Biochemistry

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# Metabolism

Thousands of chemical reactions are taking place inside a cell in an organized, well coordinated, and purposeful manner; all these reactions are collectively called as **Metabolism**.

**Purposes and characteristics:**

1. To obtain chemical energy from the degradation of energy rich nutrients.
2. To obtain building blocks (precursors) of cellular macromolecules from food materials.
3. To construct vital macromolecules, such as proteins, nucleic acids, polysaccharides, etc.

The net of metabolic processes are called **Metabolic pathways**

**Basis of Metabolism –** chemical reactions catalyzed by sequential enzyme systems.

**Regulation of metabolism –**

1. Regulation through the action of allosteric enzymes, which increase or decrease the activity under the influence of effector molecules.

2. Hormonal regulation. Hormones are chemical messengers secreted by different endocrine glands.

3. Regulation at the DNA level; the concentration of the enzyme is changed by regulation at the level of synthesis of the enzyme.

# Metabolism

**Pathways of Metabolsm –**

**CATABOLISM (degradation)**
energy rich complex macromolecules are degraded into smaller molecules. Energy released during this process is trapped as chemical energy, usually as ATP.

**ANABOLISM (biosynthesis)**
The cells synthesize complex molecules from simple precursors. Needs energy.

**AMPHIBOLIC processe**
Combination of anabolic and catabolic processes. Crossroads of metabolism

**Flow through of foodstuff:**

1 - digestion in the gastrointestinal tract converts the macromolecules into small units. **Primary metabolism**.
2 - small units are absorbed, catabolized to smaller components, and ultimately oxidized to CO2. The reducing equivalents are mainly generated in the mitochondria by the final common oxidative pathway, citric acid cycle. In this process, NADH or FADH2 are generated. **Secondary metabolism.**
3 - reduced equivalents enter into the electron transport chain (Respiratory chain), where energy is released. This is the **tertiary metabolism** or Internal respiration or cellular respiration

# Metabolism

**Pathways of Metabolsm –**

**CARBOHYDRATES – mainly around glucose**
**carbohydrates go through glycolysis pathway, are converted to acetyl-CoA**
**Further are oxidized in the citric acid cycle**
**AIM – provision of energy**

**LIPIDS – mainly around fatty acids**
**AIM – provision of energy**

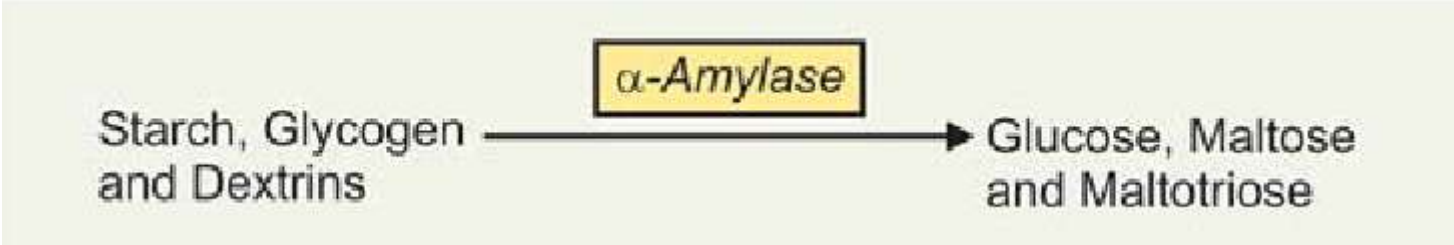
**PROTEINS - amino acds**
**Can be transaminated and oxidized at carbon skeleton**
**AIM – building blocks AND energy**

# Bioenergetics

DIGESTION OF CARBOHYDRATES

Dietary carbohydrates - polysaccharides: **starch and glycogen**.
It also contains disaccharides: **sucrose, lactose and maltose**
and in small amounts monosaccharides like **fructose** and pentoses.

**1. Digestion in mouth**




*Salivary amylase (ptyalin)* hydrolyzes α-1 →4 glycosidic linkage at random deep inside polysaccharide

**2. Digestion in stomach**
Practically no action. No enzymes in gastric juice.

**3. Digestion in duodenum**: Food reaches the duodenum where it meets the pancreatic juice. Pancreatic juice contains a carbohydrate-splitting enzyme *pancreatic amylase (also called amylopsin)* similar to salivary amylase.

# Bioenergetics

DIGESTION OF CARBOHYDRATES

**4. Digestion in Small Intestine**

Action of Intestinal Juice

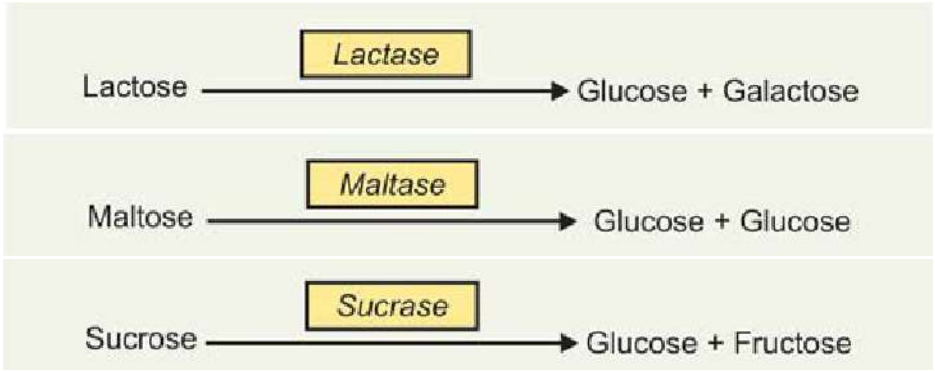
A• *Intestinal amylase*: This hydrolyses terminal α-1→4 glycosidic linkage in polysaccharides and oligosaccharide molecules liberating free glucose molecule.

B • *Lactase:* β-galactosidase. Lactose is hydrolysed to equimolar amounts of glucose and galactose.

C* *Isomaltase:* hydrolysis of α-1→6 glycosidic linkage, splitting α-limit dextrin at the branching points and producing maltose and glucose.

D • *Maltase:* The enzyme hydrolyses the α-1→4 glycosidic linkage between glucose units in maltose = two glucose molecules.

E• *Sucrase*: hydrolyses sucrose molecule = equimolar quantities of glucose and fructose.



# Bioenergetics

ABSORPTION OF CARBOHYDRATES

Carbohydrate digestion is complete when the food materials reach small intestine and all complex dietary carbohydrates like starch and glycogen and the disaccharides are ultimately converted to simpler monosaccharides. All monosaccharides, products of digestion of dietary carbohydrates, are practically completely absorbed almost entirely from the small intestine.

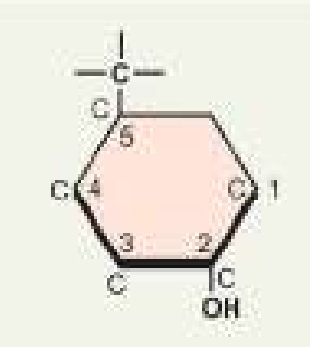
**Two mechanisms**:

1. Simple diffusion: This is dependent on sugar concentration gradients between the intestinal lumen, mucosal cells and blood plasma. (monosaccharides)

2. "Active" Transport Mechanisms (Wilson and Crane's Hypothesis)

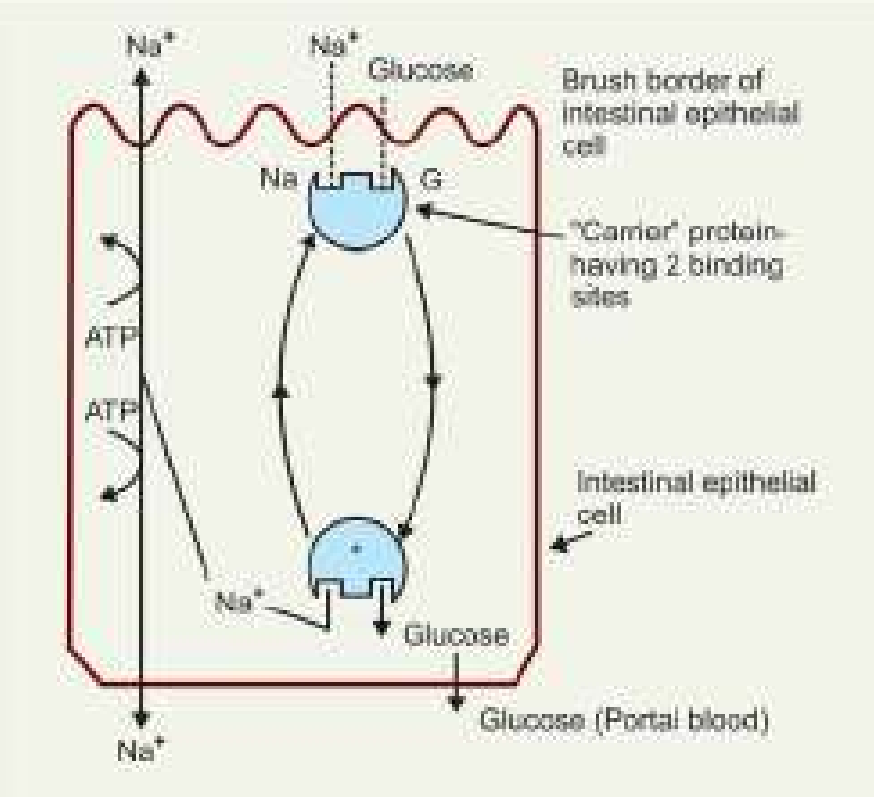
• Glucose and galactose are absorbed very rapidly and hence it has been suggested that they are absorbed actively and it requires energy.

• Fructose absorption is also rapid but not so much as compared to glucose and galactose, but it is definitely faster than pentoses. Hence fructose is not absorbed by simple diffusion alone and it is suggested that some mechanism facilitates its transport, called as facilitated transport.



# Bioenergetics

ABSORPTION OF CARBOHYDRATES




Active absorption of glucose

- COTRANSPORT

Sugars like D-fructose and D-mannose are probably absorbed by facilitated transport which requires the presence of carrier protein but does not require energy.

Other sugars like pentoses and L-isomers of glucose and galactose are absorbed passively by simple diffusion.

# Bioenergetics

FATE OF MONOSACCHARIDES

Monosaccharides with the portal blood pass through the liver (the first 'filter') before entering the systemic circulation.

In liver two mechanisms operate:

- Withdrawal of carbohydrates from blood and
- Release of glucose by liver to the blood.

The functional state of the liver will be of prime importance and will have a profound influence on the carbohydrate metabolism on the entire organism.

The intake by the organs/tissues

1. Depend on the tissue
2. Can be active/passive
3. Can be facilitated by insulin

# Bioenergetics

UTILISATION OF GLUCOSE

**OXIDATION – for Energy**

*Oxidation of glucose or glycogen to pyruvate and lactate is called glycolysis.*

Glucose is degraded to pyruvate, which in presence of O2 is completely oxidized to CO2 and H2O.

*Glycolysis occurs in all tissues.*

**OXIDATION – not for Energy**

*HMP shunt*

The pathway provides

*1 NADPH which is used for reductive synthesis and*

*2 Pentoses which are used for nucleic acids synthesis.*

*This pathway operates only in certain special tissues and not all tissues.*

**OXIDATION – not for Energy**

*Uronic Acid Pathway*

*Provides D-glucuronic acid which is used for synthesis of mucopolysaccharides and conjugation reactions.*

# Bioenergetics

UTILISATION OF GLUCOSE – **GLYCOLYSIS** ***(glucose/glycogen to pyruvate/lactate for energy)***

*Originaly described by Embden, Meyerhof and Parnas. Hence, it is also called as* ***Embden Meyerhof pathway.***

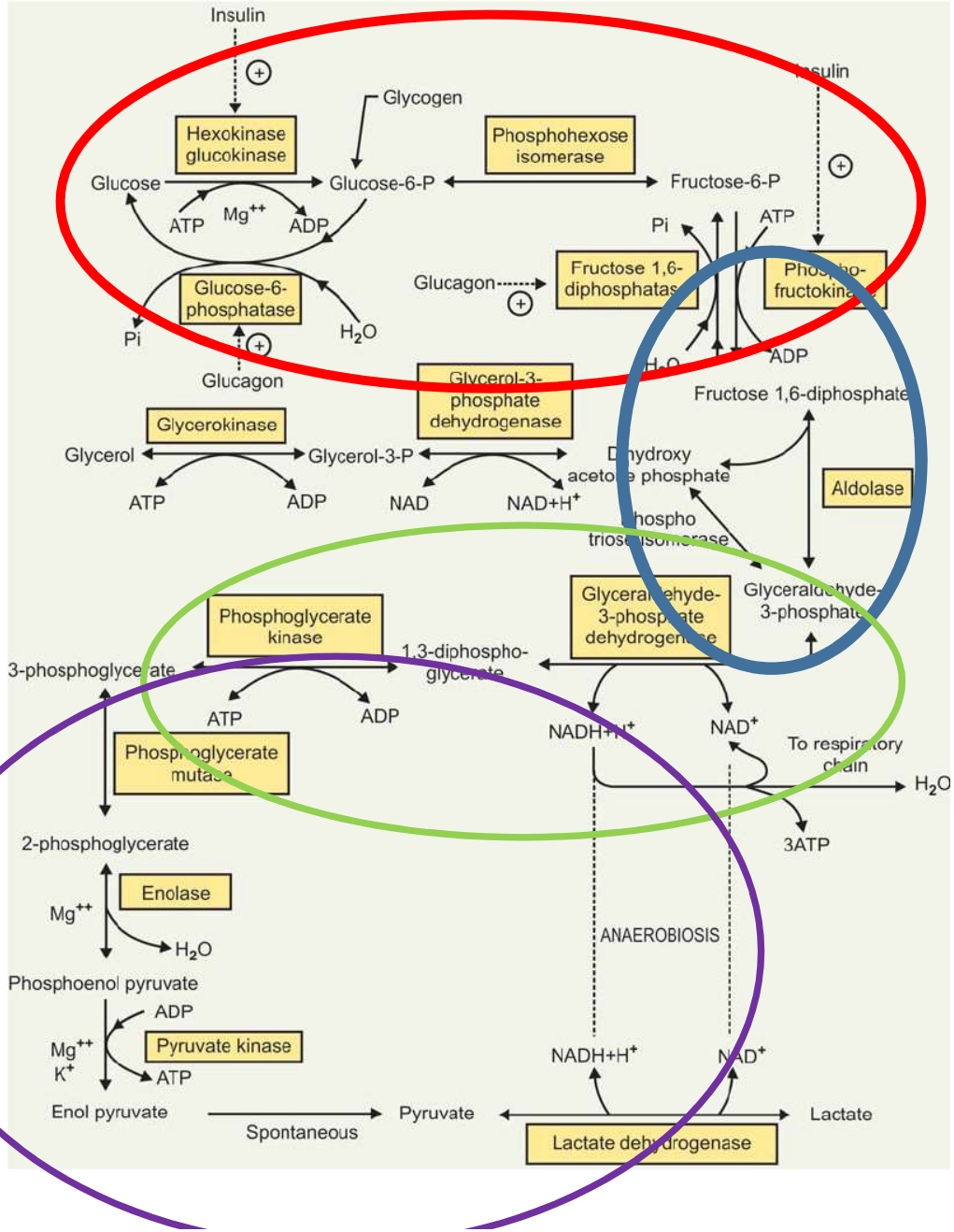
1. **Occur in all tissues**

2. **Can happen in the presence and in the absence of O2** (skeletal muscles! Cancer cells)

3. **Biphasic nature –**
   a. **Aerobic phase** *(Oxidation is carried out by dehydrogenation and reducing equivalent is transferred to NAD+. Reduced NAD in presence of O2 is oxidized in electron-transport chain producing ATP)*
   b. **Anarobic phase** *(NADH cannot be oxidised in electron transport chain, but can be oxidized to NAD+ by conversion of pyruvate to lactate, without producing ATP).*

4. **Occur extramitochondrially**

# Bioenergetics

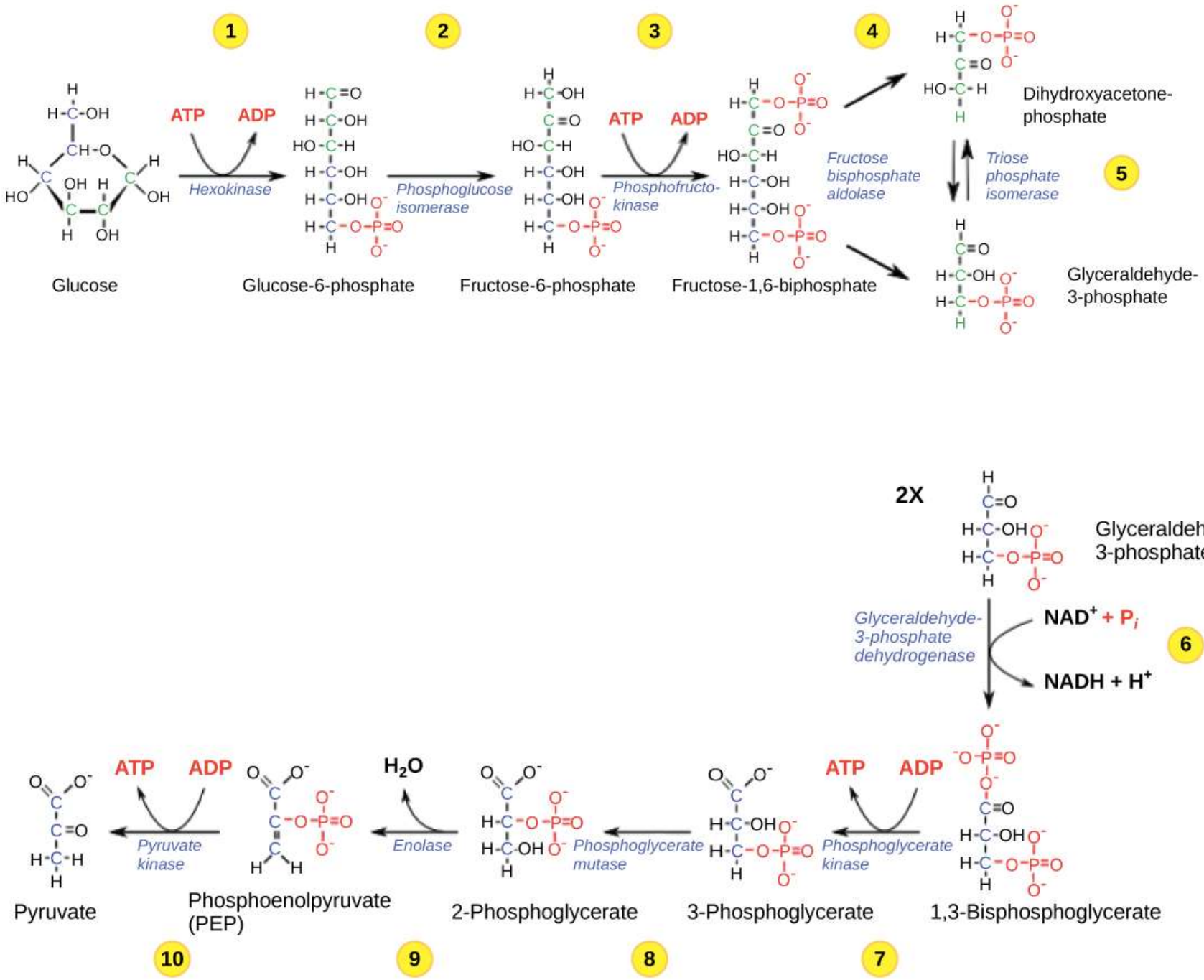



Stage I -
This is a preparatory stage.
“Doing symmetrical glucose”

Stage II –
Splitting of the molecule

Stage III –
Yielding of energy

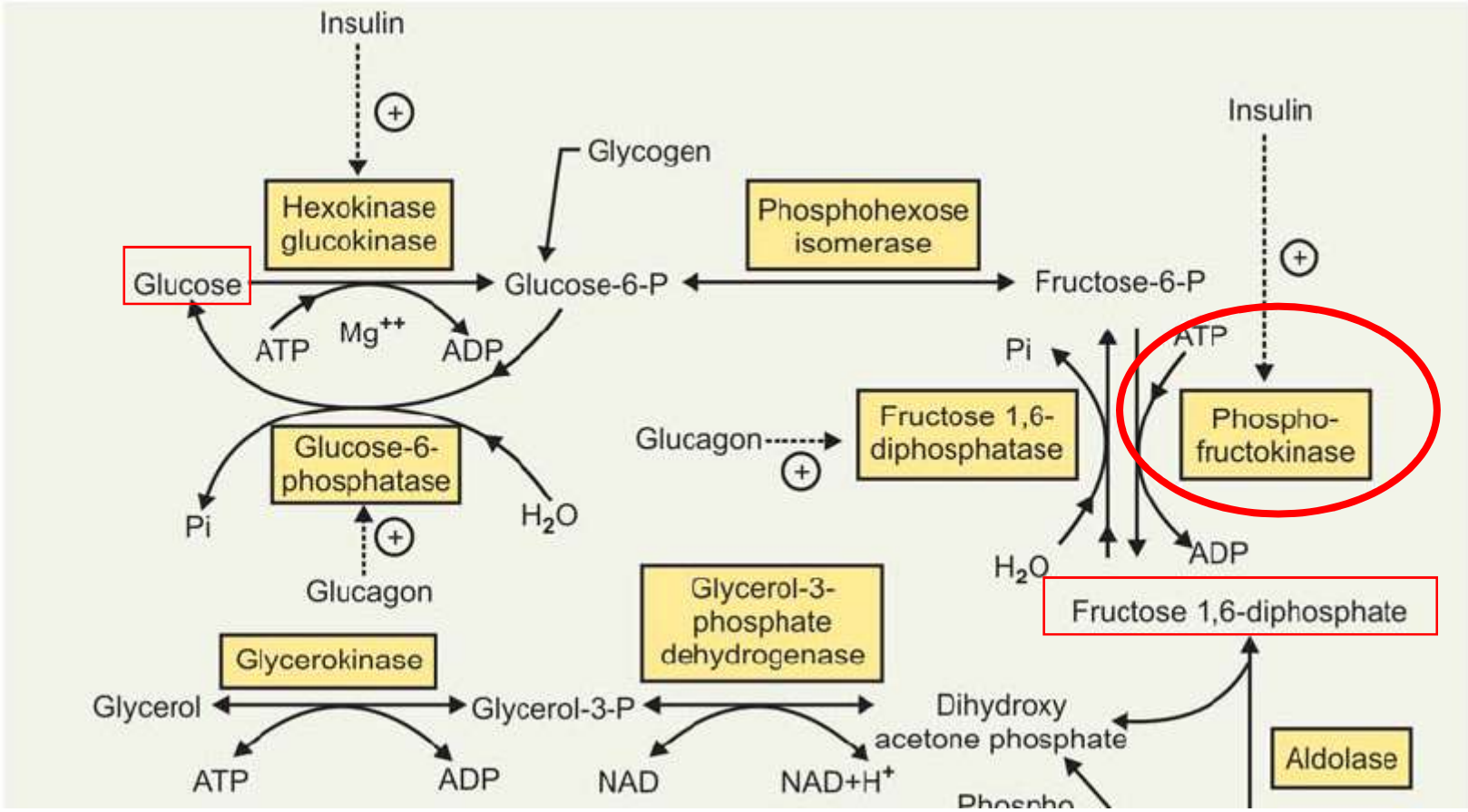
Stage IV –
Recovery of the PO4 group

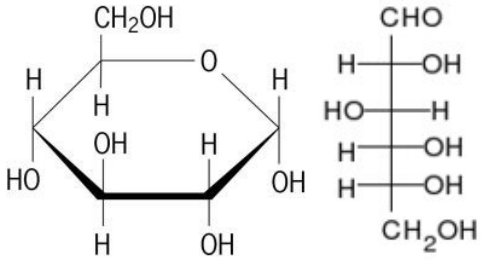

1
2
3
4
5
6
7
8
9
10
ATP
ADP
Hexokinase
Phosphoglucose isomerase
Phosphofructo-kinase
Fructose bisphosphate aldolase
Triose phosphate isomerase
Glyceraldehyde-3-phosphate dehydrogenase
Phosphoglycerate kinase
Phosphoglycerate mutase
Enolase
Pyruvate kinase
$H_2O$
$NAD^+ + P_i$
$NADH + H^+$
2X
Glucose
Glucose-6-phosphate
Fructose-6-phosphate
Fructose-1,6-biphosphate
Dihydroxyacetone-phosphate
Glyceraldehyde-3-phosphate
1,3-Bisphosphoglycerate
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate (PEP)
Pyruvate

# Bioenergetics

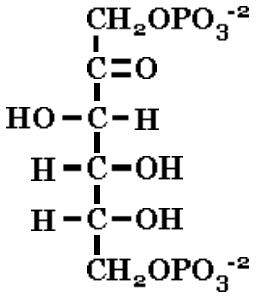
1. Glycogen → Fructose-1, 6 bi — P −1 ATP

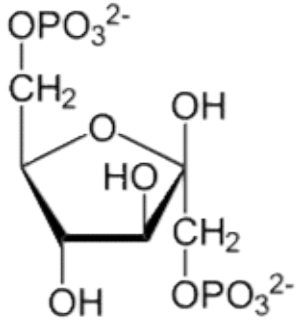
Loss of energy!



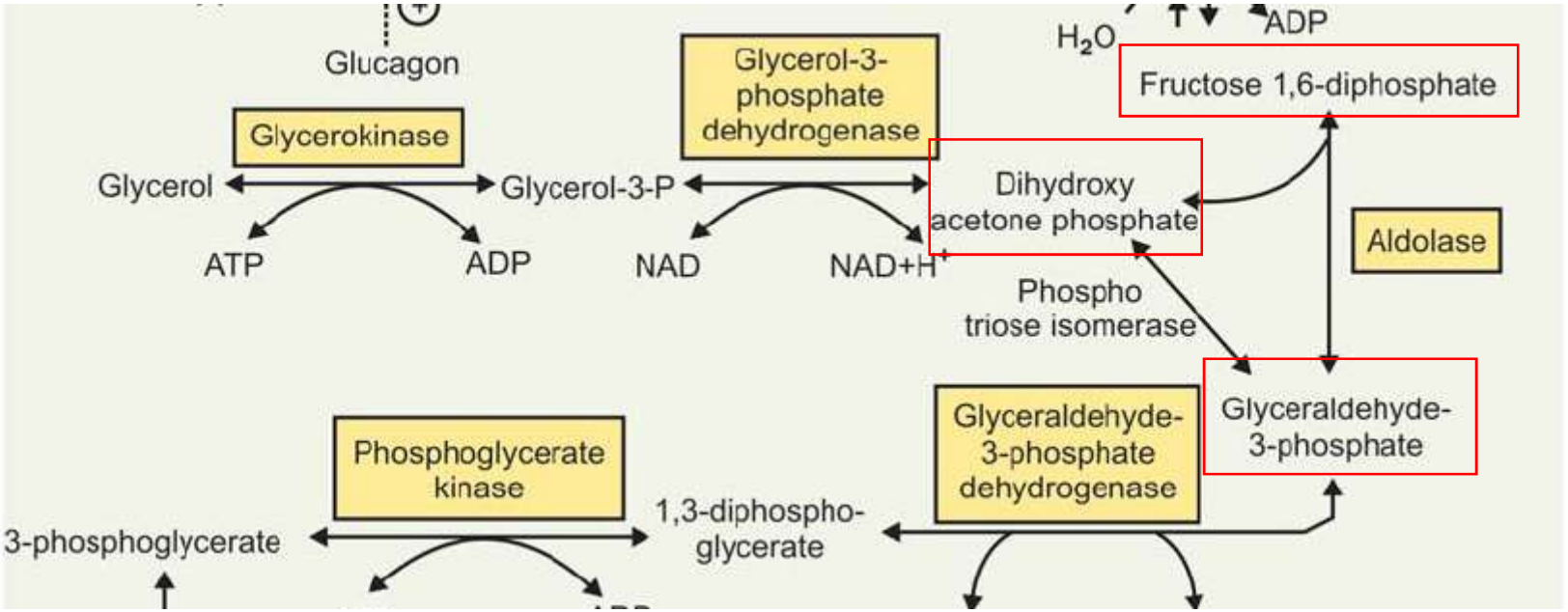



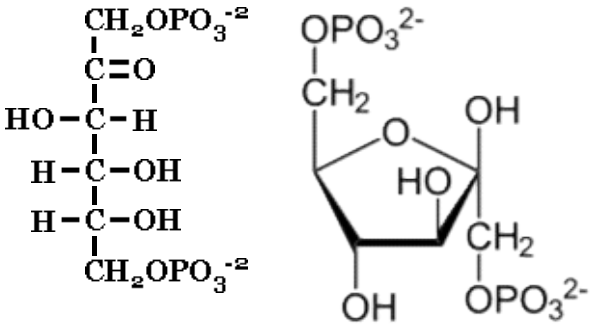

**Input** - Glucose
**Output** – fructose 1-6 diphosphate
**Intermediate** – glucose -6-phosphate, fructose-6-phosphate



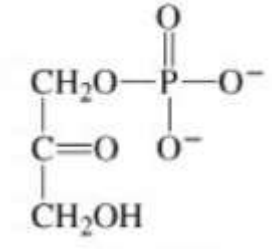
# Bioenergetics

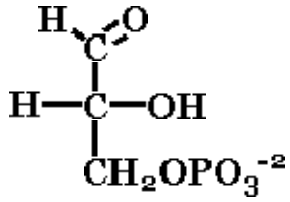


**Input** - fructose 1-6 diphosphate

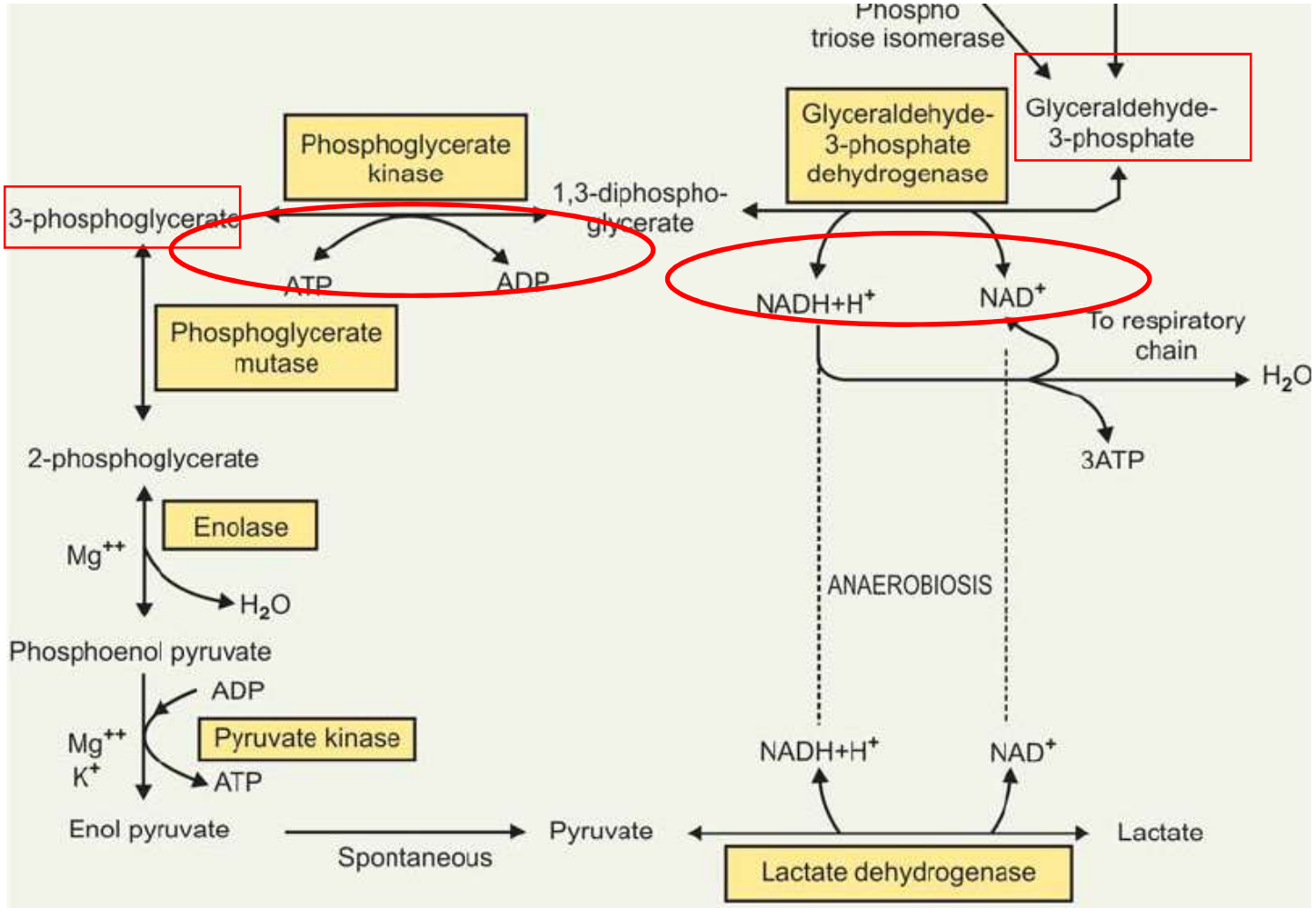
**Output** – glyceraldehyde-3-phosphate AND dihydroxy acetone-phosphate



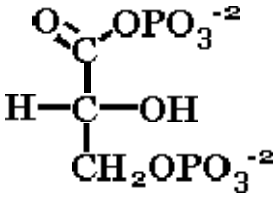


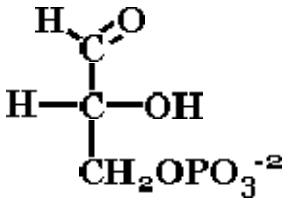
# Bioenergetics

Gain of energy !!! (ATP and NADH)

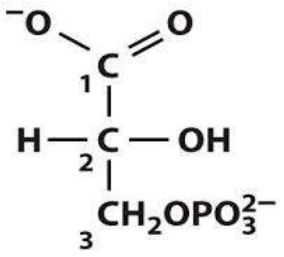



Aldehyde group is oxidized to acid



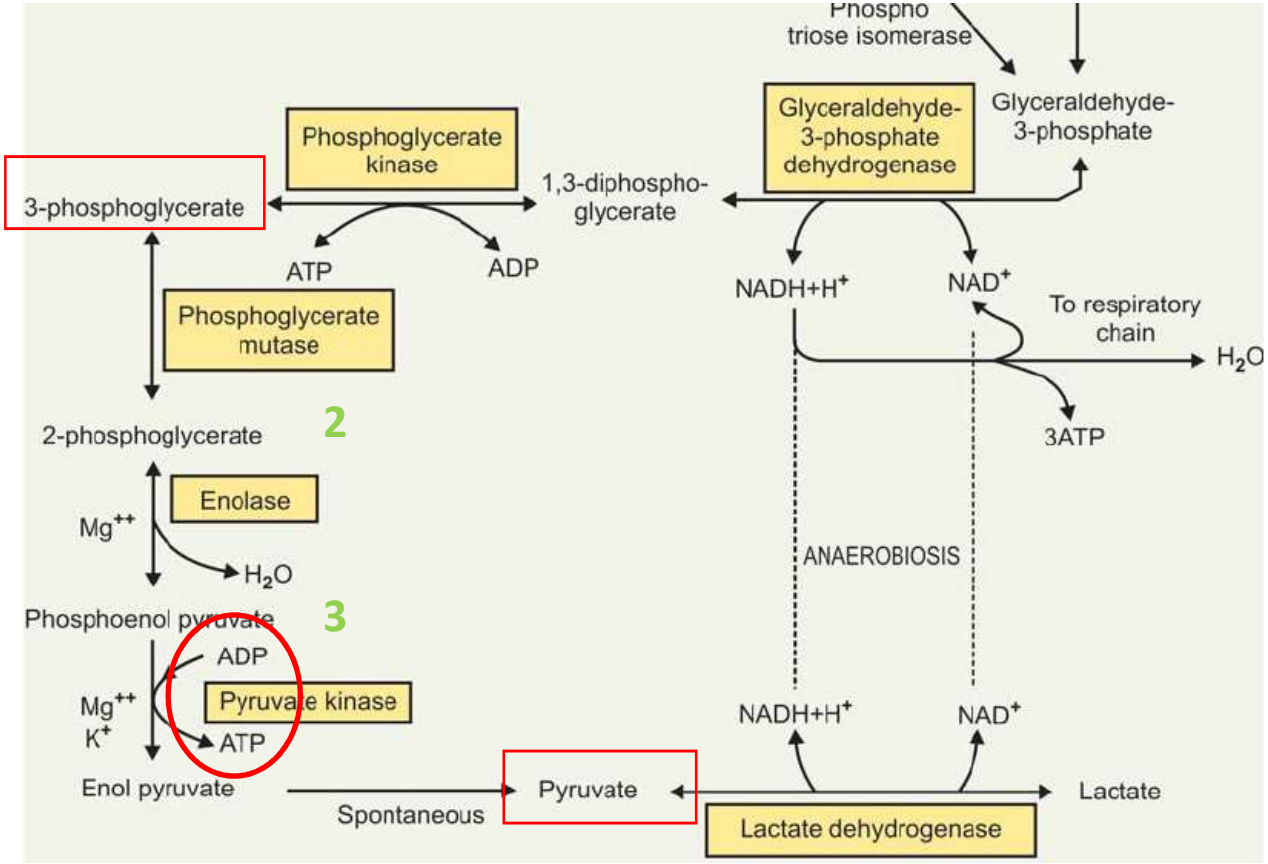


**Input** - glyceraldehyde-3-phosphate
(dihydroxy acetone-phosphate
also isomerizes to G-3-P)
**Output** – 3-phosphoglycerate
**Intermediate** – 1,3 biphospho glycerate

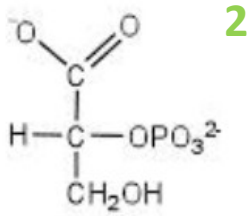


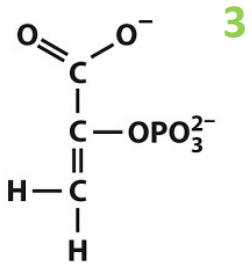
# Bioenergetics

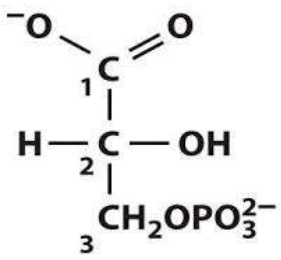
Gain of energy



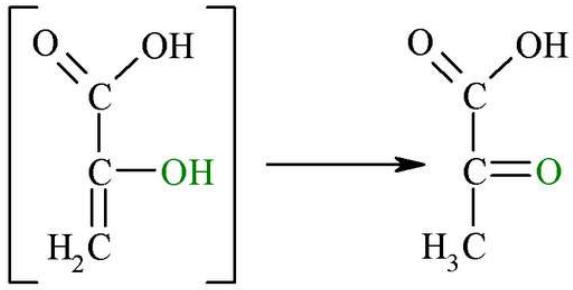

Taking back phosphates that were used in step I



**Input** - 3-phosphoglycerate
**Output** – pyruvate
**Intermediate** – 2-phosphoglycerate, phosphoenolpyruvate



# Bioenergetics

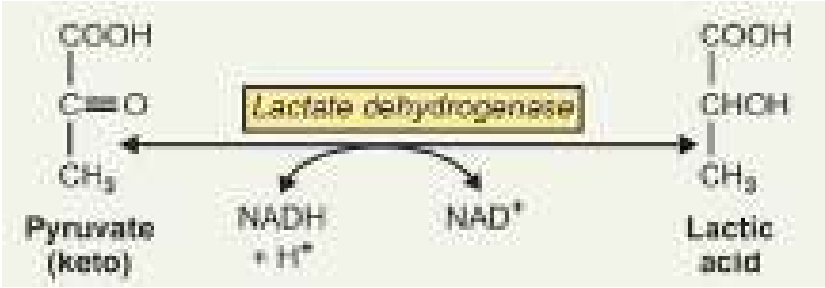
A – aerobic phase

| Reaction catalysed by | ATP Production |
|---|---|
| ***Stage I*** | |
| 1. Hexokinase/Glucokinase reaction (for phosphorylation) | – 1 ATP |
| 2. Phosphofructokinase-1 (for phosphorylation) | – 1 ATP |
| ***Stage III*** | |
| 3. Glyceraldehyde-3-P dehydrogenase (oxidation of 2 NADH in electron transport chain) | + 6 ATP |
| 4. Phosphoglycerate kinase (substrate level phosphorylation) | + 2 ATP |
| ***Stage IV*** | |
| 5. Pyruvate kinase (substrate level phosphorylation) | + 2 ATP |
| Net gain | = 10–2 = **8 ATP** |

In the presence of O2 NADH is converted back to NAD+ in electron-transport chain in mitochondria with generation of ATP (3ATP per NADH)

B – anaerobic phase

Electron-transfer chain is not working -> huge loss of NAD+
To recuperate NAD+ which can be limited pyruvate is converted to lactate




*In anaerobic phase per molecule of glucose oxidation 4 – 2 = 2 ATP will be produced.*

**+ 2 ATP**

Erythrocytes. Skeletal muscle under severe stress – acidosis. However, under harsh conditions NAD+ is further limited and glycolysis stops

# Bioenergetics

**HOW to regulate Glycolysis**

(a) Changes in the rate of enzyme synthesis, Induction/repression. (timescale – hours)

Induction of glycolysis enzymes + inhibition of gluconeogenesis
Glucokinase. Phospho-1-fructokinase, pyruvate kinase
Glucose induces several enzymes, insulin also is an inducer

(b) Covalent modification by reversible phosphorylation. (very quick)

Phosphorylation of pyruvatekinase

(c) Allosteric modification.

Phosphofructokinase – inhibited by citrate and ATP, induced by AMP
(AMP is very sensitive indicator of ATP concentration – rise metabolically amplified )

# Bioenergetics

**Conversion of pyruvate to Acetyl-CoA = oxidative decarboxylation**

1. Occurs in the presence of O2
2. 3-carbon moiety are converted to 2-carbon Acetyl-CoA and CO2
3. Occurs in mitochondria (pyruvate is transported by specific proteins)

The heart of the reaction –
***pyruvate dehydrogenase complex***

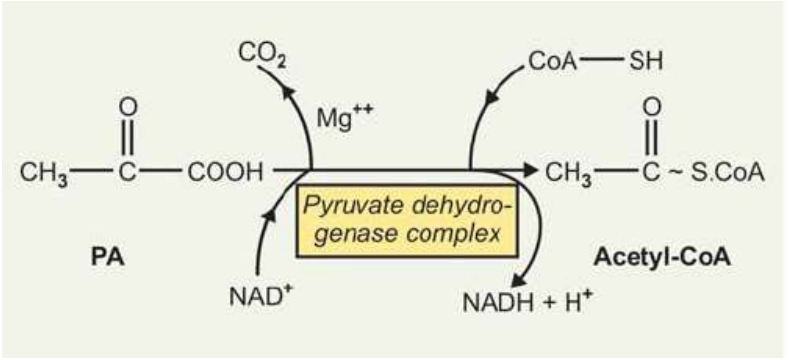
The enzyme complex consists of:

29 *molecules of Pyruvate dehydrogenase (PD)*
+ 8 *molecules of Flavoprotein containing dihydrolipoyl dehydrogenase, and*
+ 1 *molecule of dihydrolipoyl transacetylase.*

*The enzyme complex for its activity requires at least six coenzymes/cofactors:*

- Thiamine pyrophosphate (TPP)
- Lipoic acid
- CoA–SH
- FAD
- $NAD^+$ and
- $Mg^{++}$

NET REACTION




**Energy balance –**

1 pyruvate = 1 NADH
(NADH is usually oxidized to NAD+ and 3 ATP in electrontransportchain) therefore

1 pyruvate = 3 ATP

# Bioenergetics

**HOW to regulate Conversion of pyruvate to Ac-CoA?**

(a) Changes in the rate of enzyme synthesis, Induction/repression. (timescale – hours)

(b) Covalent modification by reversible phosphorylation. (very quick)

Phosphorylated pyruvate dehydrogenase complex is inactive.

Dephosphorylation of pyruvate dehydrogenase complex induced by insulin activates it. Phosphorylation is triggered by high ATP:ADP ratio, NADH:NAD+, Ac-CoA:CoA-SH, increase cyclicAMP

(c) Allosteric modification.

What else? Accumulation of pyruvate and lactate is seen if there's:

Lack of vitamin B1 (thiamine) and TPP – reduces the reaction (chronic alcoholism – chronic reduction of B1 vitamin)

# Bioenergetics

**CITRIC ACID CYCLE = trycarboxylic acid cycle, Krebs' cycle**

THE FINAL STEP!

1. Cycle (!)
2. Final breakdown/catabolism stage of carbohydrates (as well as fats and proteins)
3. If we consider carbohydrates – then **input** – Ac-CoA after PDH complex

(Input is 2-carbon moiety!)

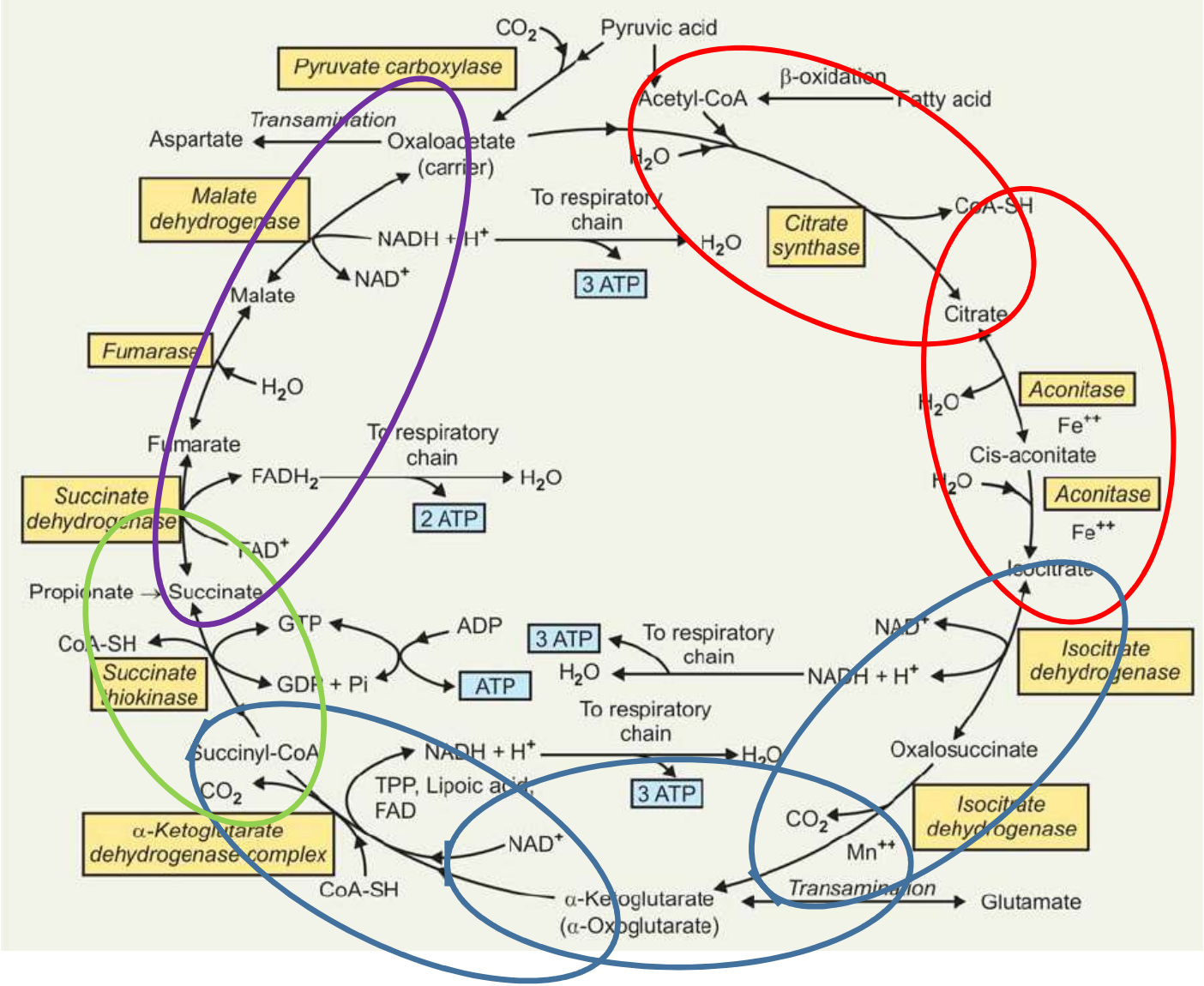
4. Strictly **AEROBIC**
4. Output – **ENERGY!** and CO2
5. Occurs in mitochondria (in matrix – majorly inner side of mt inner membrane)

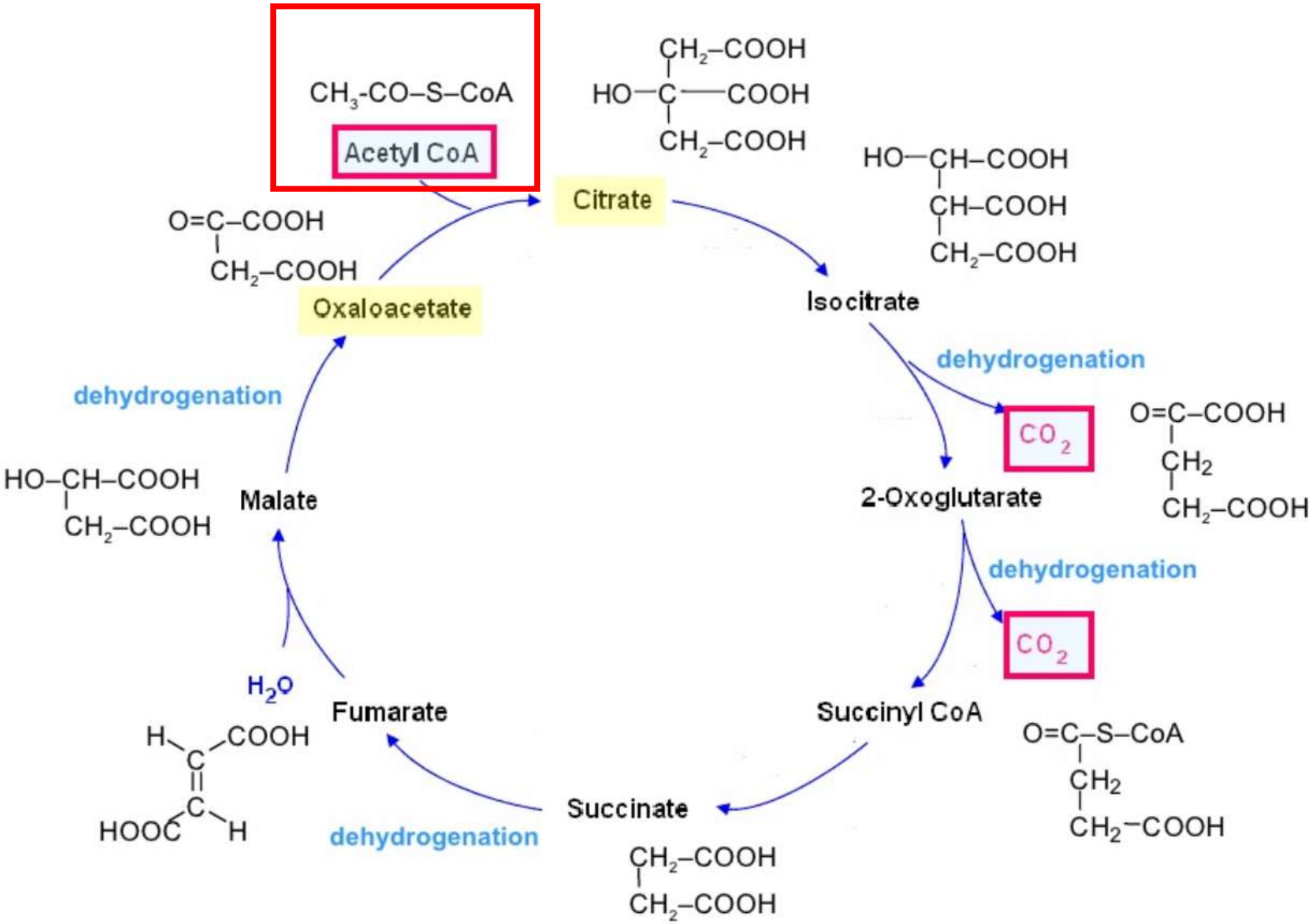
# Bioenergetics

**CITRIC ACID CYCLE = trycarboxylic acid cycle, Krebs' cycle**

4 stages:

1. Formation of citric acid and formation of isocitrate
2. Isocitrate (6C) is converted to Succinyl-CoA (CO2 is released)
3. Succinyl-CoA is converted to succinic acid
4. succinic acid is oxidized to oxaloacetate (OAA) to continue the cycle




CH3-CO–S–CoA
Acetyl CoA
Citrate
Isocitrate
dehydrogenation
CO2
2-Oxoglutarate
dehydrogenation
CO2
Succinyl CoA
Succinate
dehydrogenation
Fumarate
H2O
Malate
dehydrogenation
Oxaloacetate

# Bioenergetics

**CITRIC ACID CYCLE = trycarboxylic acid cycle, Krebs' cycle**

OUTLINE BRIEF

Acetyl-CoA (2-carbon group) brings in Acetyl group than is transferred to oxaloacetic acid (OAA). Citrate is formed.

Then dehydrogenations and loss of two molecules of CO2, accompanied by internal re-arrangements, occur.

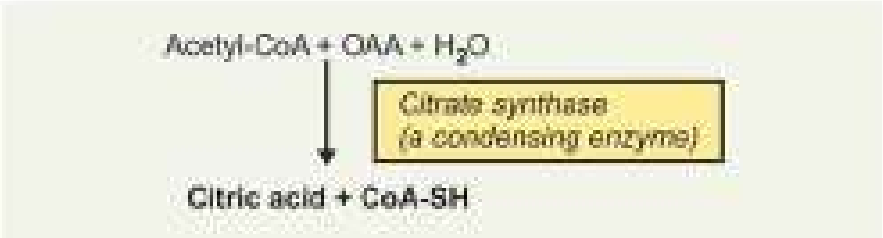
The final is oxaloacetic acid that goes to the beginning of the cycle and accepts another Acetyl group from Ac-CoA.

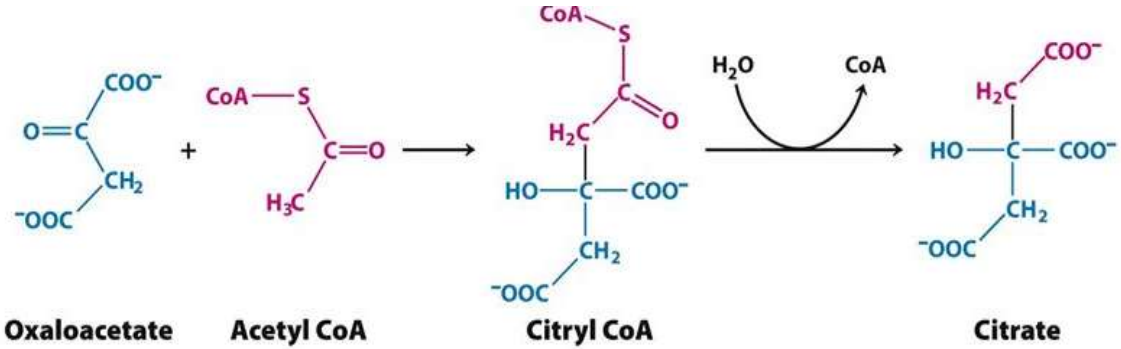
OAA – cycles!

# Bioenergetics

**CITRIC ACID CYCLE = trycarboxylic acid cycle, Krebs' cycle STAGE 1**

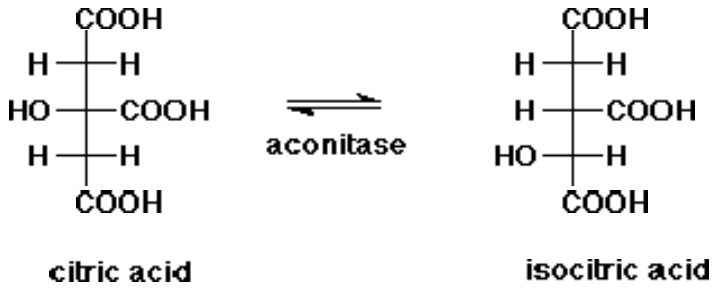
1. *Formation of Citric Acid from Acetyl-CoA and OAA*




CoA goes back to pyruvate dehydrogenase complex reaction

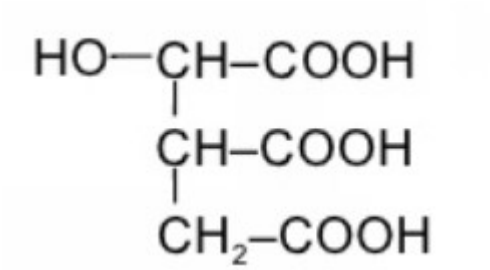
2. Formation of cis-aconitic acid and isocitric acid from citric acid:



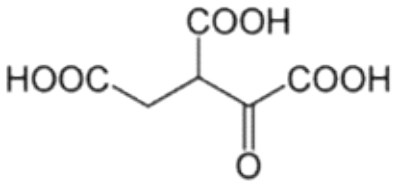
# Bioenergetics

**CITRIC ACID CYCLE = trycarboxylic acid cycle, Krebs' cycle STAGE 2**

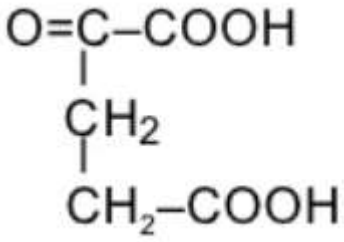
1. Formation of oxalosuccinic acid and α-oxo-glutarate from isocitric acid




isocitric acid




oxalosuccinic acid
(oxidation)

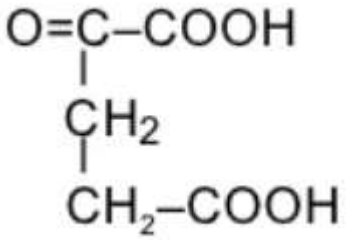



+CO2!

keto-glutarate
(decarboxylation)

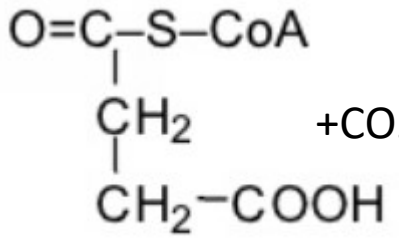
**ENERGY: NAD+ -> NADH**

2. Oxidative decarboxylation of α-oxoglutarate to succinyl-CoA




*The enzyme complex for its activity requires at least six coenzymes/cofactors:*

- Thiamine pyrophosphate (TPP)
- Lipoic acid
- CoA–SH
- FAD
- $NAD^+$ and
- $Mg^{++}$



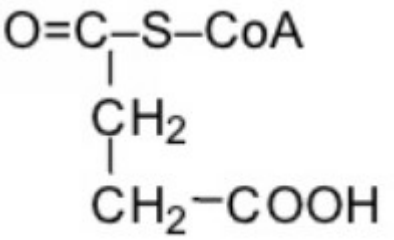

+CO2!

**ENERGY: NAD+ -> NADH**

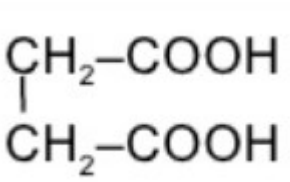
# Bioenergetics

**CITRIC ACID CYCLE = trycarboxylic acid cycle, Krebs' cycle STAGE 3**

Succinyl-CoA is converted to succinic acid.




**Input**




Reaction requires GDP or IDP, which is converted in presence of Pi to either GTP or ITP.

- The release of free energy from oxidative decarboxylation of α-oxoglutarate is sufficient to generate one high energy bond in addition to the formation of NADH (preceding and following steps).

**WHAT TO DO WITH GTP/ITP???**

- In presence of enzyme nucleoside diphosphate kinase, ATP is produced either from GTP or ITP.

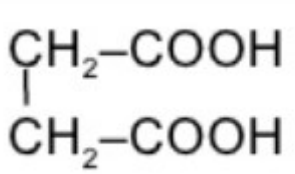
**Energetic: One ATP is produced in this reaction at substrate level.**

# Bioenergetics

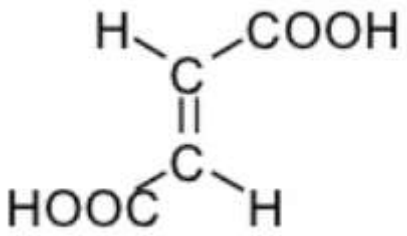
**CITRIC ACID CYCLE = trycarboxylic acid cycle, Krebs' cycle STAGE 4**

Succinyl-CoA is converted oxaloacetic acid to start the cycle

1 Oxidation of succinic acid to fumaric acid



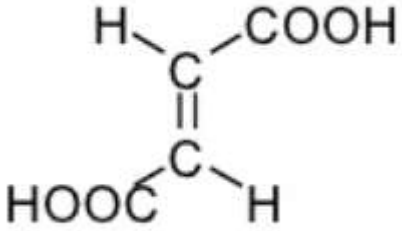




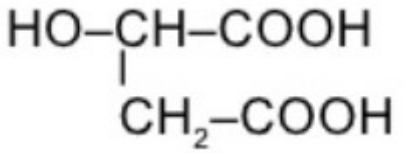

Succinate dehydrogenase – hem containing enzyme with FAD cofactor

**Energy: FAD-H2 (equals to 2 ATP)**

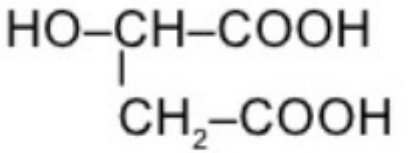
2 Formation of malic acid

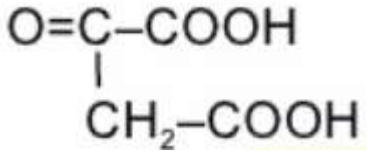







3 Oxidation of malic acid to oxaloacetate (OAA)




CYCLE!

**ENERGY: NAD+ -> NADH**

# Bioenergetics

**CITRIC ACID CYCLE = trycarboxylic acid cycle, Krebs' cycle**

ROLE OF VITAMINS IN TCA CYCLE
Five B vitamins are associated with TCA cycle essential for yielding energy.

• Riboflavin: In the form of flavin adenine dinuleotide (FAD)— a cofactor for succinate dehydrogenase enzyme.

• Niacin: In the form of nicotinamide adenine dinucleotide (NAD) the electron acceptor for isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase.

• Thiamine: As "thiamine diphosphate"—required as coenzyme for decarboxylation in the α-ketoglutarate dehydrogenase reaction.

• Lipoic acid: It is required as coenzyme for α-ketoglutarate dehydrogenase reaction.

• Pantothenic acid: As part of coenzyme A, the cofactor attached to "active" carboxylic acid residues such as acetyCoA and succinyl-CoA.

# Bioenergetics

## TCA – regulation

**KEY POINTS (which enzymes are regulated)**
**Stage 1 – citrate synthase (Ac-CoA joining -> citrate)**
**Stage 2 – isocitrate dehydrogenase (isocitrate oxidation -> oxalosuccinate(alpha-ketoglutarate))**
**Stage 3 – alpha-oxoglutarate dehydrogenase (alpha-ketoglutarate oxidative decarboxylation -> succinyl-CoA)**

HOW to regulate TCA?
(a) Changes in the rate of enzyme synthesis, Induction/repression. (timescale – hours)

(b) Covalent modification by reversible phosphorylation. (very quick)

Stage 3 enzyme is very similar to enzyme of pyruvate conversion into Ac-CoA – regulation similar. Phosphorylated enzyme is not active. Therefore, dephosphorylation of enzyme complex induced by insulin activates it. On the other hand, phosphorylation is triggered by high ATP:ADP ratio, NADH:NAD+, Ac-CoA:CoA-SH, increase cyclicAMP

(c) Allosteric modification.
High ATP – negative on stage 1, negative on stage 2
High NADH – negative on stage 2
High ADP – positive on stage 2

# Bioenergetics

## TCA – anabolic/catabolic?

TCA – AMPHIBOLIC

Catabolic property = 2Ac-CoA is coverted into energy (ATP/NADH/FADH2), CO2 etc (Ac-CoA comes from carbohydrates/lipids/proteins)

Anabolic property = intermediates used in the biosynthesis

Heme synthesis – uses succinyl-CoA
Cholesterol followed by steroids synthesis – uses Ac-CoA
Long chain fatty acids synthesis – uses citrate flow from mitochondria (citrate is converted to Ac-CoA extramitochondrially)
Aminoacid synthesis –

- alpha-ketoglutarate – Arginine, Histidine, Glutamine, Proline, Glutamate
- fumarate – phenylalanine, tyrosine
- Succinyl-CoA – Valine, Isoleucine, Methionine
- oxaloacetate – Aspartate

AA that depends on pyruvate - Glycine, alanine, serine, cysteine/cystine, threonine, Hydroxy-Proline and tryptophan

# Bioenergetics

## ENERGY FROM Glucose (The net score)

1. **Glucogenesis**
   in: *1glucose*
   out: 2ATP + 2NADH + *2pyruvate* (for glycogen 3ATP +2NADH +*2pyruvate*)

2. P**yruvate dehydrogenase complex**
   in: *2pyruvate*
   out: 2NADH + *2Ac-CoA*

3. **TCA**
   in: *2Ac-CoA*
   out: 6NADH + 2GTP/ITP + 2FADH2

--- (energy currency rate) ---
NADH = 3ATP (in electron-transfer chain)
FADH2 = 2ATP (in electron-transfer chain)
GTP/ITP = ATP (substrate level phosphorylation)

**TOTAL SCORE**
1glucose = 2 + 30 + 2 + 4 = 38ATP (39ATP for glycogen) AEROBICALLY!!
(this is in case of Malate shuttle, 36 for alphaglycerophosphate shuttle)

1glucose = 2 ATP ANAEROBICALLY!

# Bioenergetics

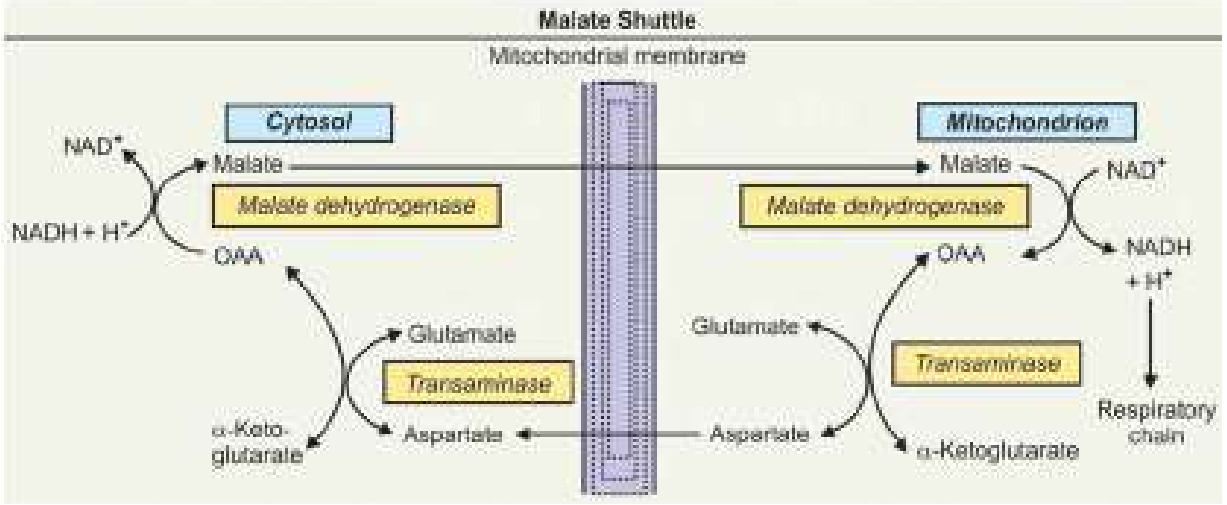
**WHAT AFFECTS THE NET BALANCE OF ENERGY REACTION from glucose???**

**Transfer of compounds through membrane**

Glycolysis – extramitochondrial, pyruvate-to-AcCoA – intramitochondrial, TCA – intramitochondrial. NADH/FADH2 are utilized in electrontransfer chain (mitochondrial), so NADH from glycolysis should be transferred to electron-transfer chain. Mitochondrial membrane is not permeable for NADH!!!!

*Shuttle systems* ("brothers"-enzyme systems are present on both sides on mt membrane)

- Trough transfer of Malate to mt/Aspartate from mt (theres intermediate of OAA and alpha-ketoglutarate)




*Another way:* Through transfer of glycerophosphate to mt (dihydroxeacetonephosphate back). This shuttle uses FADH2-generating enzymes inside the mt – therefore there will be loss of energy during transfer.

WHAT else uses shuttle systems?

(Citrate shuttle – for Ac-CoA)

# Bioenergetics

## Effects

**Pasteur effect –**

**AERATION decreases glycolysis (less glucose is used by bacteria and less alcohol is formed)**
**With working TCA a lot of ATP is formed – inhibits glycolysis at 1$^{st}$ stage**

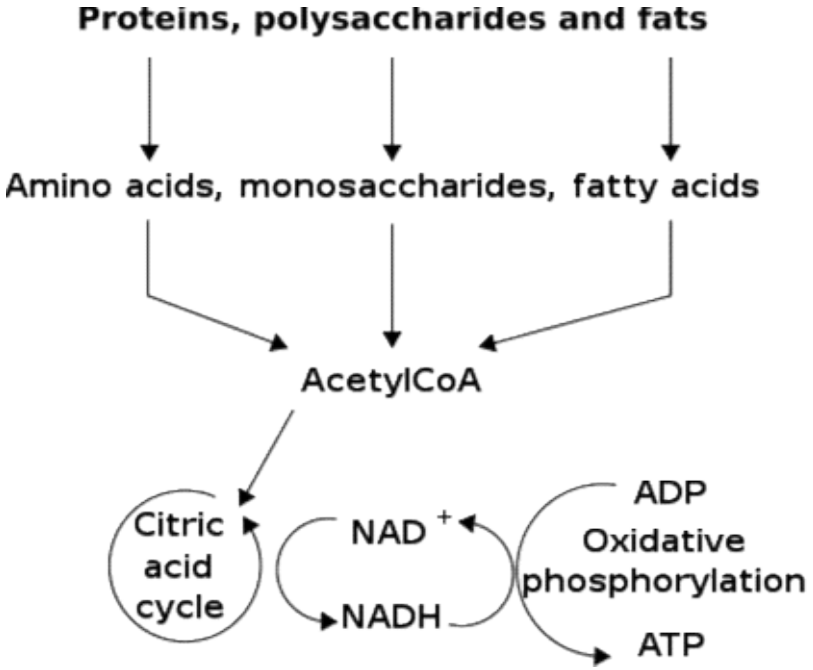
# Bioenergetics

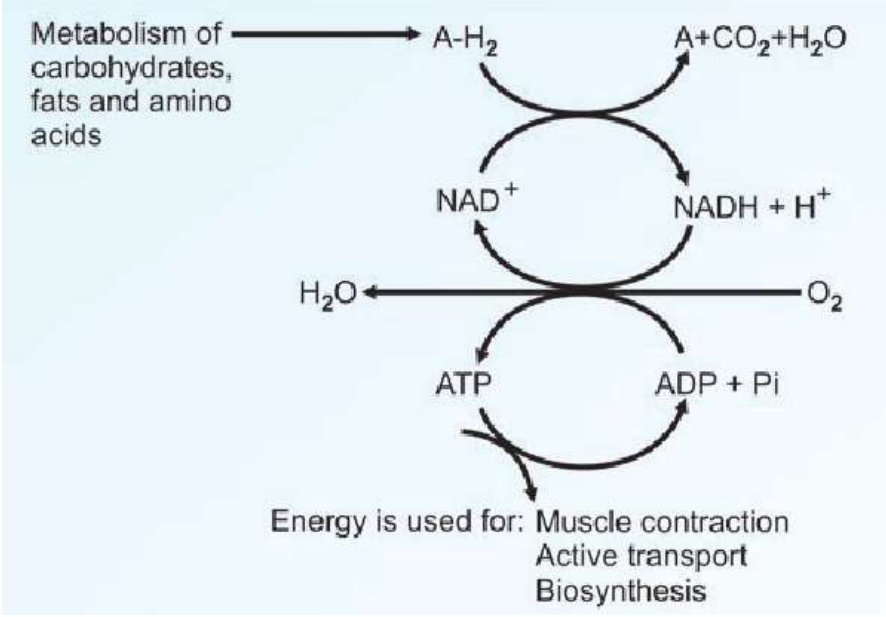
## Biological oxidation and electron transfer chain

Metabolism

1. Primary – digestion of macromolecular food into monomers
2. Secondary – catabolization of monomers/derivatives –
   generation of O2 and reduced equivalents NADH, FADH2 (majorly in TCA)

3. Tertiary (**internal respiration**) – reduced equivalents go to electron transfer chain (ETC) and release energy, which is captured into ATP



Literature biochemistry

1. Lehninger Principles of Biochemistry (Nelson D.L., Cox M.M.)
2. Principles and Techiniques of Biochemistry and Molecular Biology (Wilson K., Walker J.)