CC 8, SEM – 4 (HONS.)

METABOLISM

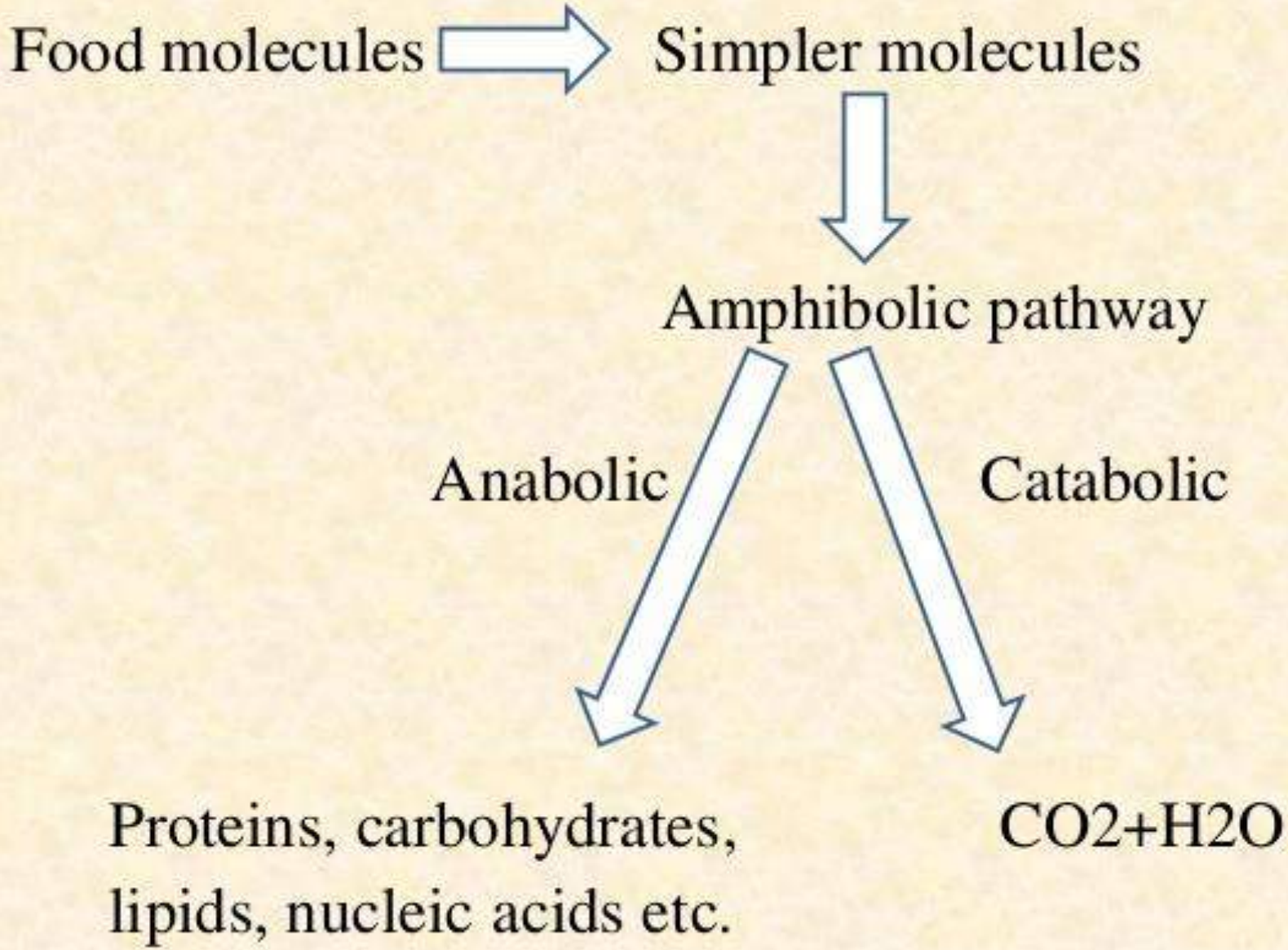
Thousands of chemical reactions are taking place inside a cell in an organized, well co-ordinated and purposeful manner; all these reactions are called as METABOLISM.

TYPES OF METABOLIC PATHWAY:

- ✓Catabolic Pathway
- ✓Anabolic Pathway
- ✓Amphibolic Pathway

STAGES AND PHASES OF METABOLISM:

- ✓Primary
- ✓Secondary
- ✓Tertiary


Food molecules
Simpler molecules
Amphibolic pathway
Anabolic
Catabolic
Proteins, carbohydrates,
lipids, nucleic acids etc.
CO2+H2O

MAJOR PATHWAYS OF CARBOHYDRATE METABOLISM

1) Glycolysis

2) Citric Acid Cycle

3) Gluconeogenesis

4) Glycogenesis

5) Glycogenolysis

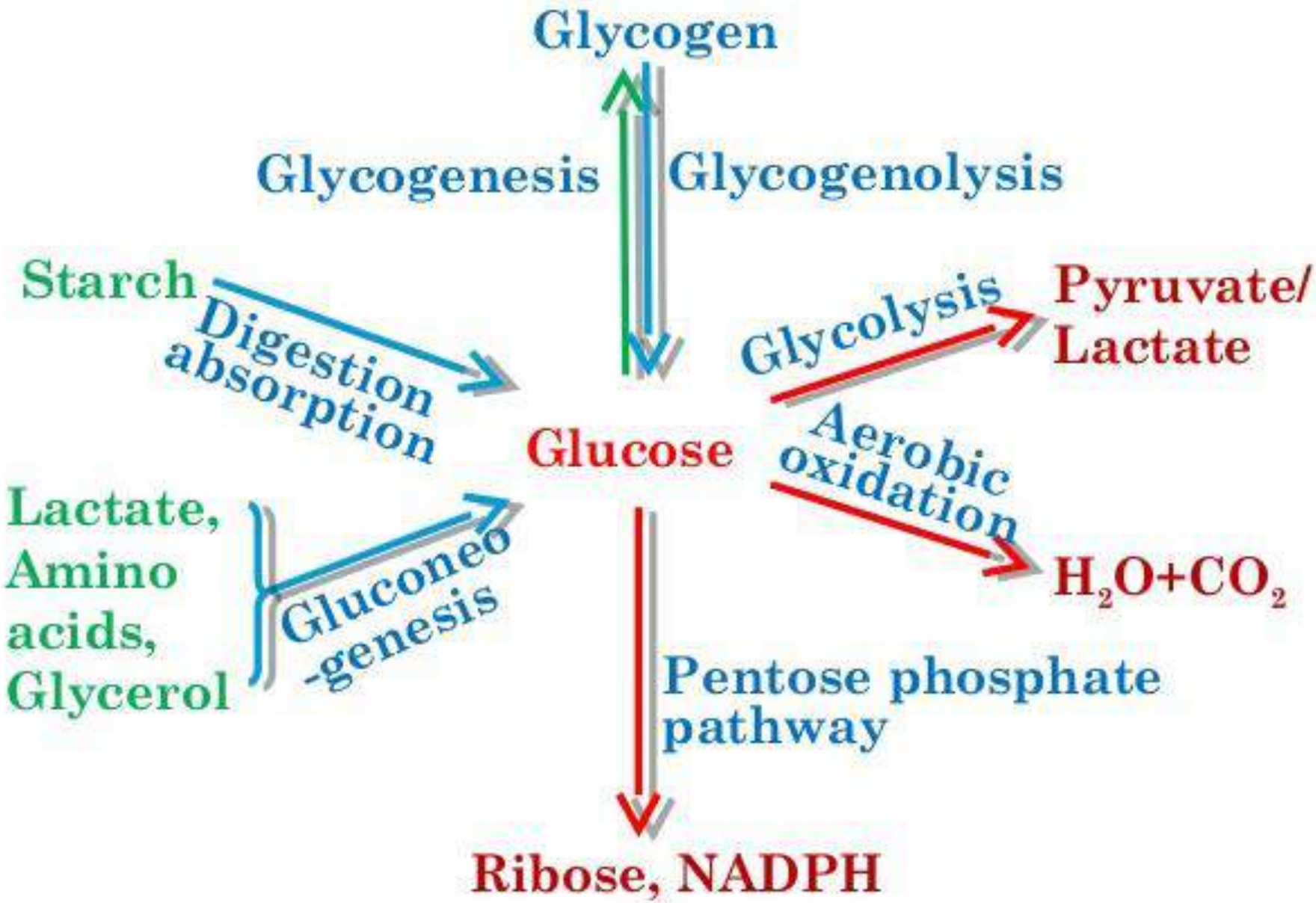
6) Hexose monophosphate shunt

7) Uronic Acid Pathway

8) Galactose Metabolism

9) Fructose Metabolism

10) Amino sugar metabolism


Glycogen
Glycogenesis
Glycogenolysis
Starch
Digestion
absorption
Glycolysis
Pyruvate/
Lactate
Glucose
Aerobic
oxidation
Lactate,
Amino
acids,
Glycerol
Gluconeo
-genesis
H2O+CO2
Pentose phosphate
pathway
Ribose, NADPH

GLYCOLYSIS

EMBDEN-MEYERHOF PATHWAY

(OR)

E.M.PATHWAY

Definition:

Glycolysis is defined as the sequence of reactions converting glucose (or glycogen) to pyruvate or lactate, with the production of ATP

Salient features:

1) Takes place in all cells of the body.
2) Enzymes present in "cytosomal fraction" of the cell.
3) Lactate – end product – anaerobic condition.
4) Pyruvate(finally oxidized to CO_2 & H_2O) – end product of aerobic condition.
5) Tissues lacking mitochondria – major pathway – ATP synthesis.
6) Very essential for brain – dependent on glucose for energy.
7) Central metabolic pathway
8) Reversal of glycolysis – results in gluconeogenesis.

Reactions of Glycolysis

1) Energy Investment phase (or) priming phase

2) Splitting phase

3) Energy generation phase

Energy Investment Phase

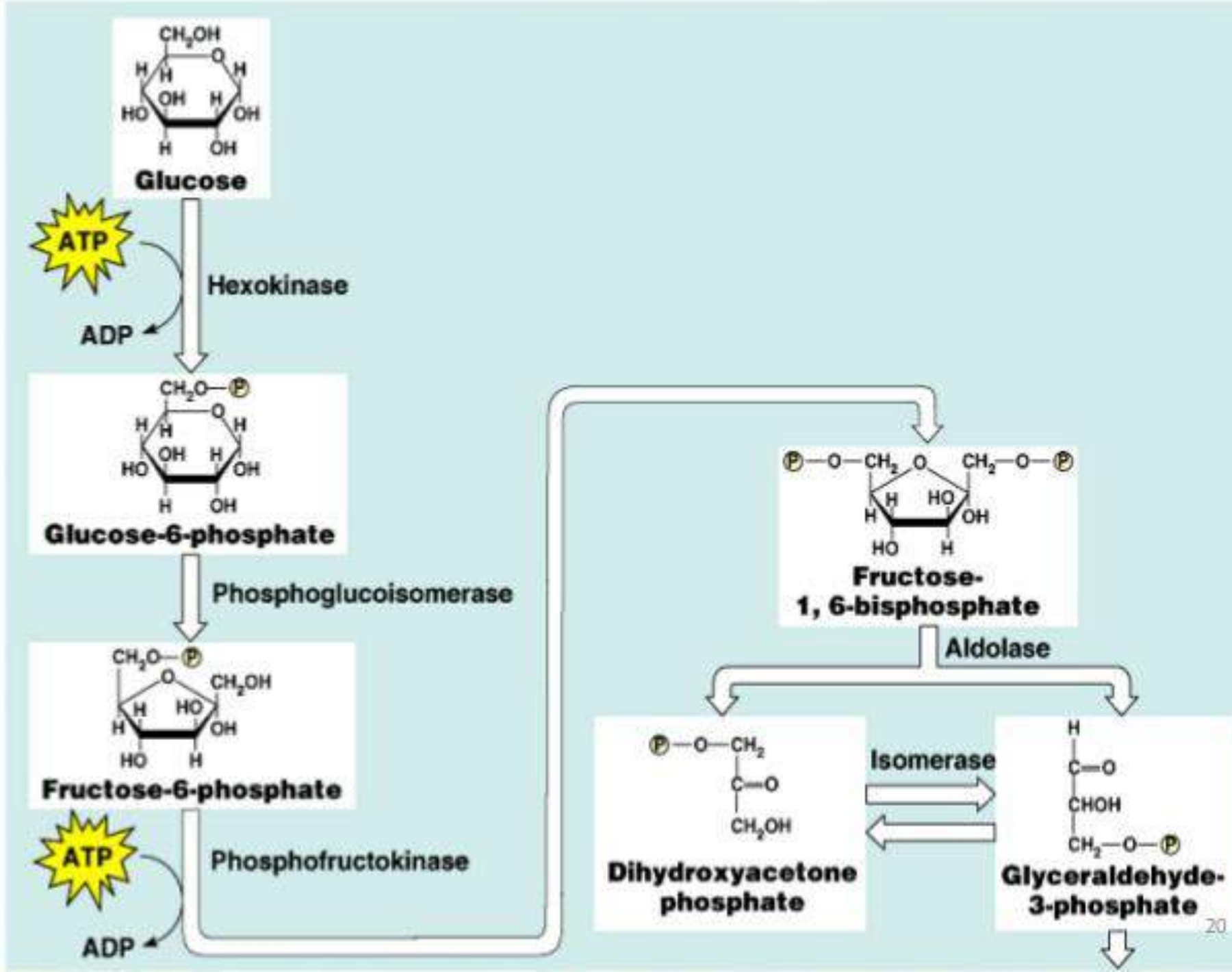
- **Glucose** is phosphorylated to **glucose-6-phosphate** by *hexokinase (or) glucokinase.*
- Glucose-6-phosphate undergoes isomerization to give **fructose -6- phosphate** in the presense of *phospho-hexose isomerase* and Mg^{2+}
- Fructose-6-phosphate is phoshorylated to **fructose 1,6-bisphosphate** by *phosphofructokinase.*

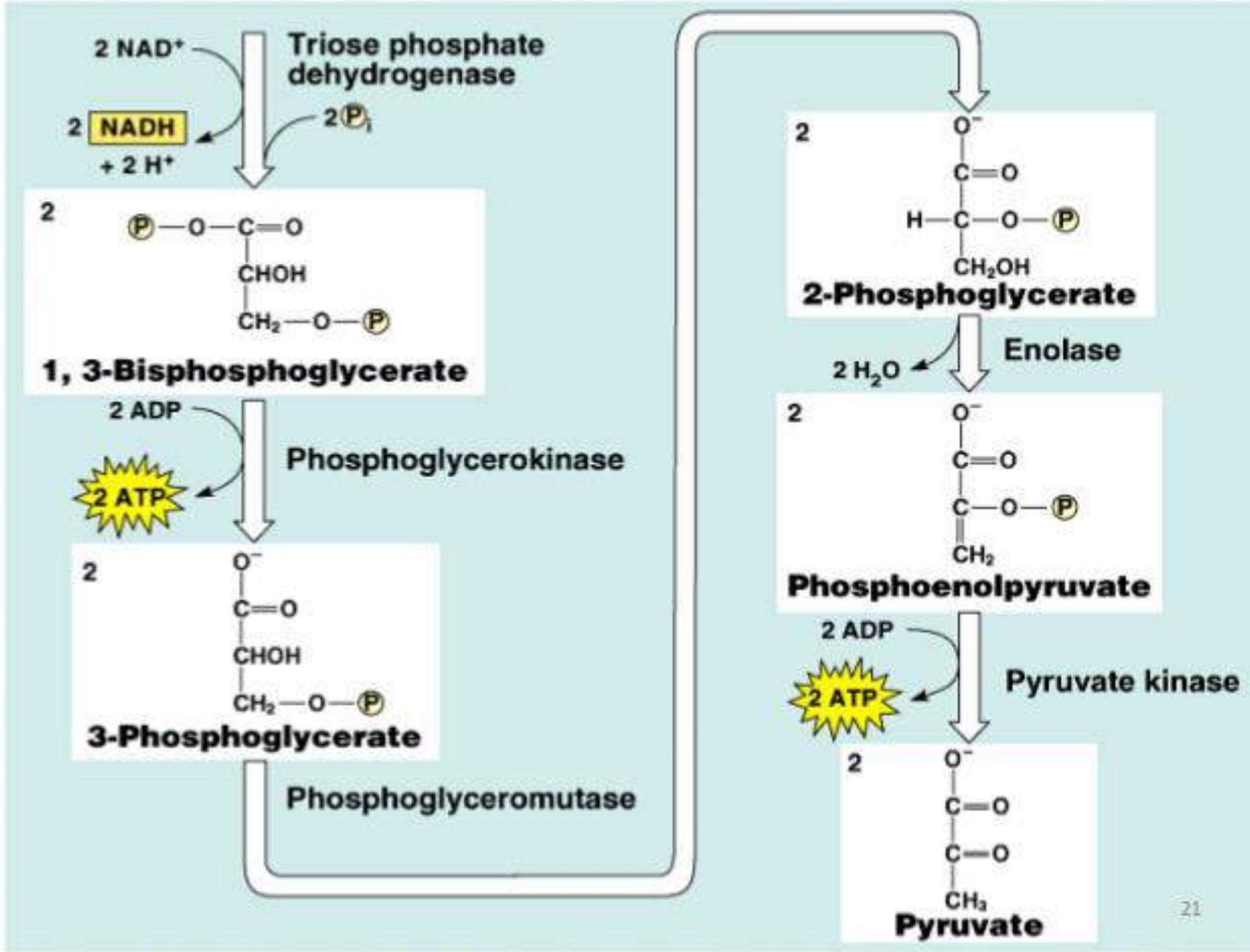
Splitting Phase

- Fructose 1,6-bisphosphate → **glyceraldehyde 3-phosphate + dihydroxyacetone phosphate.**(*aldolase enzyme*)
- **2 molecules** of glyceraldehyde 3-phosphate are obtained from 1 molecule of glucose

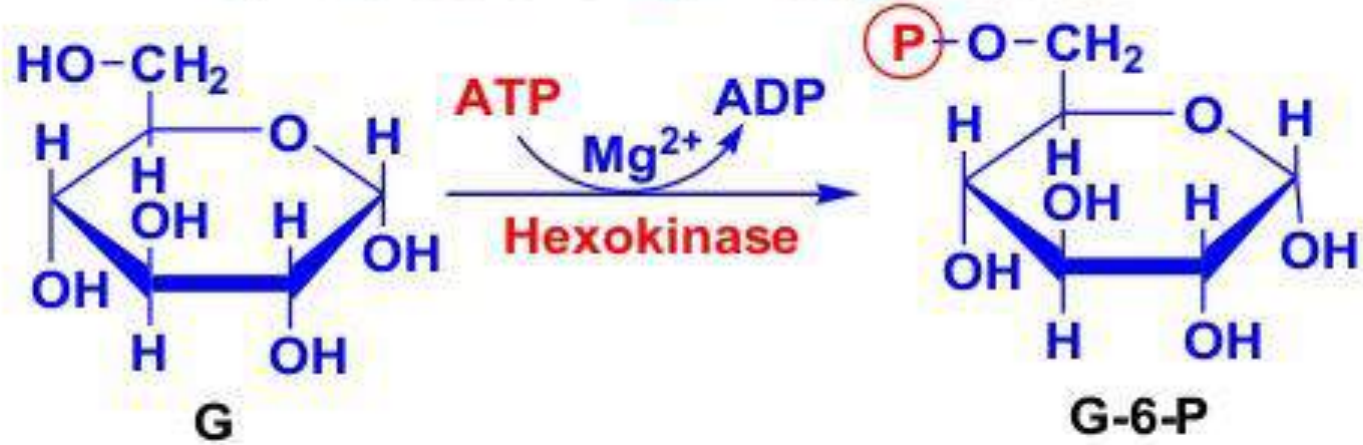
Energy Generation Phase

- Glyceraldehyde 3-phosphate → **1,3-bisphosphoglycerate**(*glyceraldehyde 3-phosphate hydrogenase*)
- 1,3-bisphosphoglycerate → **3-phosphoglycerate** (*phosphoglycerate kinase*)
- 3-phosphoglycerate → **2-phosphoglycerate** (*phosphoglycerate mutase*)
- 2-phosphoglycerate → **phosphoenol pyruvate** (*enolase* + Mg^{2+} & Mn^{2+})
- Phosphoenol pyruvate → **pyruvate [enol]** (*pyruvate kinase*) → **pyruvate [keto]** → **L-Lactate** *(lactate dehydrogenase)*


Glucose
ATP
ADP
Hexokinase
Glucose-6-phosphate
Phosphoglucoisomerase
Fructose-6-phosphate
ATP
ADP
Phosphofructokinase
Fructose-
1, 6-bisphosphate
Aldolase
Dihydroxyacetone
phosphate
Isomerase
Glyceraldehyde-
3-phosphate


2 NAD+
Triose phosphate dehydrogenase
2 NADH
+ 2 H+
2 Pi
2
1, 3-Bisphosphoglycerate
2 ADP
2 ATP
Phosphoglycerokinase
2
3-Phosphoglycerate
Phosphoglyceromutase
2
2-Phosphoglycerate
2 H2O
Enolase
2
Phosphoenolpyruvate
2 ADP
2 ATP
Pyruvate kinase
2
Pyruvate

(1) Glucose is phosphorylated to Glucose 6-phosphate

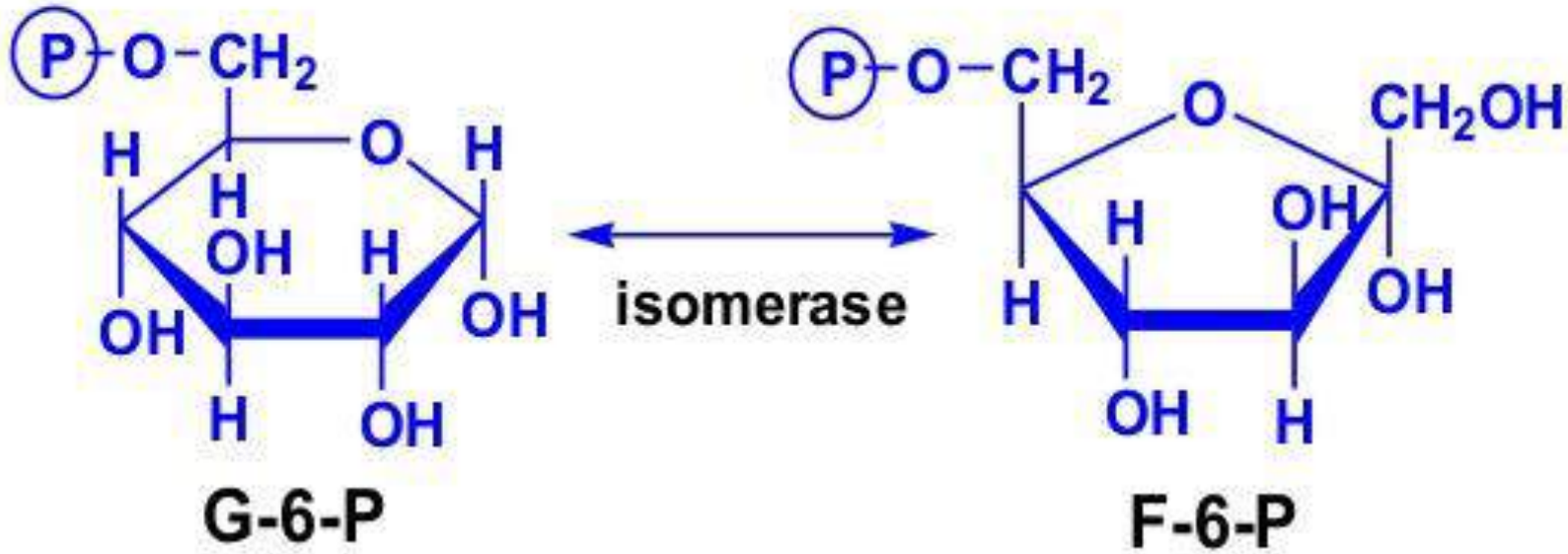


- Phosphorylated Glucose cannot get out from cell.
- Hexokinase (having 4 Isoenzymes).
- Glucokinase, GK in liver.
- Irreversible.

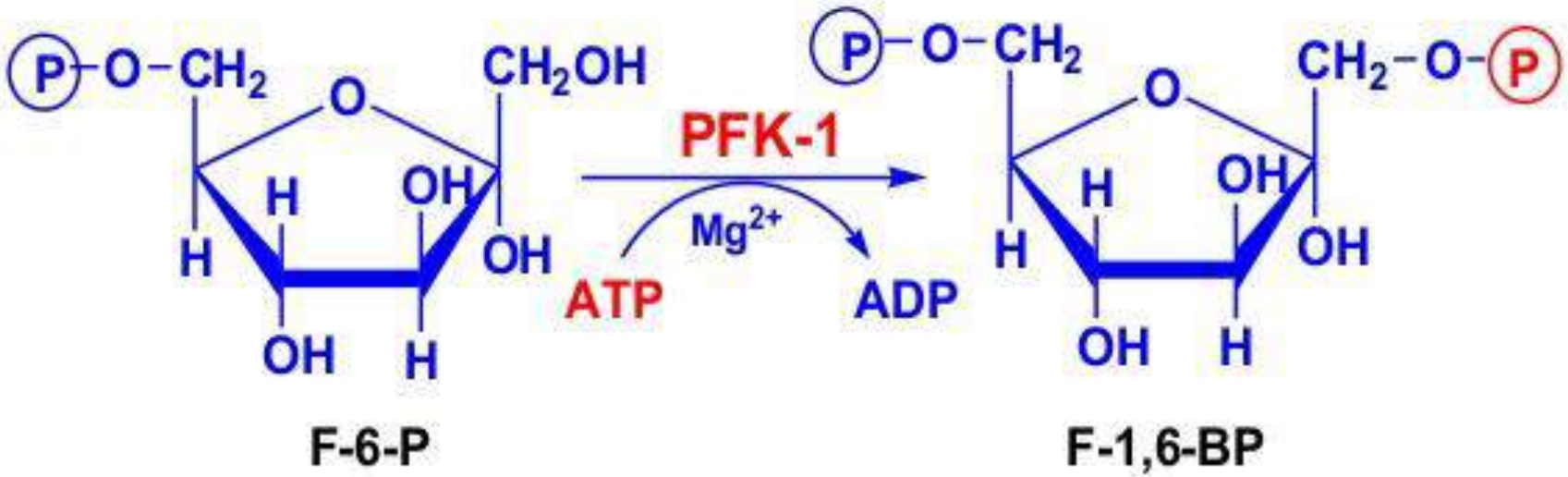
Comparison of Hexokinase and Glucokinase

Particulars	Hexokinase	Glucokinase
Occurrence	in all tissues	only in liver
K_m value	0.1mmol/L	10mmol/L
Substrate	Glucose, Fructose, Mannose	Glucose
Regulation	G-6-P	Insulin

(2) G-6-P is isomerised to Fructose 6-P

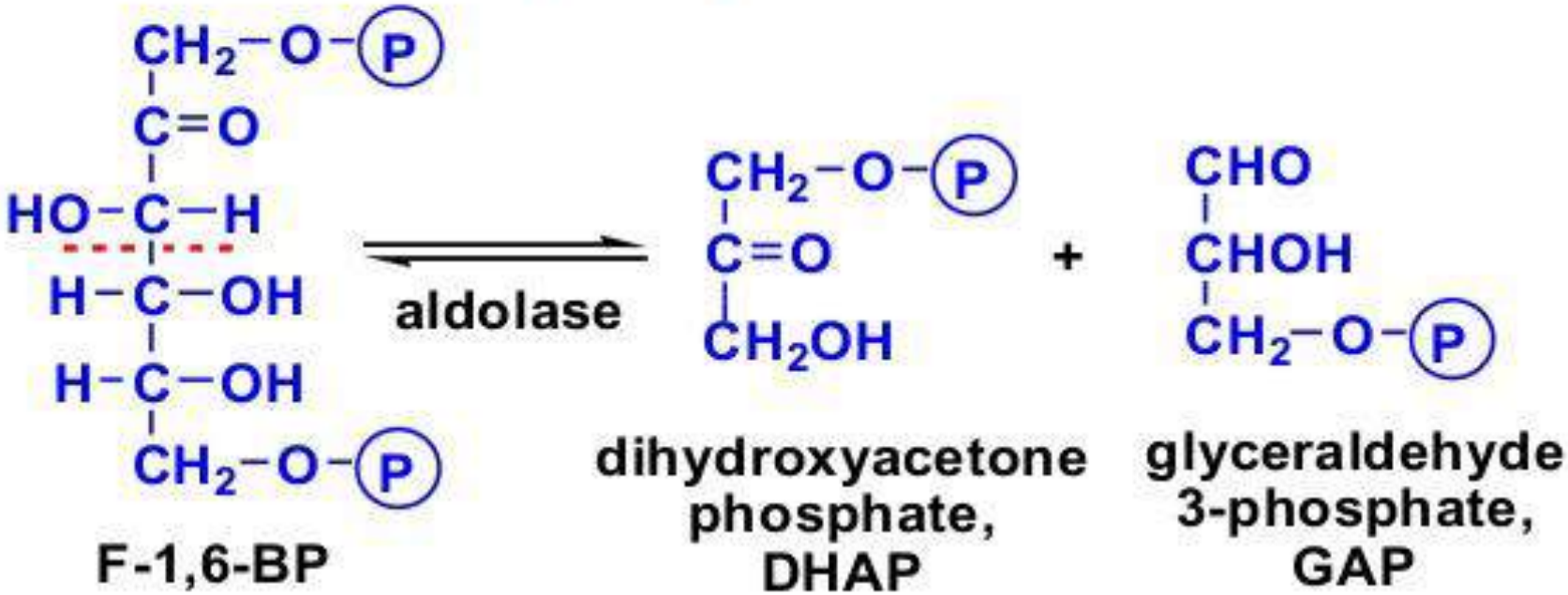


(3) F-6-P is phosphorylated Fructose 1,6-bisphosphate



- The second phosphorylation
- Phosphofructokinase-1, PFK-1

(4) F-1,6-BP cleaved to 2 Triose phosphates

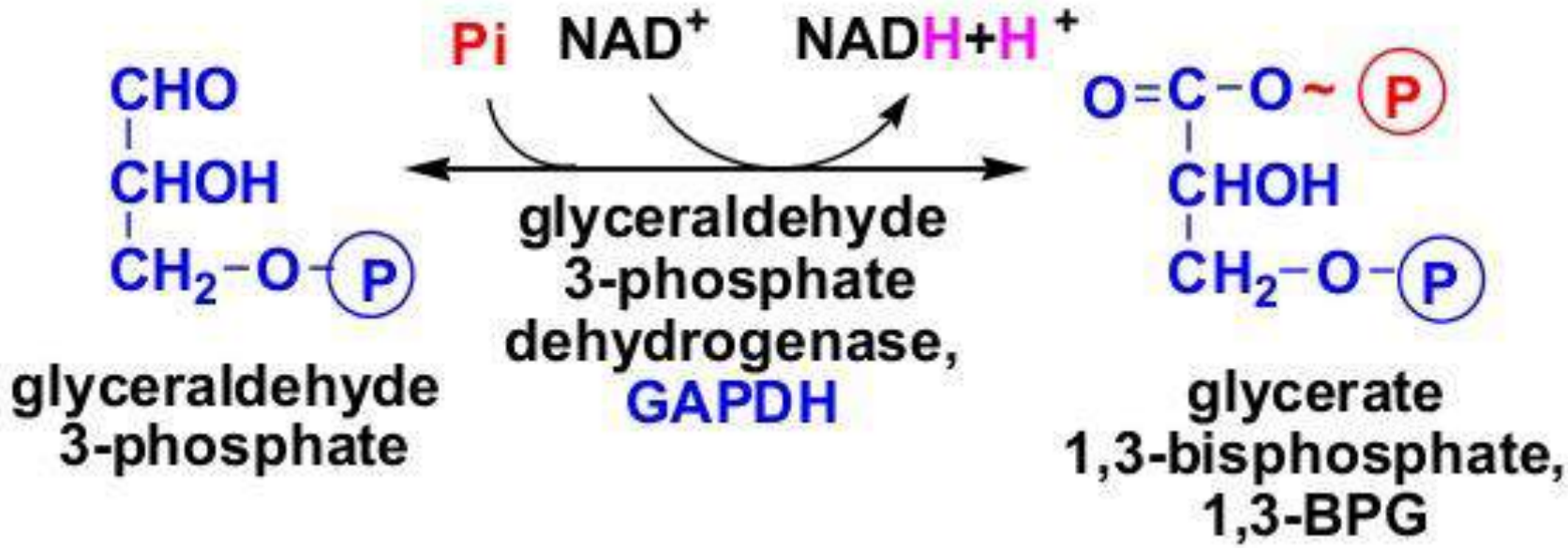


- Reversible

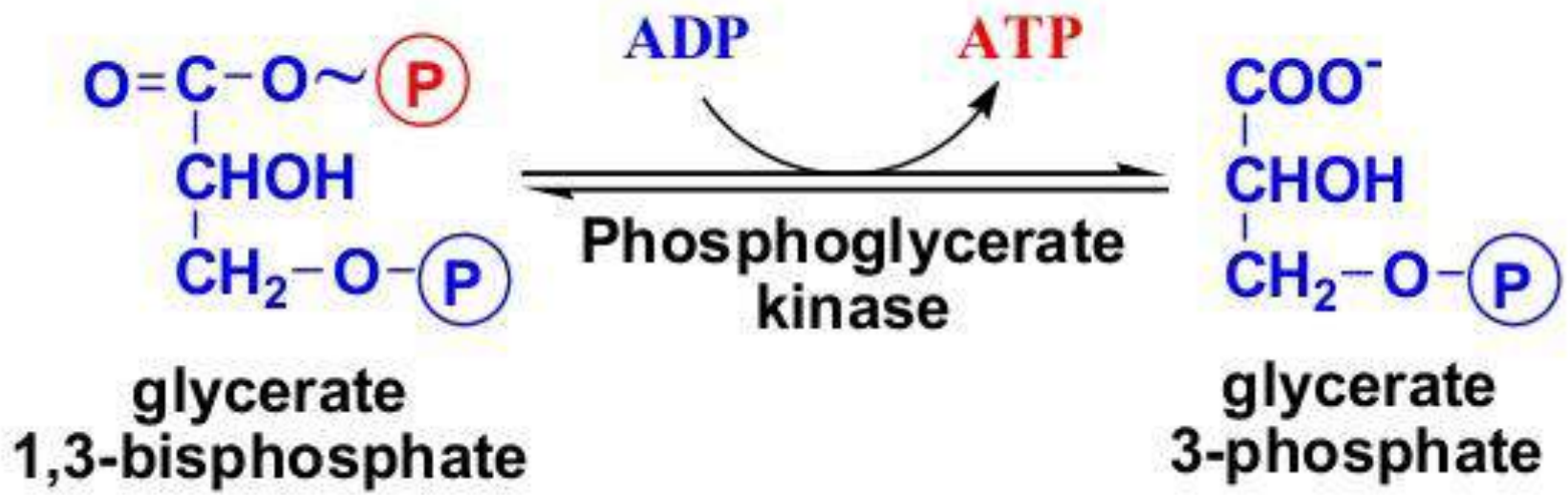
(5) Triose phosphate isomerization

G→2 molecule glyceraldehyde-3-phosphate, consume 2 ATP .

(6) Glyceraldehyde 3-phosphate oxidised 1,3-bisphospho glycerate

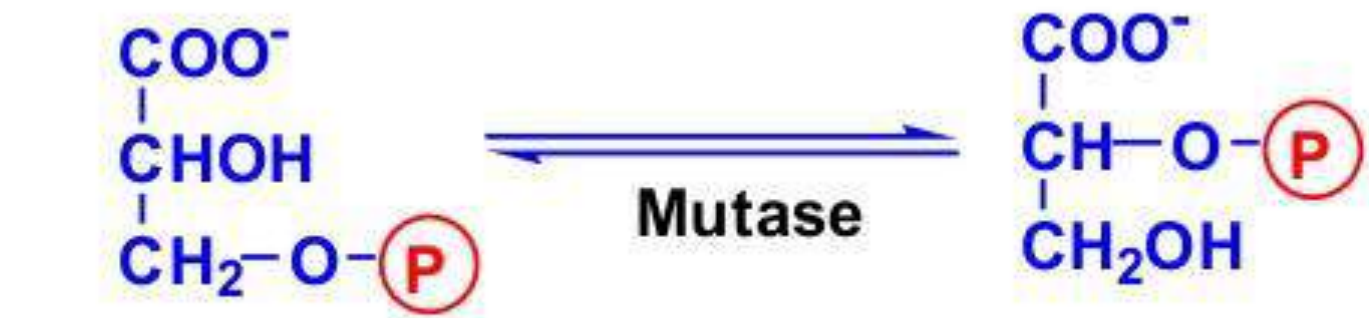


(7) 1,3-BPG dephosphorylated to 3-phospho glycerate



- Substrate level phosphorylation

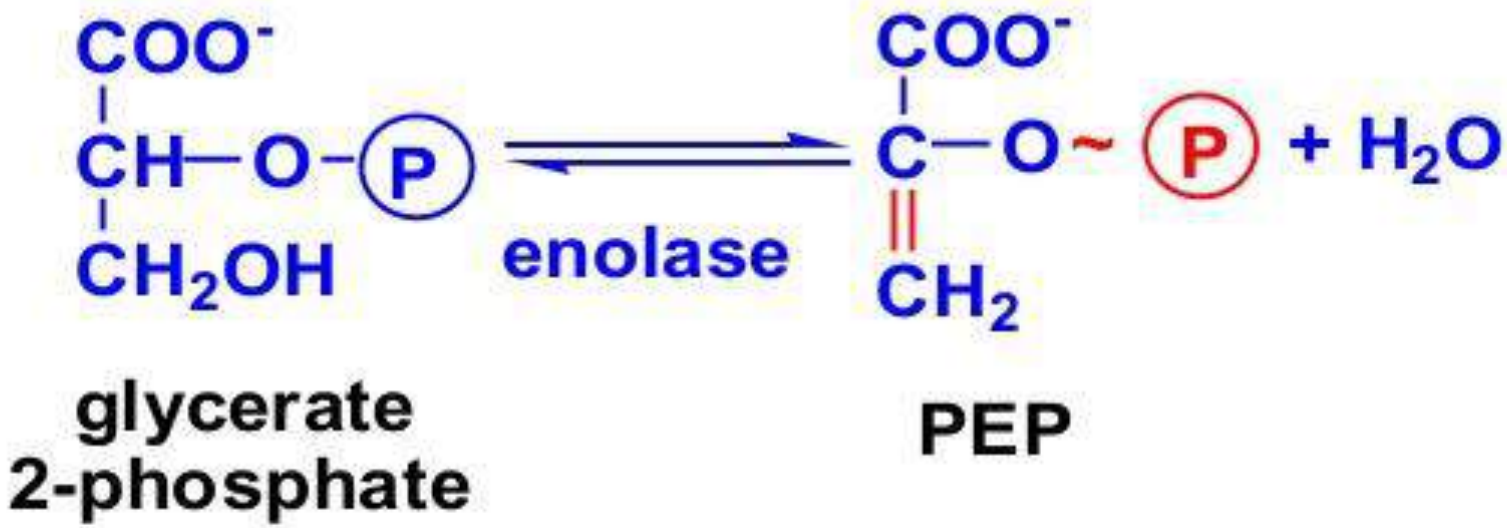
(8) Glycerate 3-phosphate mutated glycerate 2-phosphate



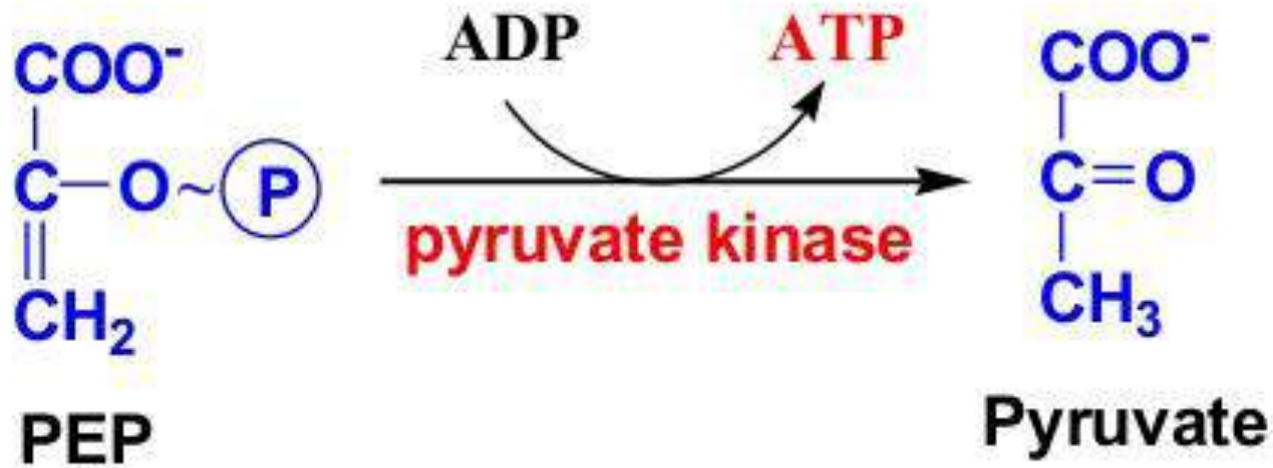
glycerate 3-phosphate

glycerate 2-phosphate

(9) Glycerate 2-phosphate → phosphoenol pyruvate

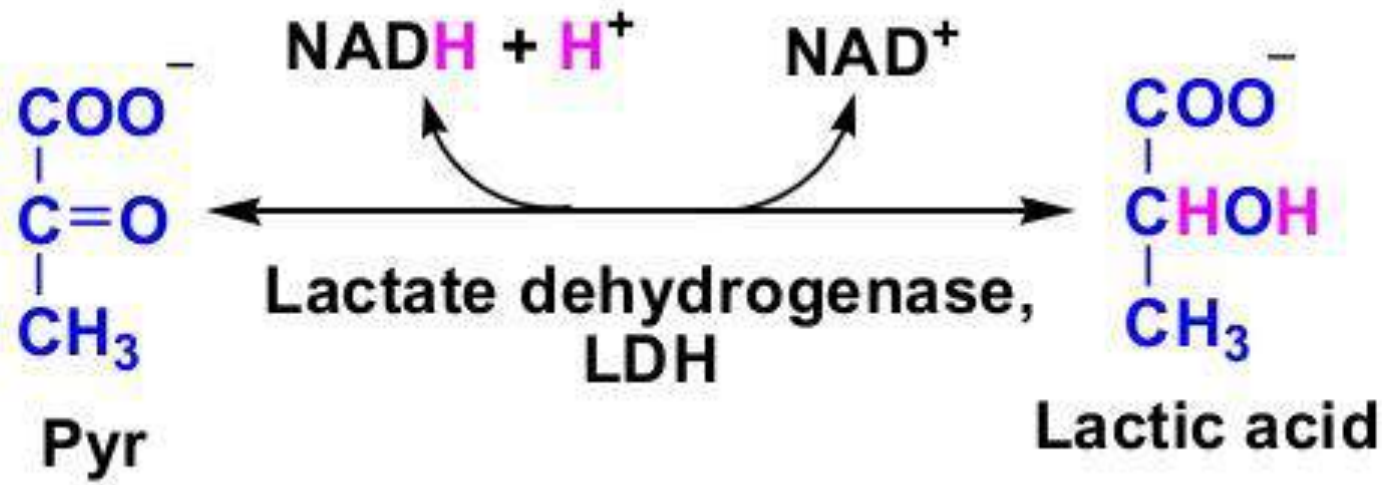


(10) PEP →pyruvate

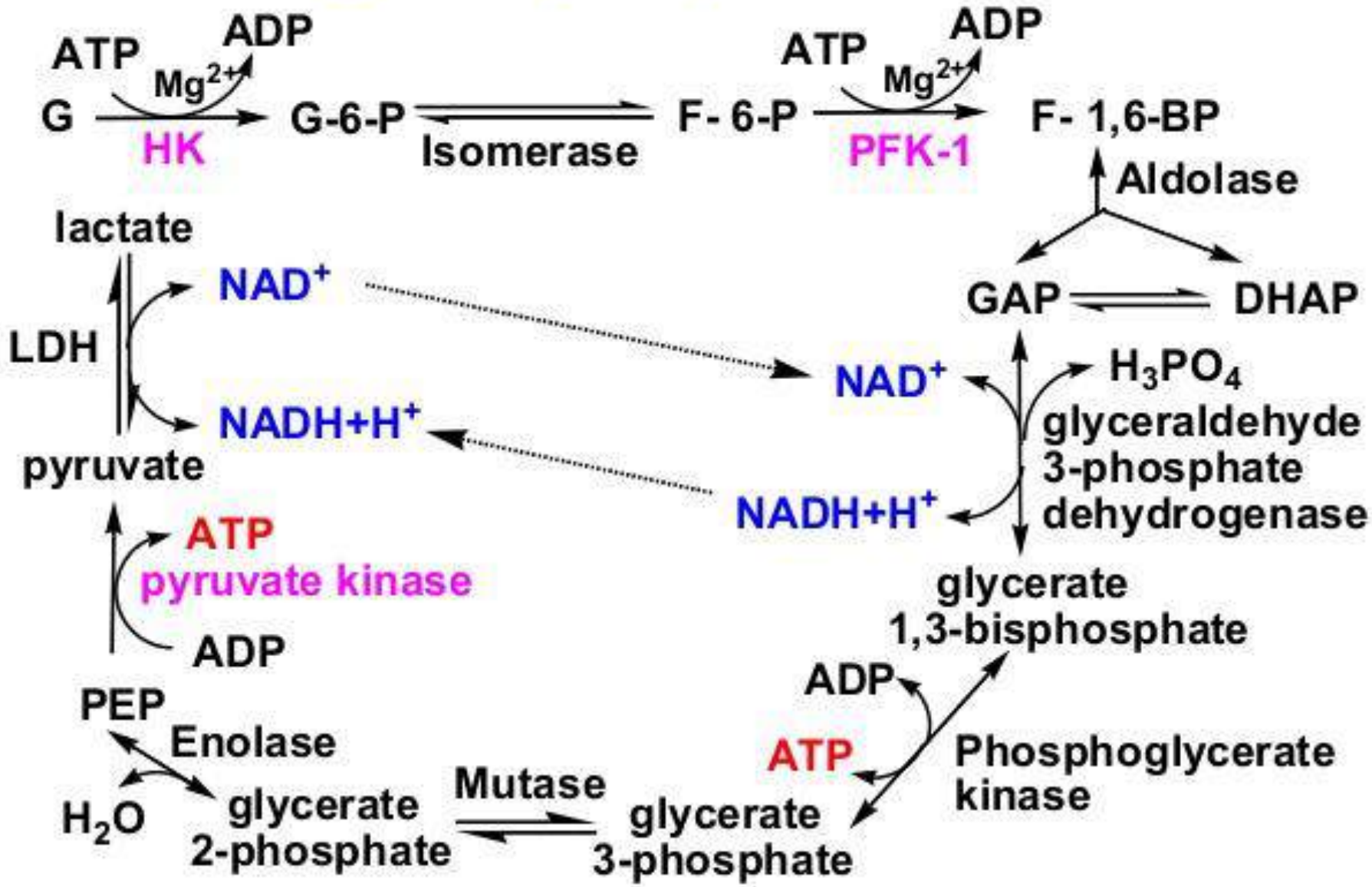


- Second substrate level phosphorylation
- irreversible

2) Pyruvate → lactate



Summary of Glycolysis



Energy production of glycolysis:

ATP production = ATP produced - ATP utilized

	ATP produced	ATP utilized	Net energy
In absence of oxygen (anaerobic glycolysis)	4 ATP (Substrate level phosphorylation) 2ATP from 1,3 DPG. 2ATP from phosphoenol pyruvate	2ATP From glucose to glucose - 6-p. From fructose -6-p to fructose 1,6 p.	2 ATP
In presence of oxygen (aerobic glycolysis)	4 ATP (substrate level phosphorylation) 2ATP from 1,3 BPG. 2ATP from phosphoenol pyruvate. + 4ATP or 6ATP (from oxidation of 2 NADH + H in mitochondria).	2ATP -From glucose to glucose - 6-p. From fructose -6-p to fructose 1,6 p.	8 ATP / 6 ATP (Pyruvate dehydrogenase 2NADH,ETC, Oxidative phosphorylation)

2. Regulation of Glycolysis

- Three key enzymes catalyze irreversible reactions : Hexokinase, Phosphofructokinase & Pyruvate Kinase.

1) PFK-1

The reaction catalyzed by PFK-1 is usually the rate-limiting step of the Glycolysis pathway.

This enzyme is regulated by covalent modification, allosteric regulation.

2) Pyruvate kinase

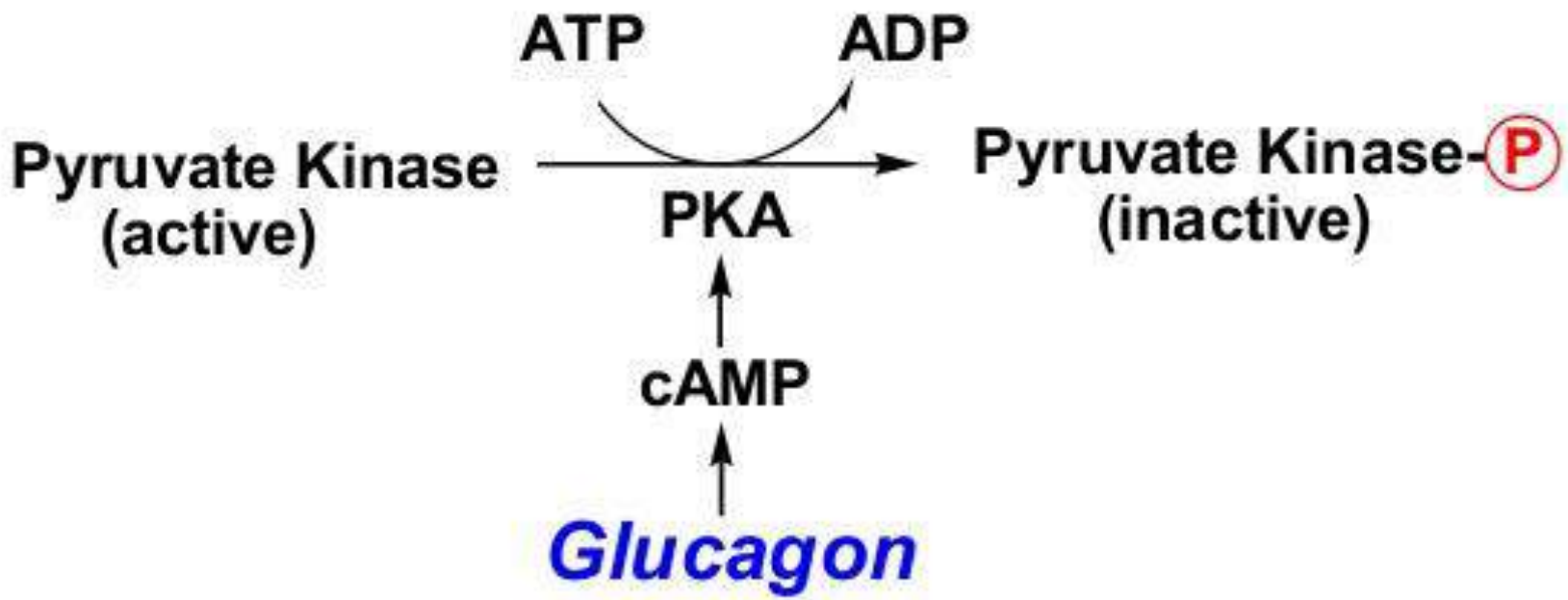
- **Allosteric regulation:**

F-1,6-BP acts as allosteric activator ;

ATP and Ala in liver act as allosteric inhibitors;

- **Covalent modification:**

phosphorylated by Glucagon through cAMP and PKA and inhibited.



3) Hexokinase and glucokinase

- This enzyme is regulated by covalent modification, allosteric regulation and isoenzyme regulation.
- Inhibited by its product G-6-P.
- Insulin induces synthesis of glucokinase.

3. Significance of glycolysis

1) Glycolysis is the emergency energy-yielding pathway.

2) Glycolysis is the main way to produce ATP in some tissues, even though the oxygen supply is sufficient, such as red blood cells, retina, testis, skin, medulla of kidney.

- In glycolysis, 1mol G produces 2mol lactic acid and 2mol ATP.

- The process of complete oxidation of glucose to CO_2 and water with liberation of energy as the form of ATP is named **aerobic oxidation**.
- The main pathway of G oxidation.

CLINICAL ASPECT

1) Lactic acidosis

- Normal value – 4 to 15 mg/dl.
- Mild forms – strenous exercise, shock, respiratory diseases, cancers
- Severe forms – Impairment/collapse of circulatory system – myocardial infarction, pulmonary embolism, uncontrolled hemmorrhage and severe shock.

2) Cancer and glycolysis :

- Cancer cells – increased uptake of glucose and glycolysis.

- Blood vessels unable to supply adequate oxygen – HYPOXIC condition – Anaerobic glycolysis / hypoxic glycolysis – Involvement of Hypoxic inducible transcription factor (HIF).

- Treatment : Use drugs that inhibit vascularization of tumours

- ✓ Pasteur effect : Inhibition of glycolysis by oxygen (Phosphofructokinase) .

- ✓ Crabtree effect : The phenomenon of inhibition of oxygen consumption by the addition of glucose to tissues having high aerobic glycolysis.

CITRIC ACID CYCLE

KREBS CYCLE / TRICARBOXYLIC ACID/ TCA CYCLE

Essentially involves the oxidation of acetyl CoA to CO_2 and H_2O.
This Cycle utilizes about two-third of total oxygen consumed by the body.

Brief History:

- Hans Adolf Krebs
- 1937
- Studies of oxygen consumptiom in pigeon breast muscle.

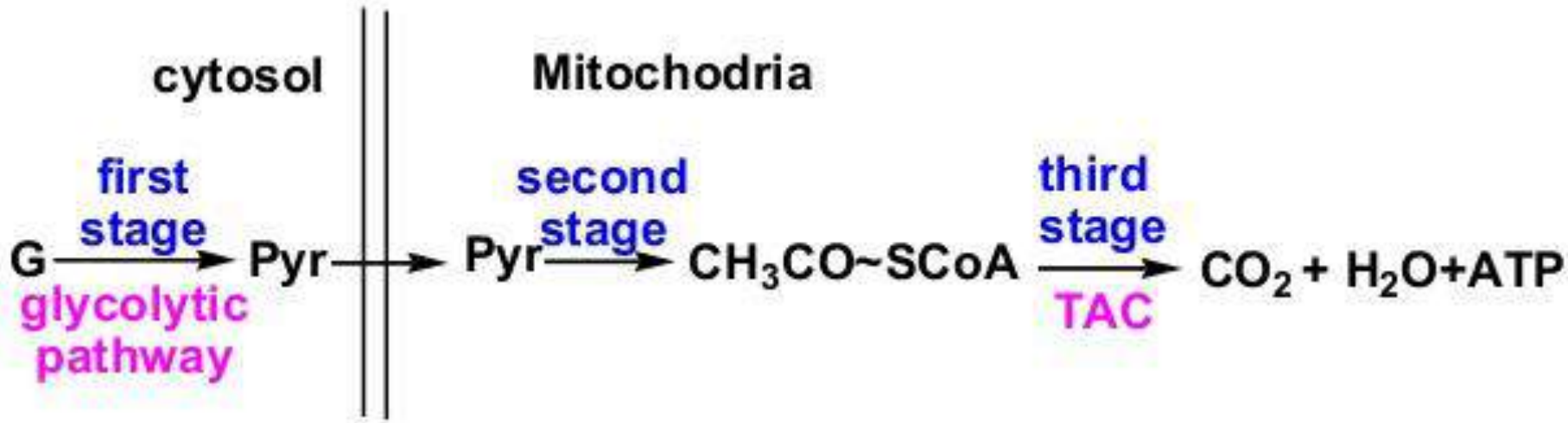
Location of TCA

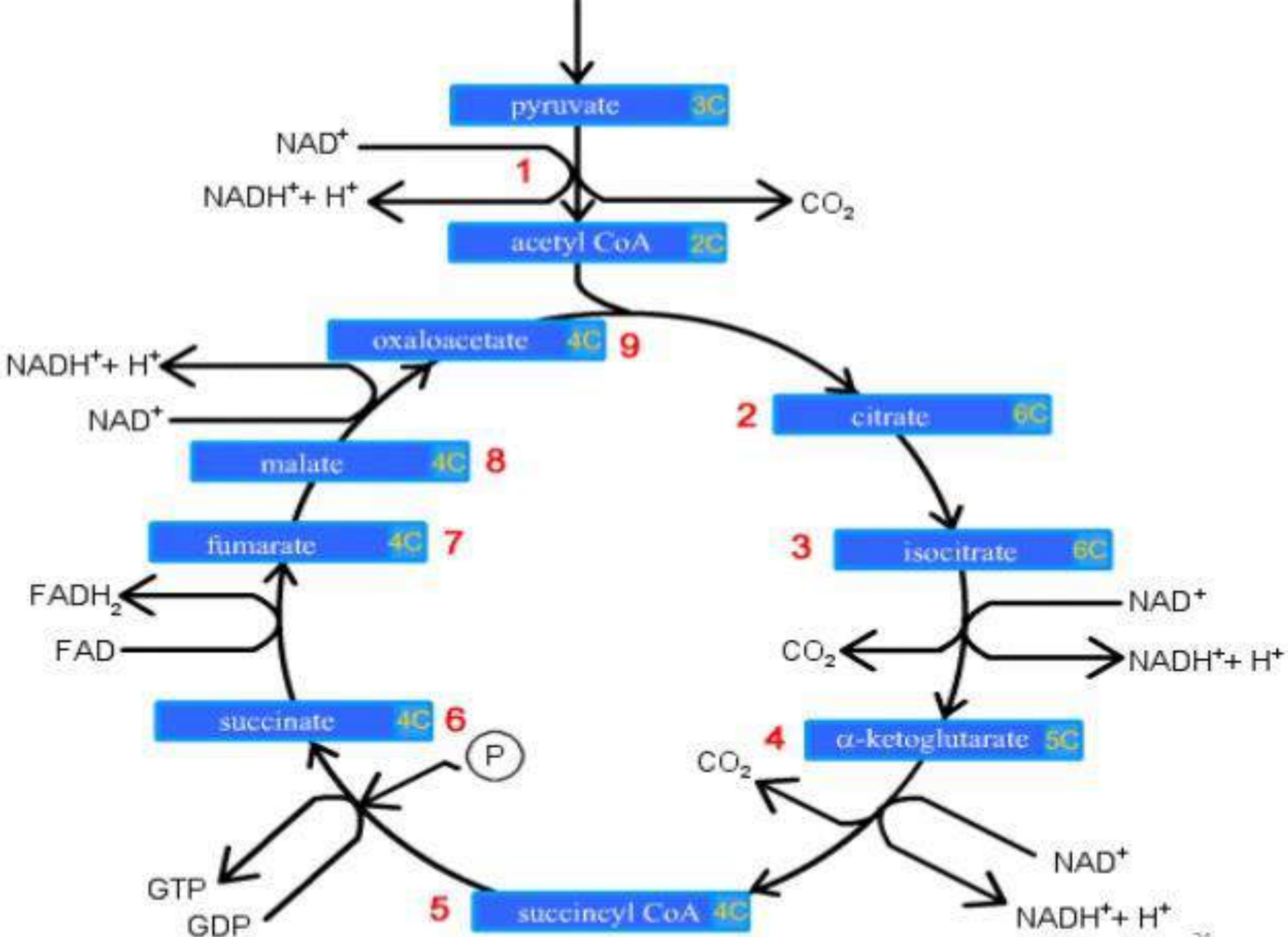
- Mitochondrial matrix
- In close proximity to the electronic transport chain.

Overview

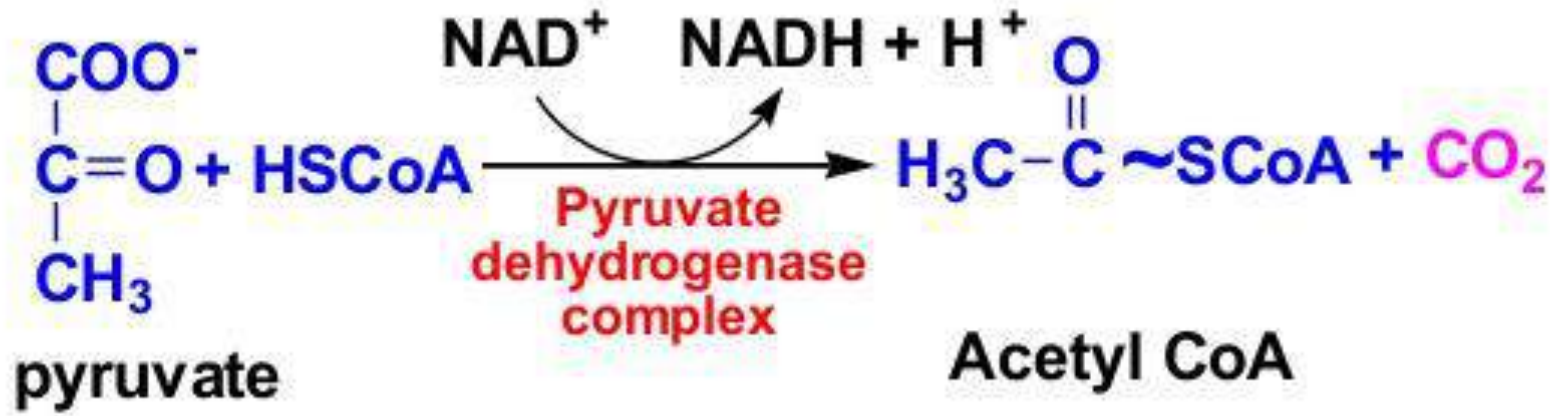
- 65-70% of the ATP is synthesized
- Name : TCA used because at the ouset of the cycle tricarboxylic acids participate.

1. Process of aerobic oxidation




pyruvate 3C
NAD+
1
NADH+ H+
CO2
acetyl CoA 2C
oxaloacetate 4C
9
NADH+ H+
NAD+
2
citrate 6C
malate 4C
8
fumarate 4C
7
3
isocitrate 6C
FADH2
FAD
NAD+
CO2
NADH+ H+
succinate 4C
6
P
4
α-ketoglutarate 5C
CO2
GTP
GDP
5
succinyl CoA 4C
NAD+
NADH+ H+

1) Oxidative decarboxylation of Pyruvate to Acetyl CoA



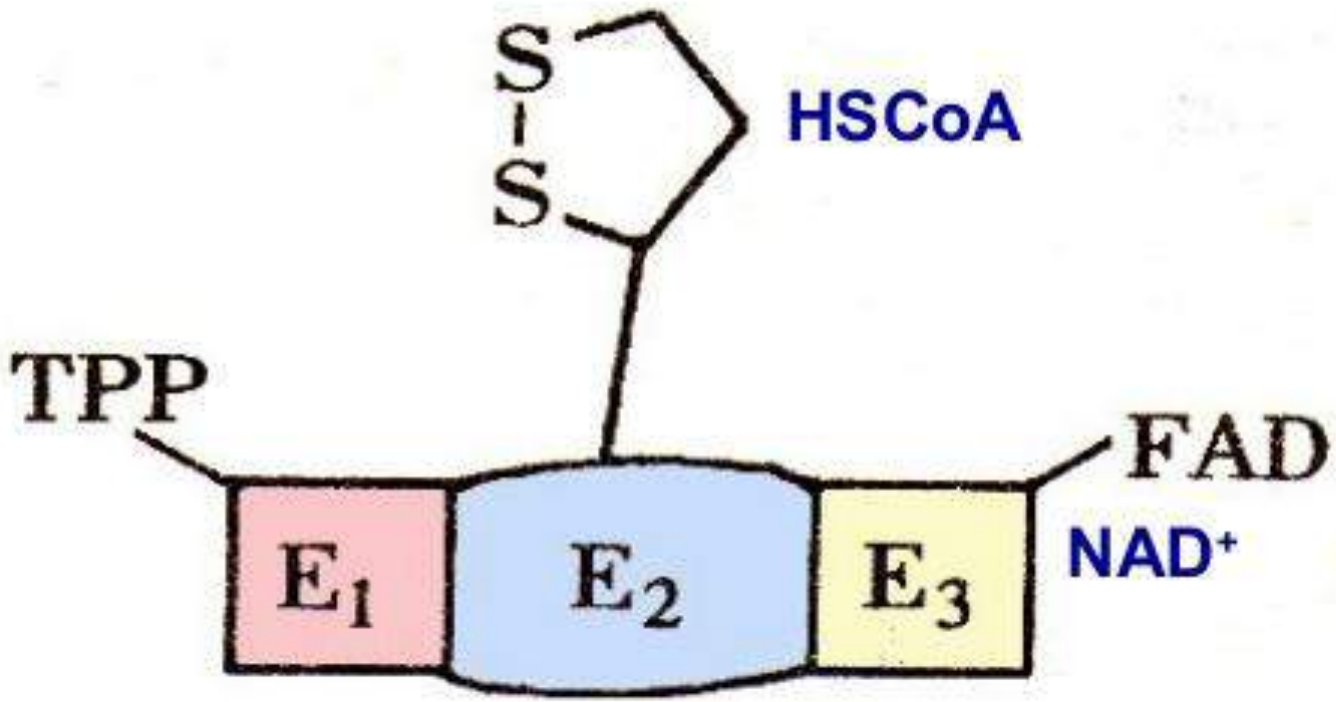
- irreversible;
- in mitochodria.

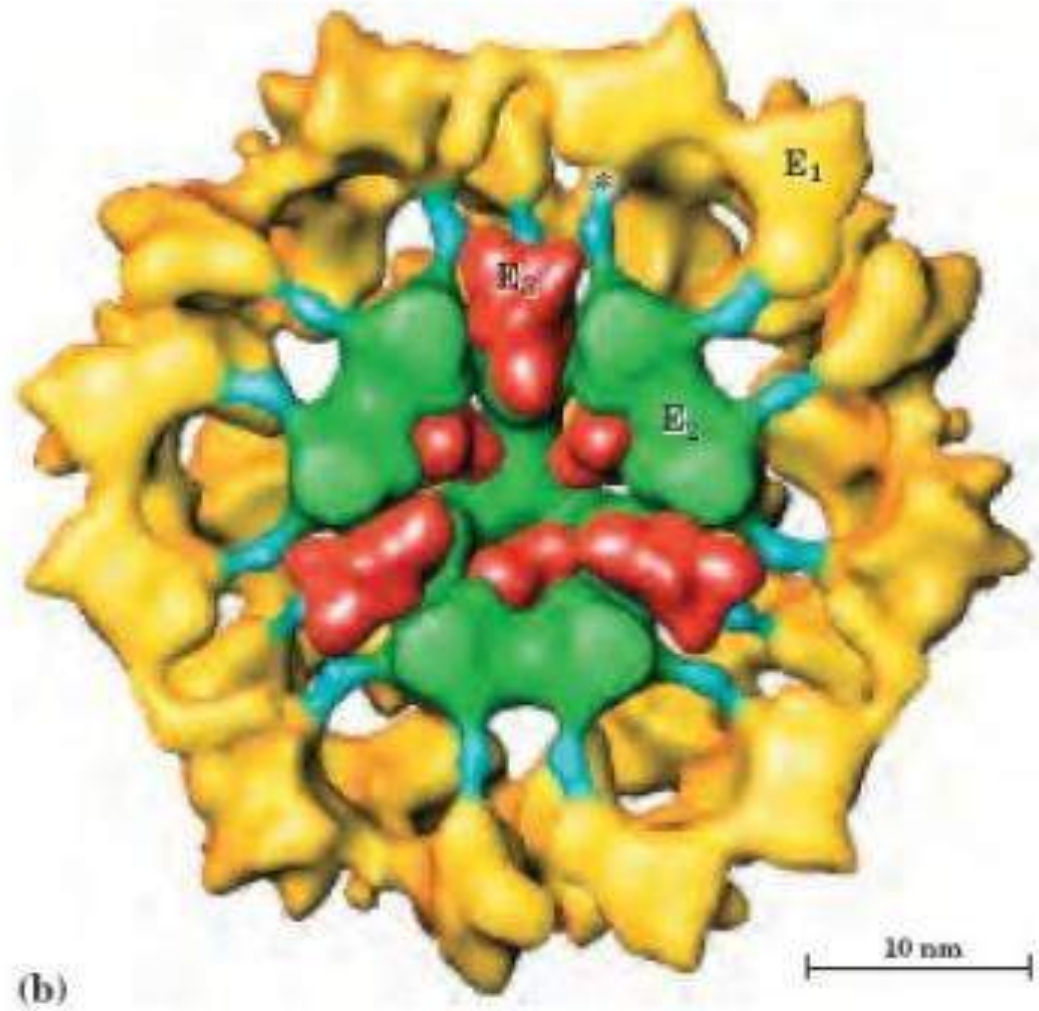
Pyruvate dehydrogenase complex:

Es
- E_1 pyruvate dehydrogenase
- E_2 dihydrolipoyl transacetylase
- E_3 dihydrolipoyl dehydrogenase

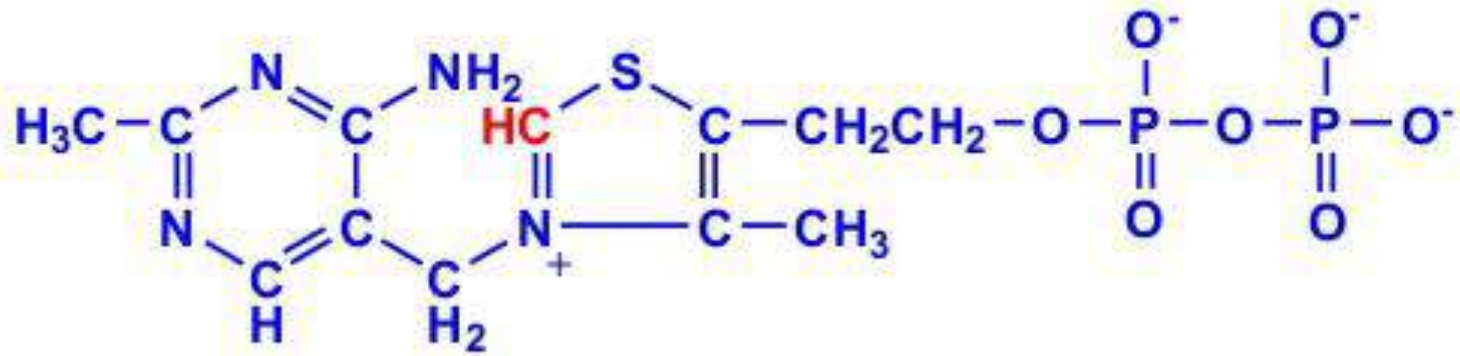
cofactors
- thiamine pyrophosphate, TPP (VB_1)
- HSCoA (pantothenic acid)
- lipoic Acid
- NAD^+ (Vpp)
- FAD (VB_2)

Pyruvate dehydrogenase complex:

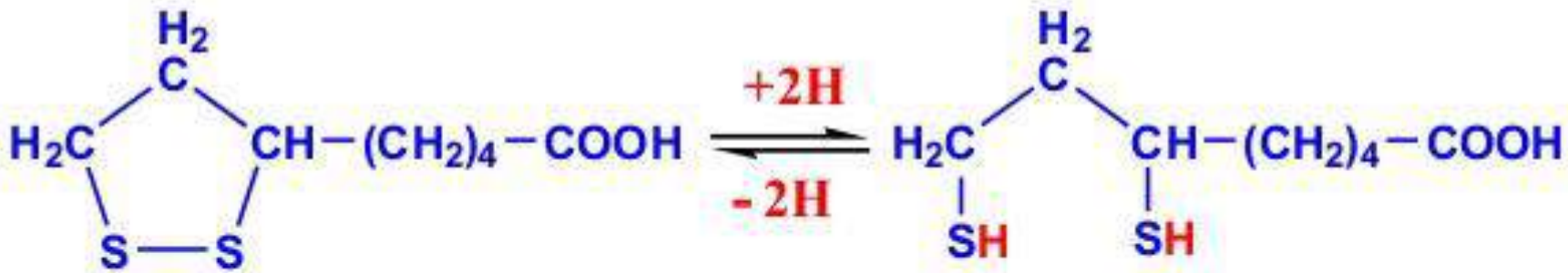




The structure of pyruvate dehydrogenase complex

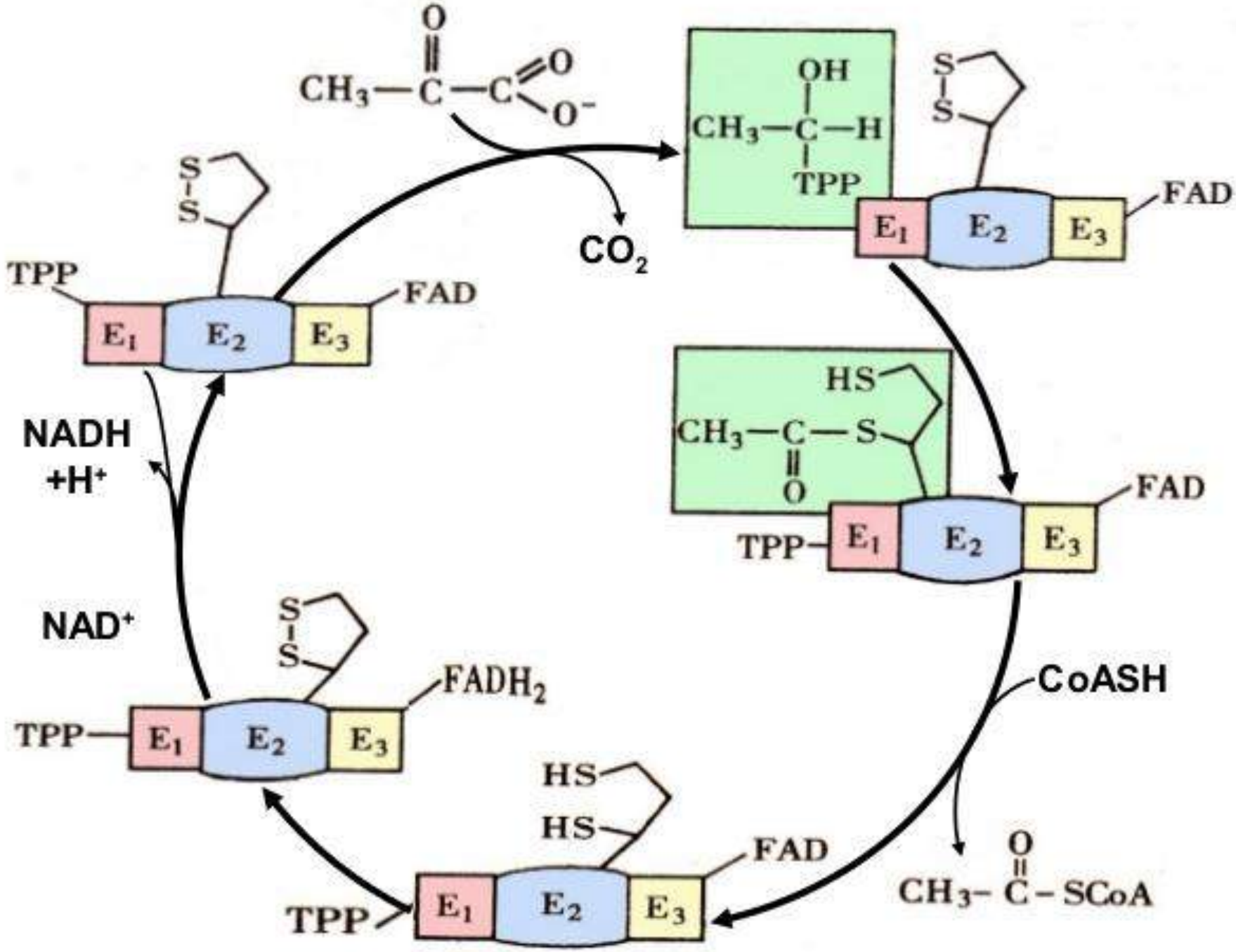


TPP



lipoic acid

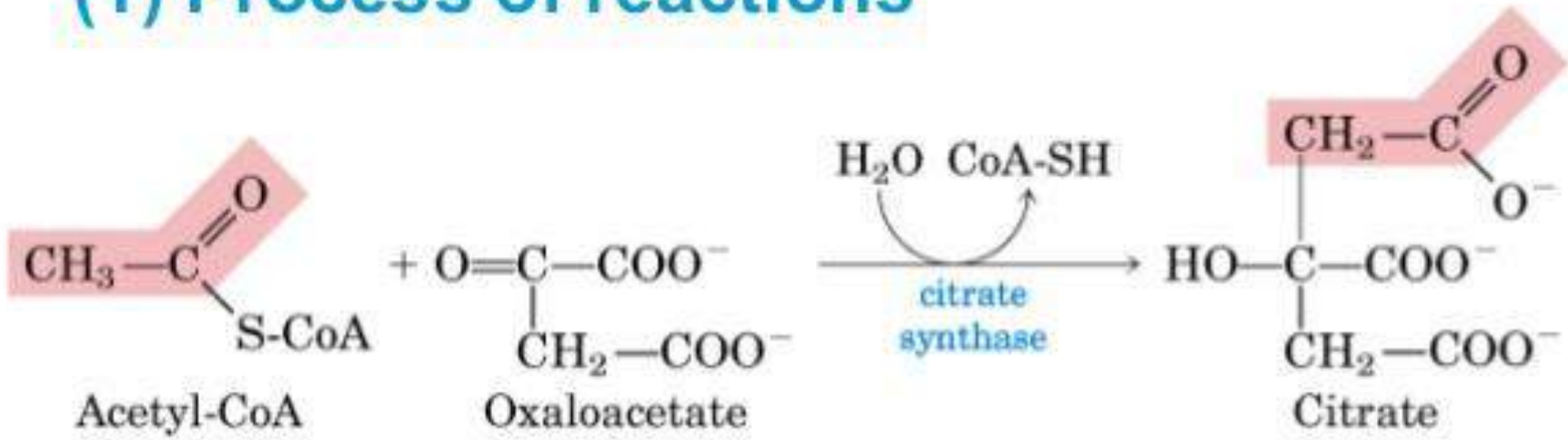
dihydrolipoic acid

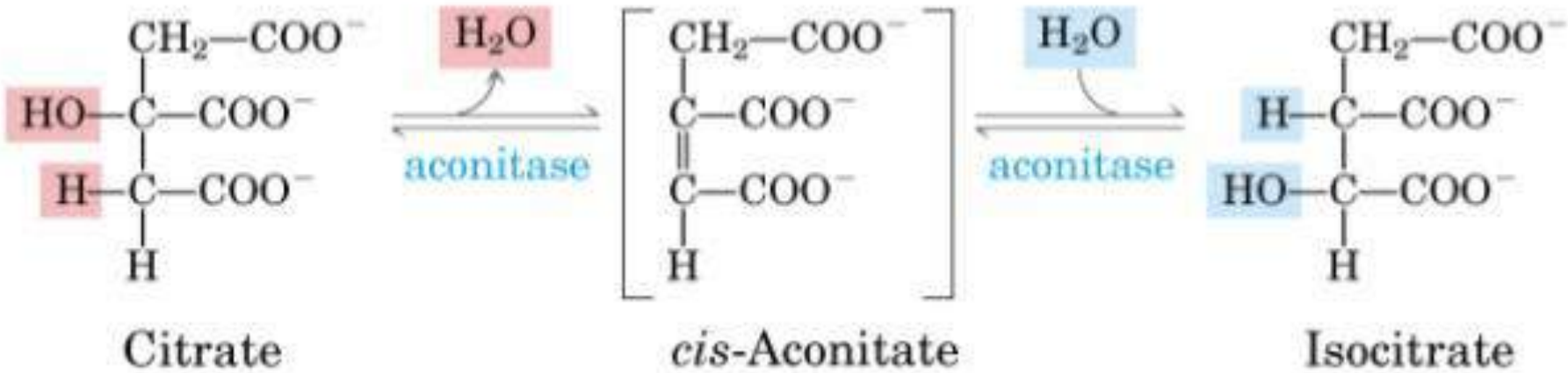

CH3—C—C(=O)—O⁻
CO2
OH
CH3—C—H
TPP
S
S
FAD
E1
E2
E3
HS
CH3—C—S
O
TPP
CoASH
CH3– C – SCoA
NADH
+H+
NAD+
FADH2
HS
HS

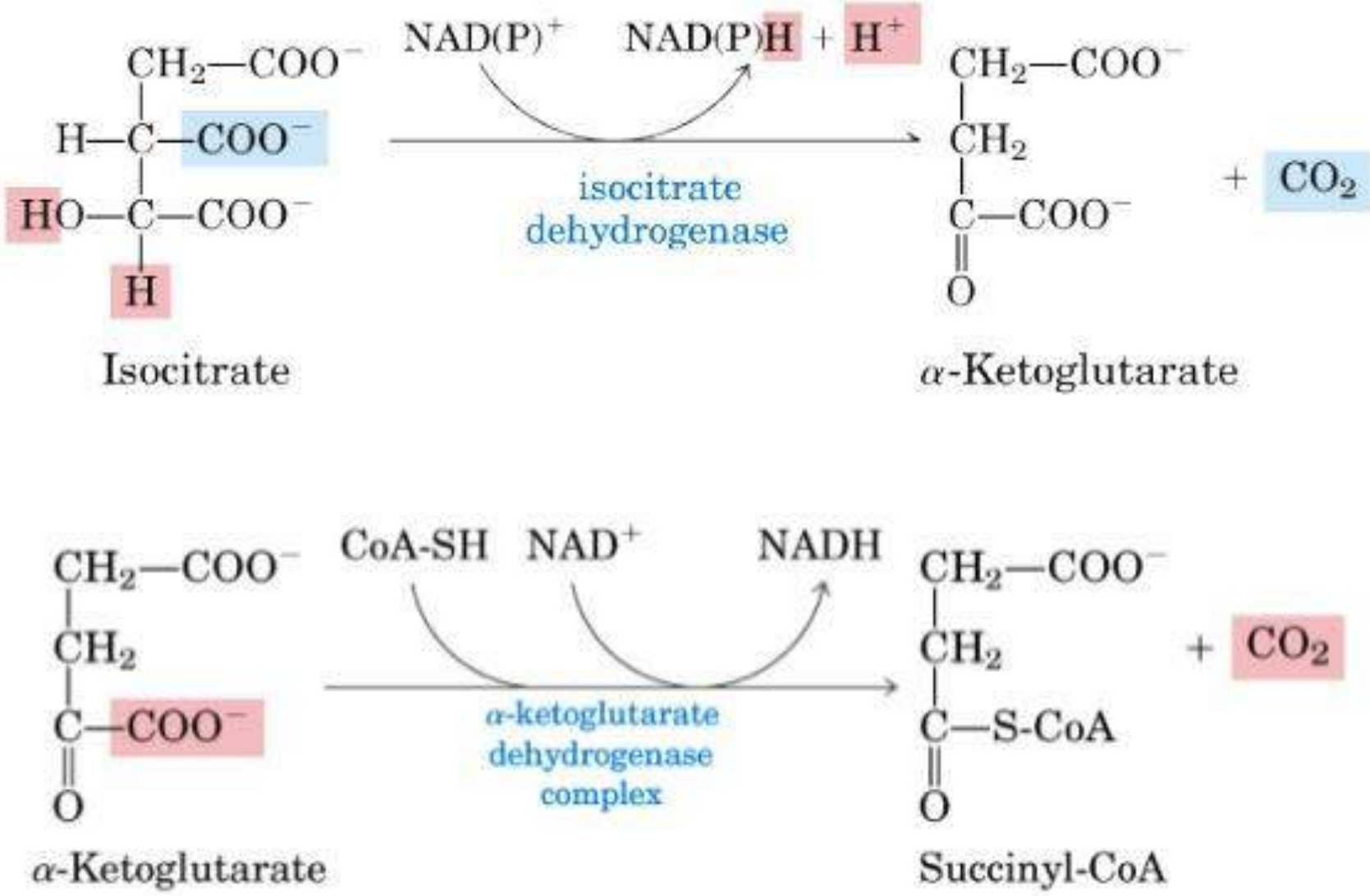
2) Tricarboxylic acid cycle, TCAC

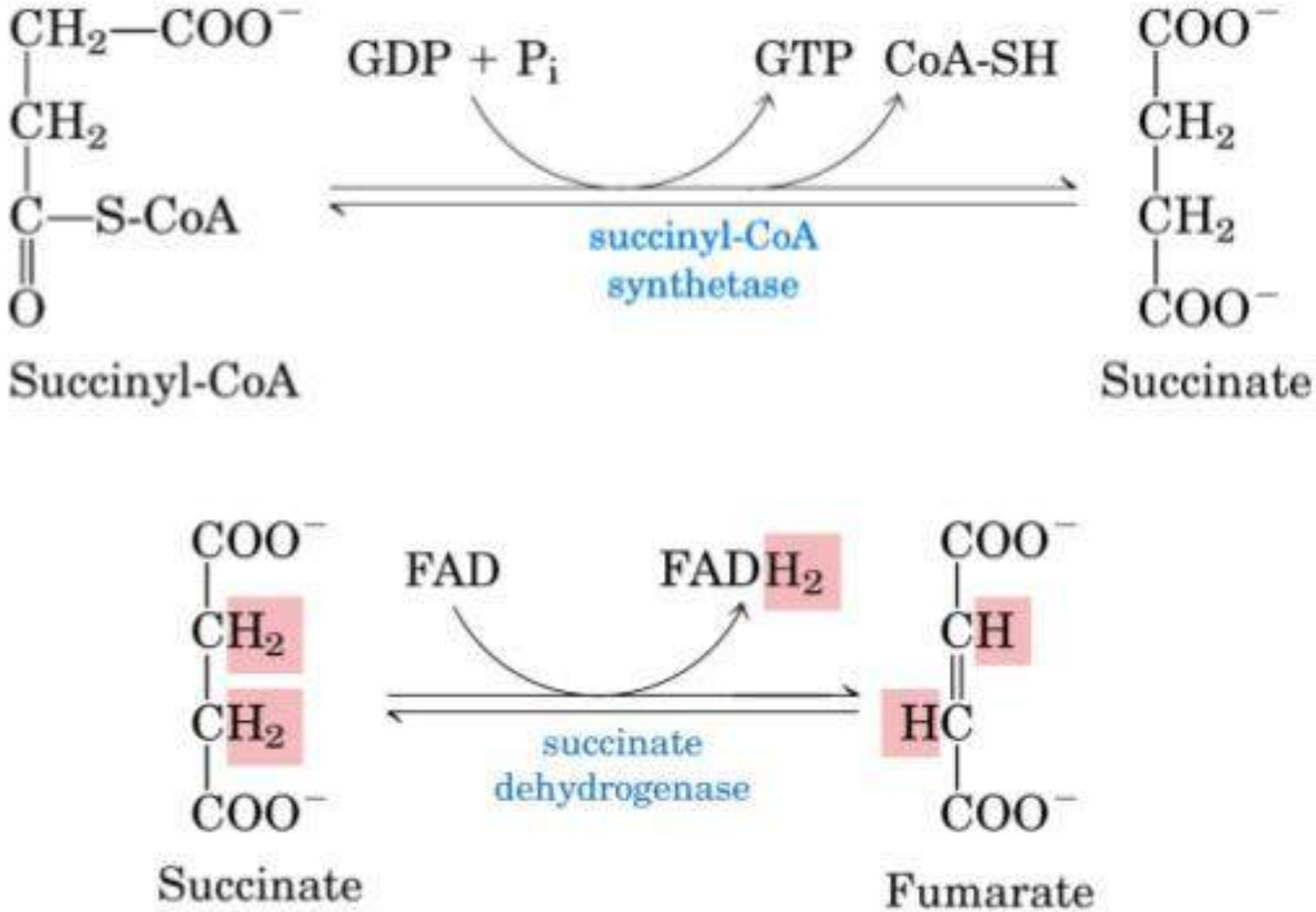
- The cycle comprises the combination of a molecule of acetyl-CoA with oxaloacetate, resulting in the formation of a six-carbon tricarboxylic acid, citrate. There follows a series of reactions in the course of which two molecules of CO_2 are released and oxaloacetate is regenerated.
- Also called citrate cycle or Krebs cycle.

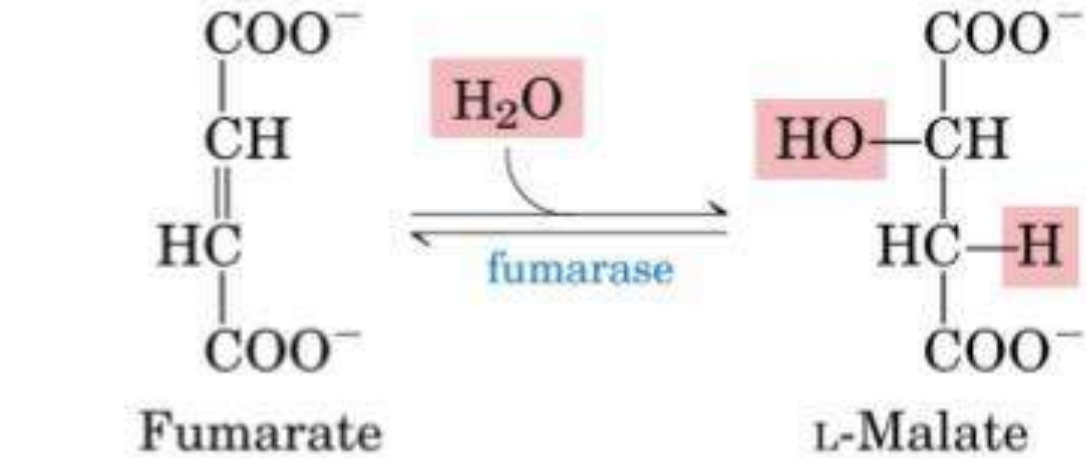
(1) Process of reactions



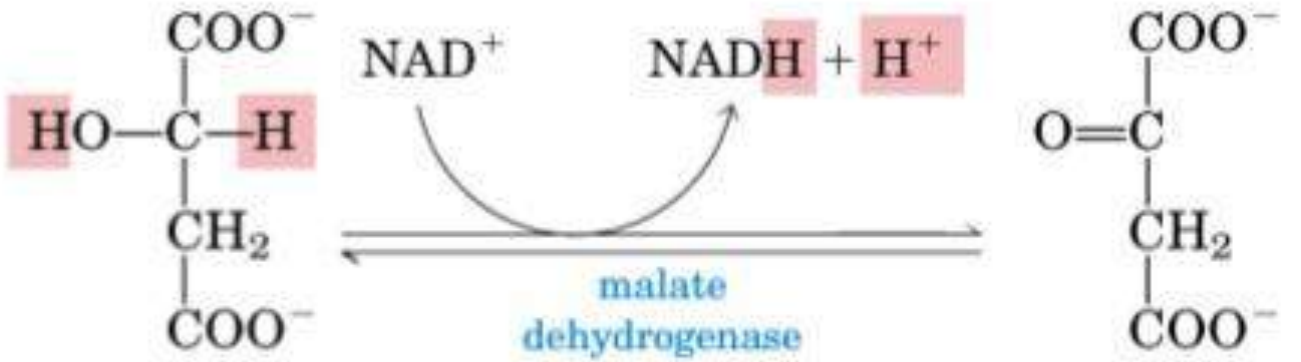



NAD(P)+
NAD(P)H + H+
isocitrate dehydrogenase
+ CO2
Isocitrate
α-Ketoglutarate
CoA-SH
NAD+
NADH
α-ketoglutarate dehydrogenase complex
+ CO2
α-Ketoglutarate
Succinyl-CoA


CH2—COO⁻
CH2
C—S-CoA
O
GDP + Pi
GTP
CoA-SH
succinyl-CoA synthetase
COO⁻
CH2
CH2
COO⁻
Succinyl-CoA
Succinate
COO⁻
CH2
CH2
COO⁻
FAD
FADH2
succinate dehydrogenase
COO⁻
CH
HC
COO⁻
Succinate
Fumarate

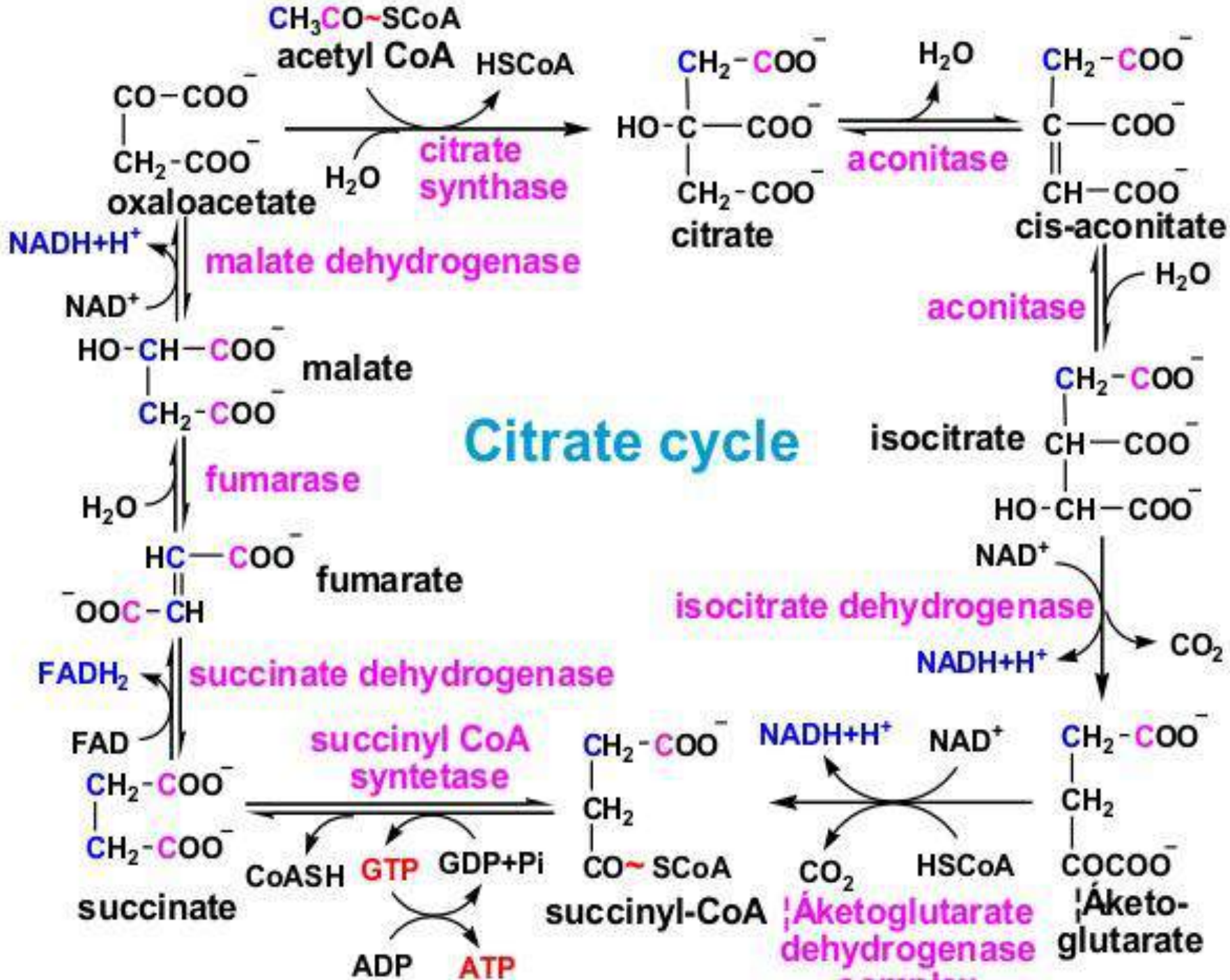


Fumarate L-Malate



L-Malate Oxaloacetate

Citrate cycle



Reactions of citric acid cycle

1) **Formation of citrate** : Condensation of acetyl CoA and oxaloacetate → catalysed by citrate synthase.

2) & 3) **Citrate is isomerized to isocitrate** → aconitase (two steps).

4) & 5) **Formation of ḍ-ketoglutarate** : enzyme isocitrate dehydrogenase.

6) **Conversion of ḍ-ketoglutarate to succinyl CoA** : through oxidative decarboxylation, catalysed by ḍ-ketoglutarate dehydrogenase complex.

7)**Formation of succinate** : enzyme succinate thiokinase

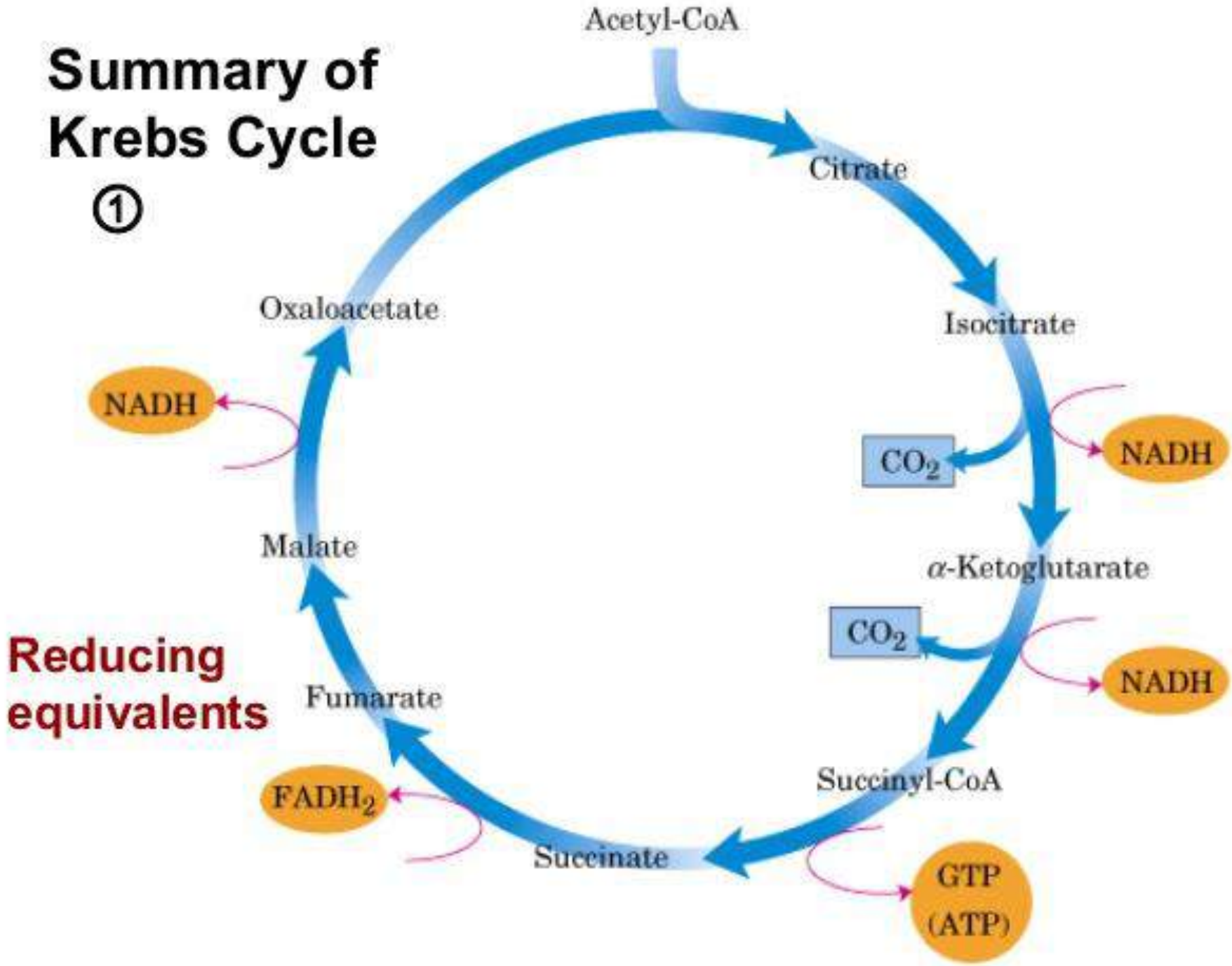
GTP + ADP ←→ ATP + GDP (nucleoside diphosphate kinase)

8)**Conversion of succinate to fumarase** : enzyme succinate dehydrogenase

9)**Formation of malate** : enzyme fumarase

10)**Conversion of malate to oxaloacetate** : enzyme malate dehydrogenase.

Summary of Krebs Cycle ①



Reducing equivalents

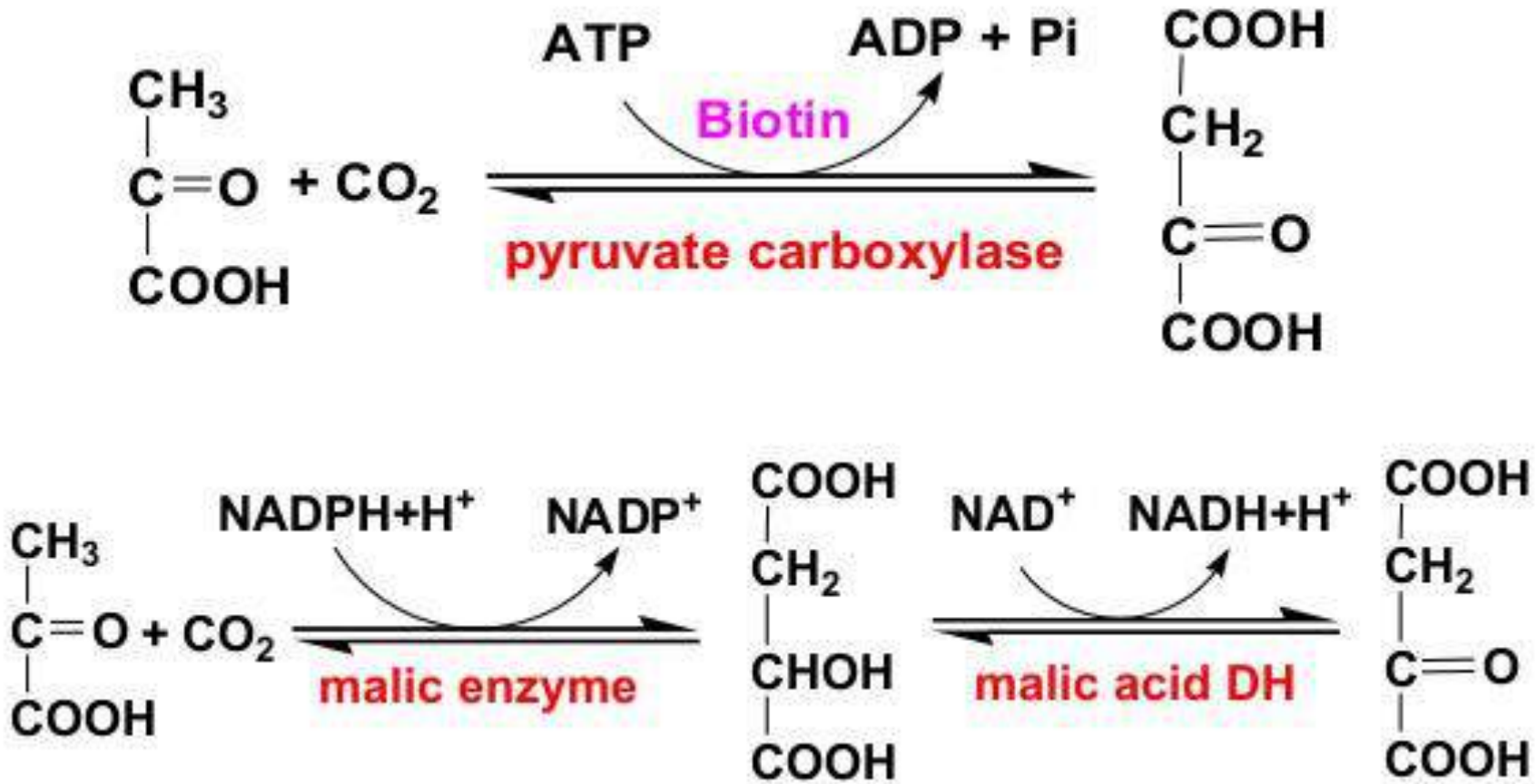
② The net reaction of the TCAC:

$$acetylCoA+3NAD^{+}+FAD+GDP+Pi+2H_2O \rightarrow 2CO_2+3NADH+3H^{+}+FADH_2+GTP+HSCoA$$

③ Irreversible and aerobic reaction

④ The enzymes are located in the mitochondrial matrix.

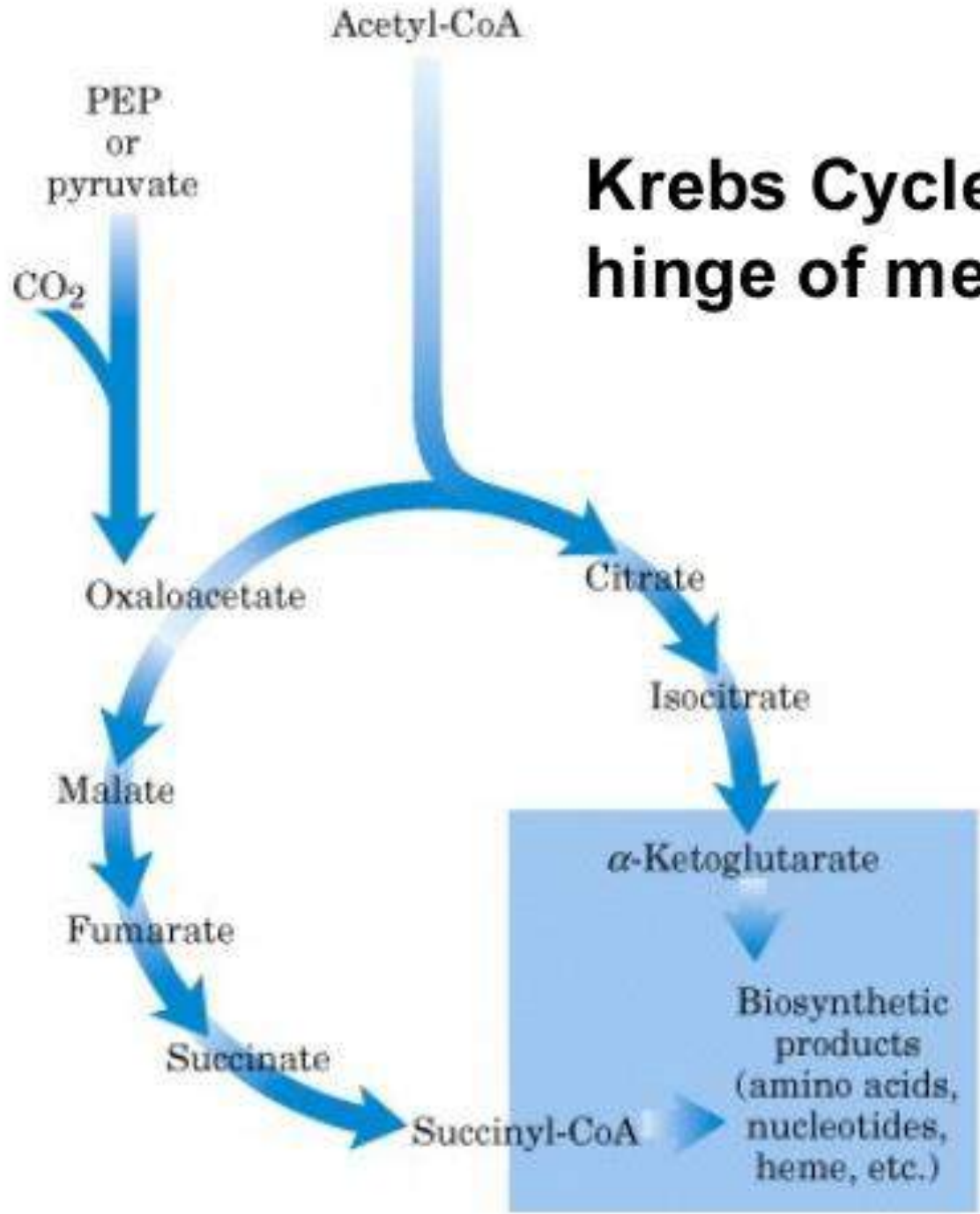
⑤ Anaplerotic reaction of oxaloacetate



(2) Bio-significance of TCAC

① Acts as the final common pathway for the oxidation of carbohydrates, lipids, and proteins.

② Serves as the crossroad for the interconversion among carbohydrates, lipids, and non-essential amino acids, and as a source of biosynthetic intermediates.



Krebs Cycle is at the hinge of metabolism.

- TCA cycle is strictly **aerobic** in contrast to glycolysis.
- Total of **12 ATP** are produced from one acetyl CoA :-

✓ During the process of oxidation of acetyl CoA via citric acid cycle → 3 NADH & 1 FADH2.

✓ Oxidation of 3 NADH by electron transport chain coupled with oxidative phosphorylation results in 9 ATP, FADH2 → 2 ATP.

✓ One substrate level phosphorylation.

2. ATP produced in the aerobic oxidation

- acetyl CoA → TCAC : 3 ($NADH+H^+$) + $FADH_2$ + 1GTP → 12 ATP.
- pyruvate →acetyl CoA: $NADH+H^+$ → 3 ATP
- 1 G → 2 pyruvate : 2($NADH+H^+$) → 6 or 8ATP

1mol G : 36 or 38mol ATP

(12 + 3) ×2 + 6 (8) = 36 (38)

3. The regulation of aerobic oxidation

- **The Key Enzymes of aerobic oxidation**

  The Key Enzymes of glycolysis

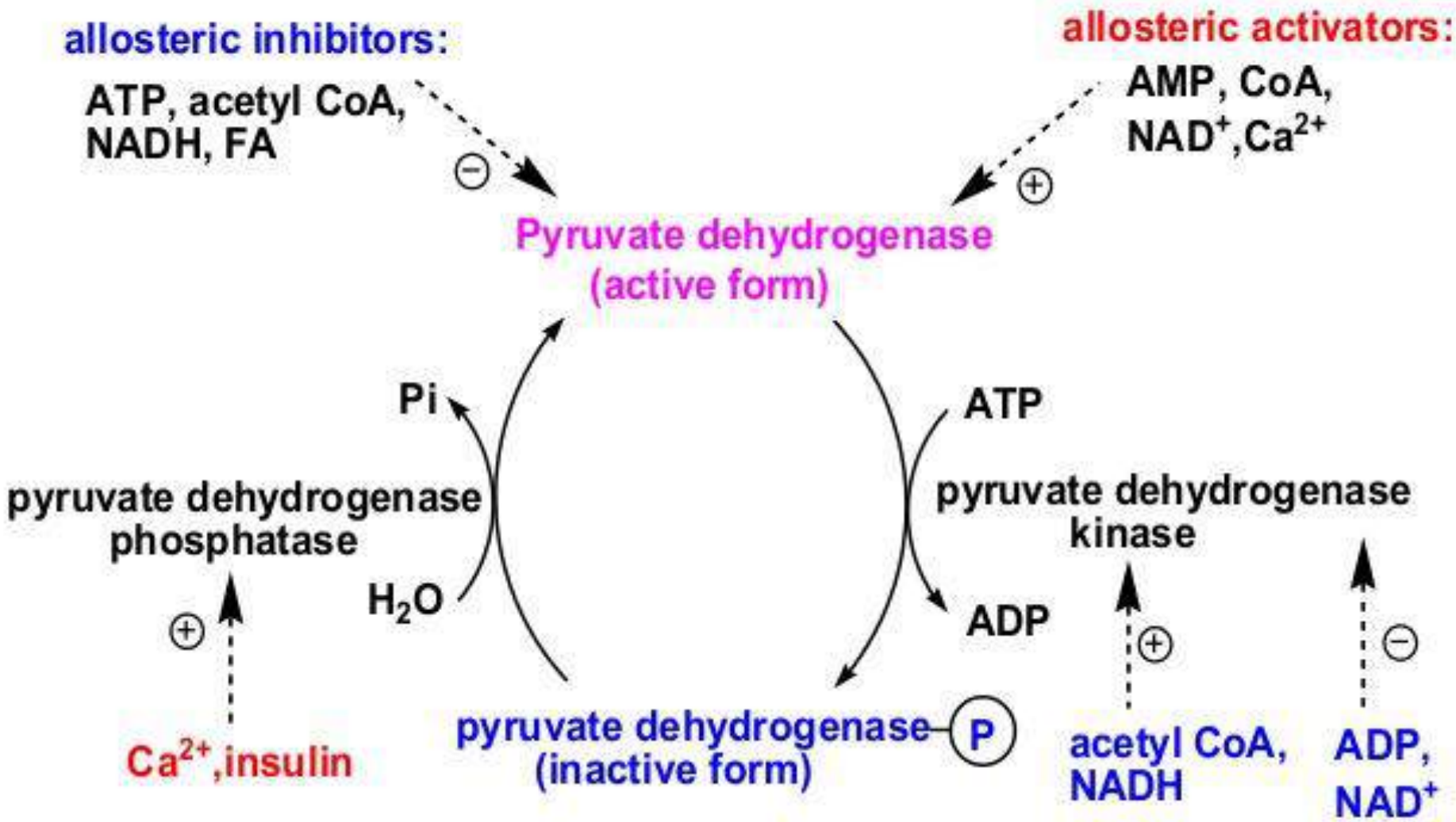
  Pyruvate Dehydrogenase Complex

  Citrate synthase

  Isocitrate dehydrogenase (rate-limiting)

  α-Ketoglutarate dehydrogenase

(1) Pyruvate dehydrogenase complex



(2) Citrate synthase

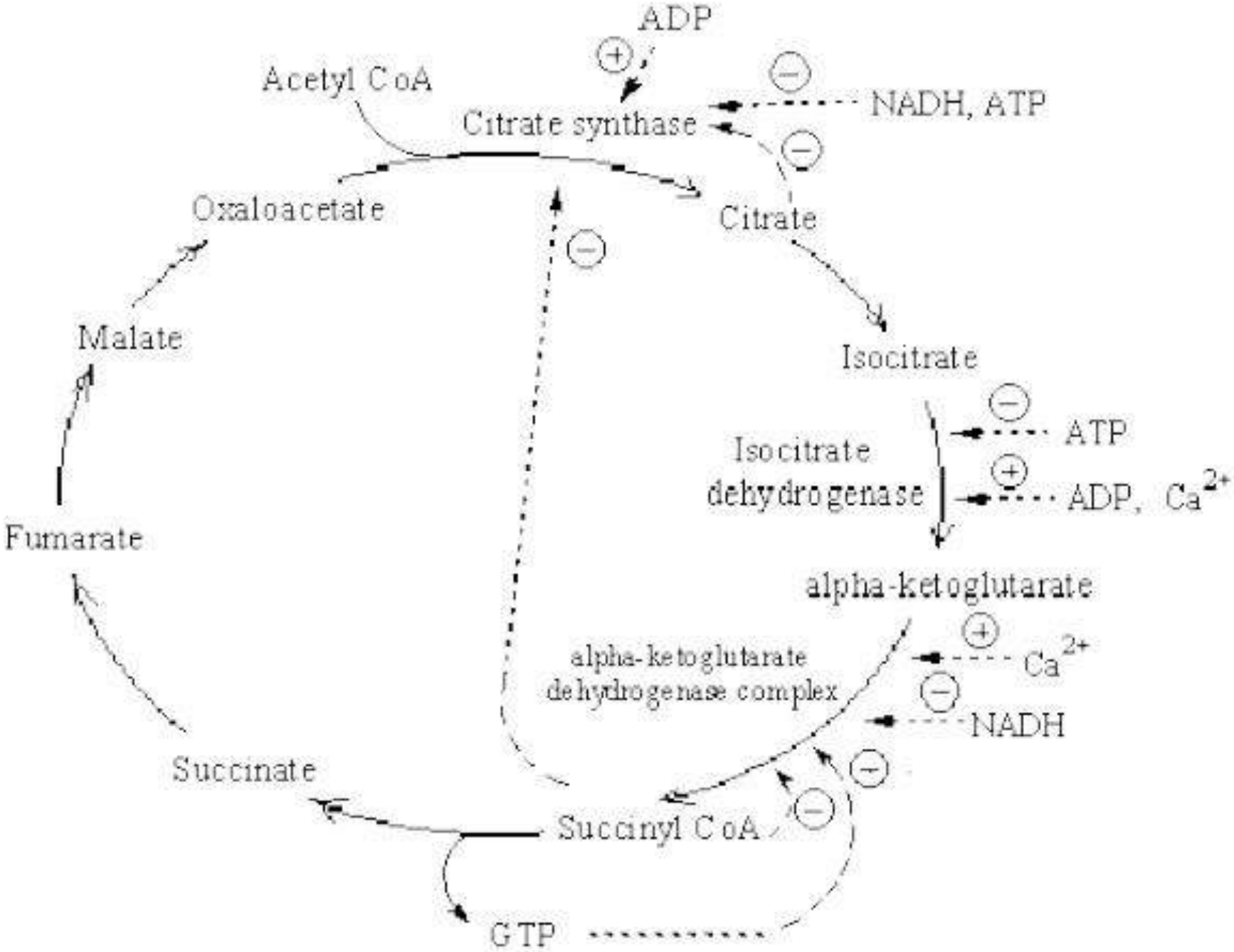
- Allosteric activator: ADP
- Allosteric inhibitor: NADH, succinyl CoA, citrate, ATP

(3) Isocitrate dehydrogenase

- Allosteric activator: ADP, Ca^{2+}
- Allosteric inhibitor: ATP

(4) α-Ketoglutarate dehydrogenase

- Similar with Pyruvate dehydrogenase complex


ADP
Acetyl CoA
NADH, ATP
Citrate synthase
Oxaloacetate
Citrate
Malate
Isocitrate
ATP
Isocitrate
dehydrogenase
ADP, Ca2+
Fumarate
alpha-ketoglutarate
alpha-ketoglutarate
dehydrogenase complex
Ca2+
NADH
Succinate
Succinyl CoA
GTP

Oxidative phosphorylation→TCAC↑

- **ATP/ADP↑ inhibit TCAC, Oxidative phosphorylation ↓**
- **ATP/ADP↓ , promote TCAC , Oxidative phosphorylation ↑**

4. Pasteur Effect

- Under aerobic conditions, glycolysis is inhibited and this inhibitory effect of oxygen on glycolysis is known as **Pasteur effect**.
- The key point is NADH :

  NADH $\longrightarrow$ mitochondria

  Pyr $\longrightarrow$ TCAC $\longrightarrow$ CO_2 + H_2O

  Pyr can't produce to lactate.

APPLIED ASPECTS OF TCA CYCLE

Mitochondrial encephalopathy occurs due to fumarase deficiency .

It is a mitochondrial myopathy affecting both the skeletal muscles and brain .