabolic processes – intermediates for synthesis

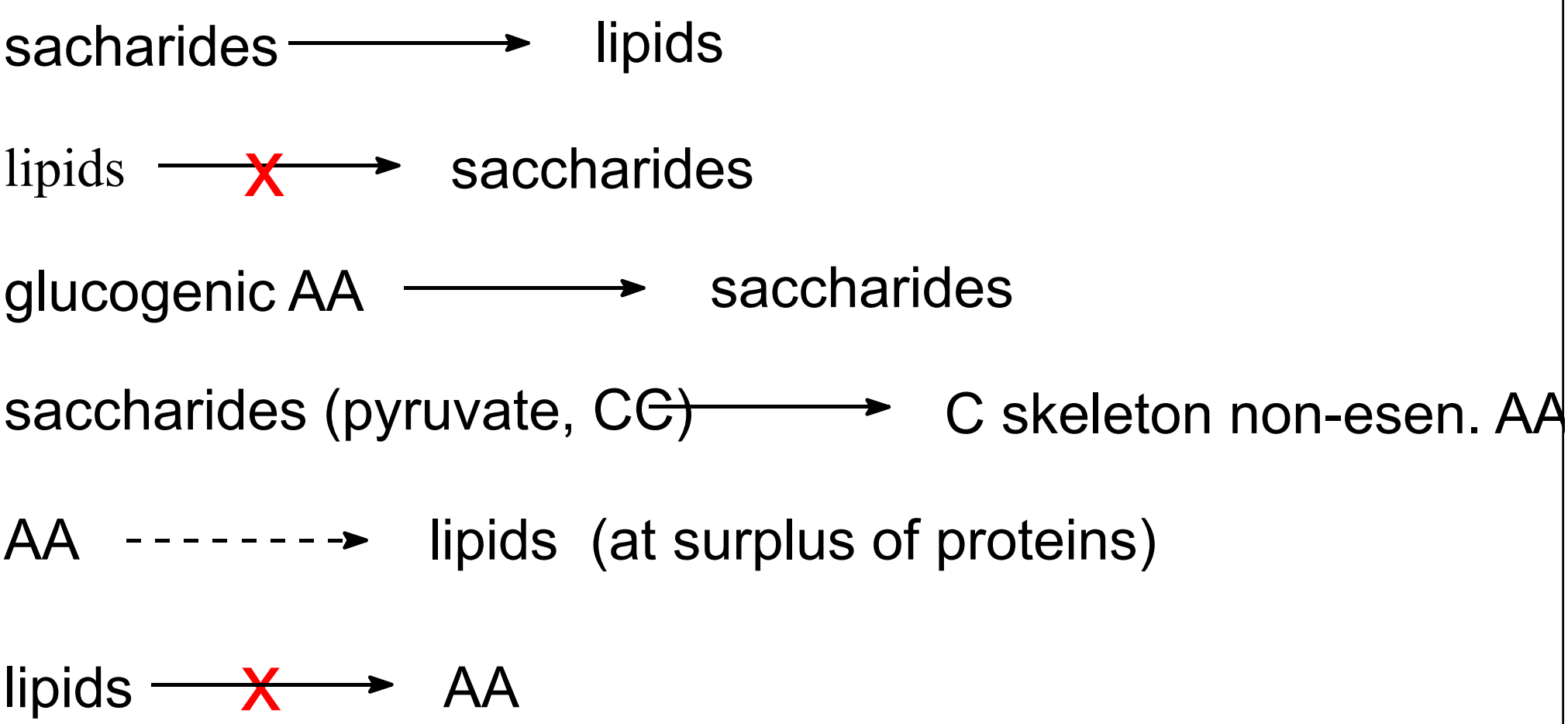


Intermediates drawn off for biosyntheses are replenished by the **anaplerotic reactions**.

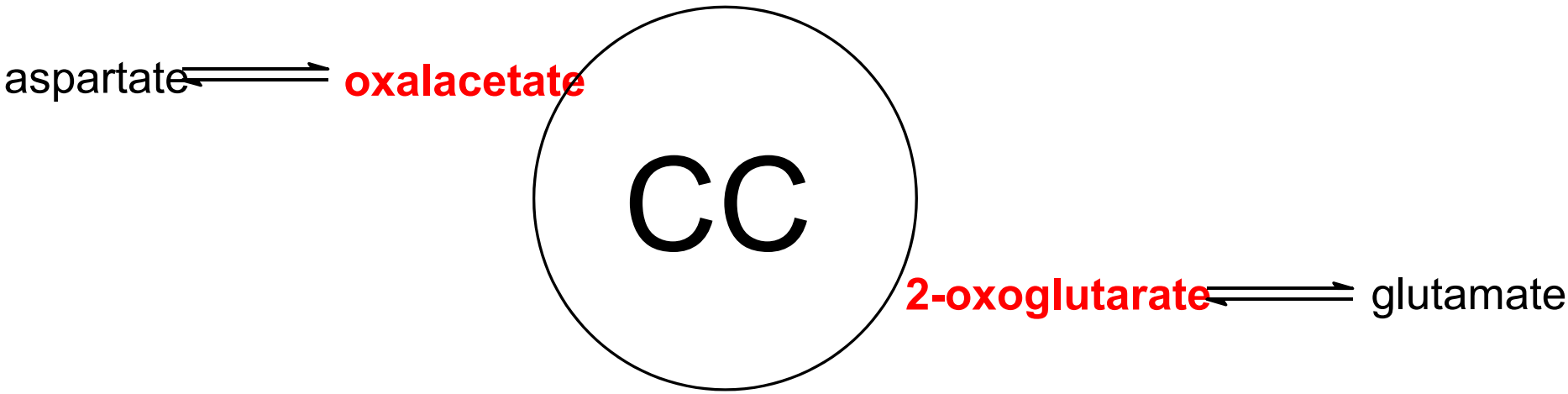
Interrelations among the metabolism pathways



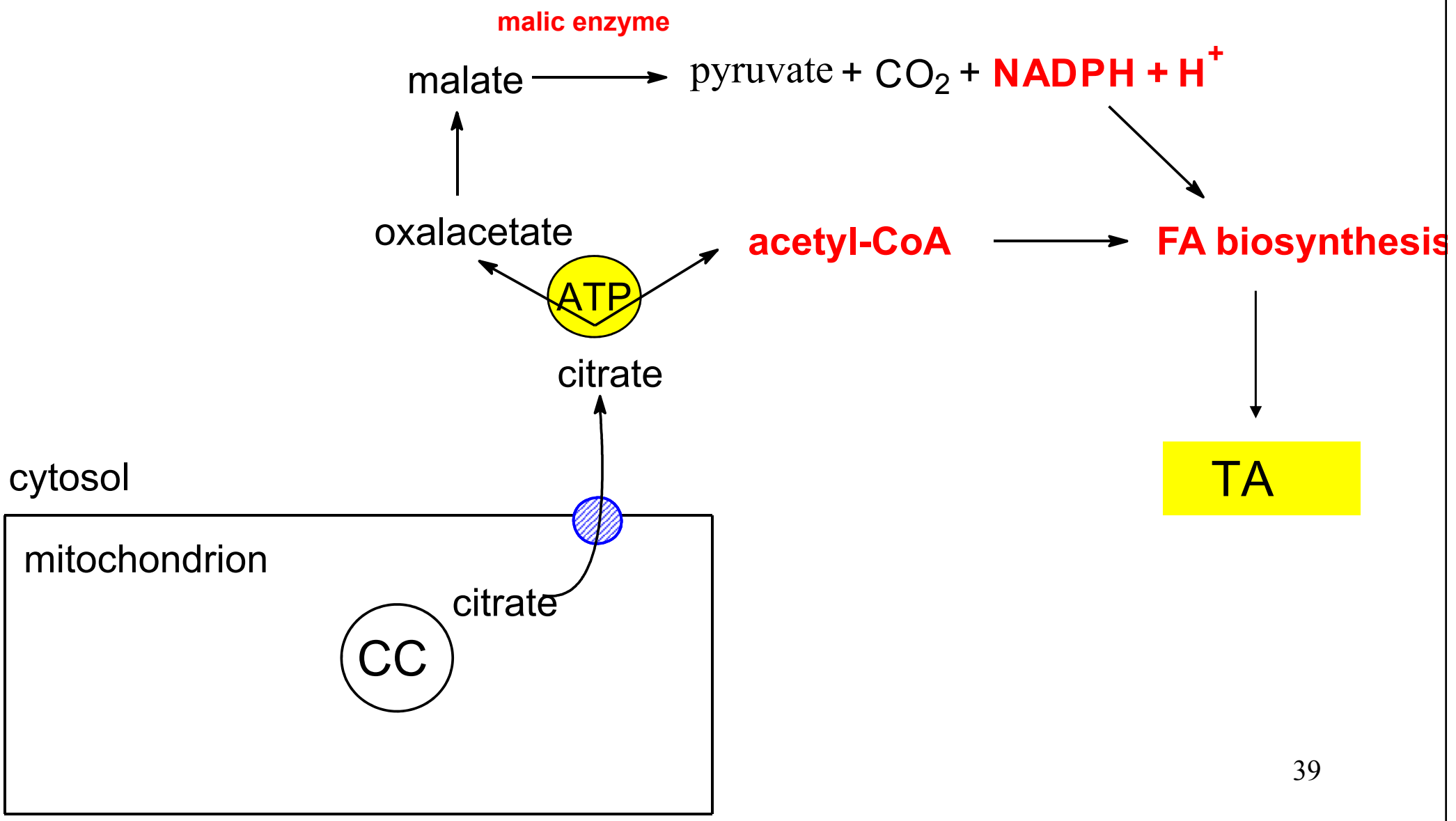
Interrelations among the metabolism of nutrients



TCA and transamination



TCA and synthesis of lipids



Vitamins necessary for TCA

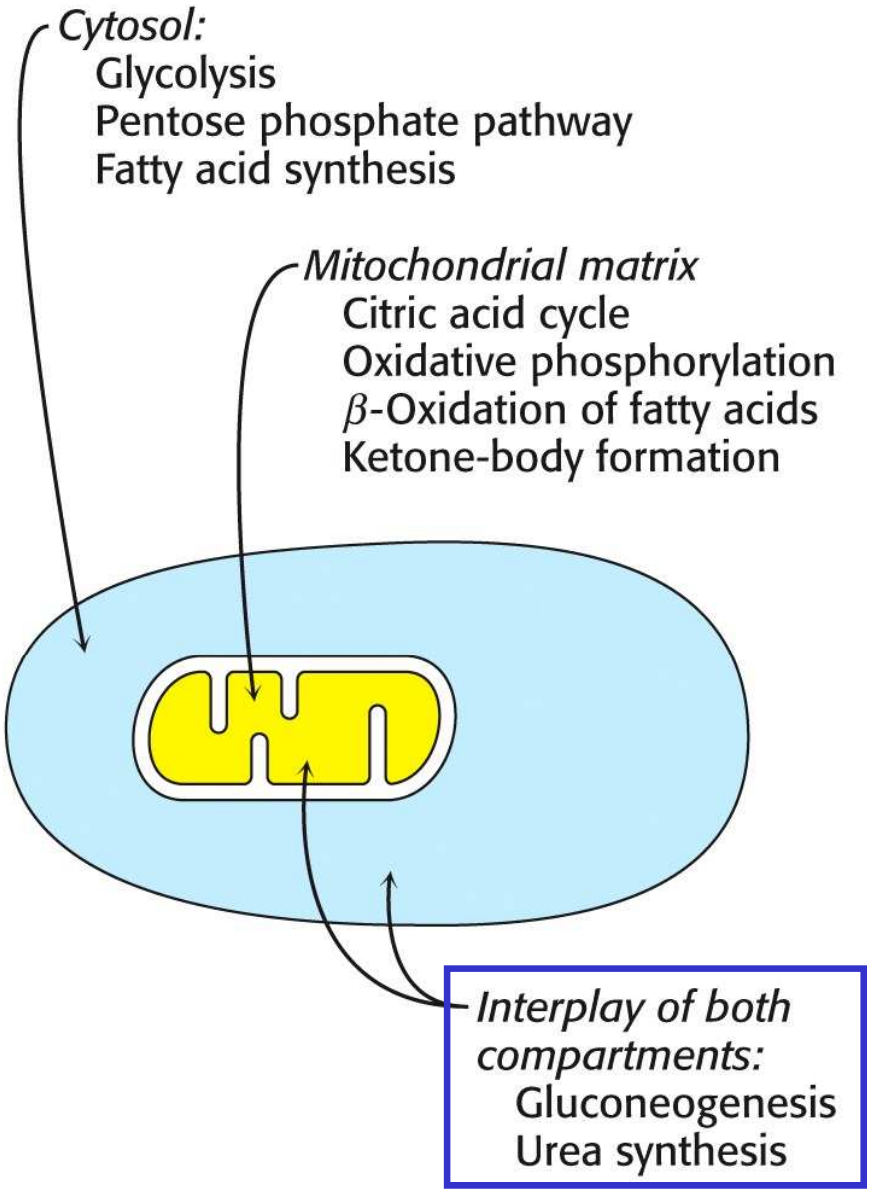
Try to complete

Vitamin	Reaction in TCA
riboflavin	
niacin	
thiamin	
Pantothenic acid	

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	**+**

Compartmentation of the major pathways of metabolism



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

Recommended intake of nutrients

Nutrient	Percentage of daily intake
Starch	55 – 60 %
Lipids	≤ 30 %
Proteins	10 -15 %

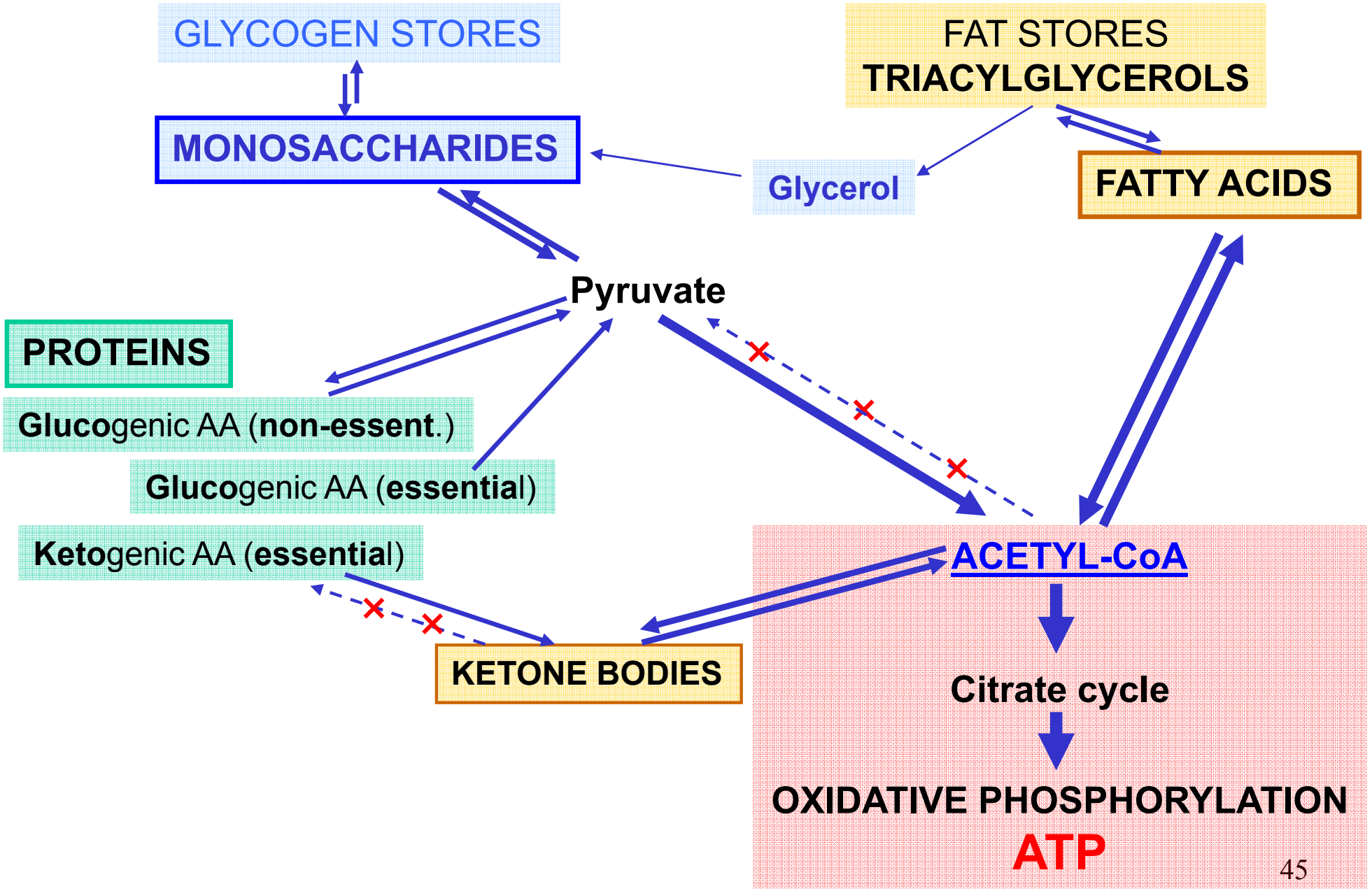
SAFA ≈ 5 %

MUFA ≈ 20 % *

PUFA ≈ 5 %

Essential FA:	linoleic, α-linolenic
Cond. esenc. FA:	arachidonic
Essencial AA:	Phe, Trp, Val, Leu, Ile, Met, Thr, Lys, His
Cond. esenc. AK:	Arg (childhood), Ala, Gln (metab. stress)

Relationships among the major energy metabolism pathways



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).

Metabolic effects of insulin

Tissue	Affected pathaway
Liver	↑ Glucose phosphorylation
	↑ Glycolysis
	↓ Gluconeogenezis
	↑Synthesis of glycogen
	↓ Glycogenolysis
	↑Synthesis of fatty acids
	↑ Pentose phosphate pathway

Tissue	Affected pathaway
Adipocytes	↑ Glucose uptake
	↑ Glycolysis
	↑ Pentose phosphate pathway
	↑Oxidation of pyruvate
	↑Cleavage of TG in lipoproteins
	↑ Synthesis of TG
	↓ Lipolysis

Tissue	Affected pathaway
Muscle	↑Glucose uptake
	↑Glycolysis
	↑Synthesis of glycogen
	↓ Glycogenolysis
	↑ Synthesis of proteins

Metabolic effects of glucagon

Tissue	Affected pathaway
Liver	↓ Glycolysis
	↑ Gluconeogenesis
	↓ Synthesis of glycogen
	↑ glykogenolysis
	↓ Synthesis of fatty acids
	↑ Oxidation of fatty acids
Adipocytes	↑Lipolysis

No receptors for glucagon are in muscles → metabolism is not afected by glucagon