# Glycolysis

- Color Index:

- Important.
- Extra Information.
- **Doctors slides.**

436 Biochemistry team

# Objectives :

- Recognize glycolysis as the major oxidative pathway of glucose
- List the main reactions of glycolytic pathway
- Discuss the rate-limiting enzymes/Regulation
- Assess the ATP production (aerobic/anaerobic)
- Define pyruvate kinase deficiency hemolytic anemia
- Discuss the unique nature of glycolysis in RBCs.

# Glycolysis

It is the **major** pathway for **glucose oxidation**, in the cytosol of all cells .

It works **aerobically or anaerobically**, depending on the availability of oxygen and intact mitochondria .

It allows tissues to **survive** in presence or absence of oxygen, e.g. Skeletal muscle.

RBCs rely completely on glucose as their metabolic fuel "metabolized by anaerobic glycolysis.

❖ We highly recommend you watch this video:

**1-Aerobic**
**(Available both : O2 & mitochondria)**

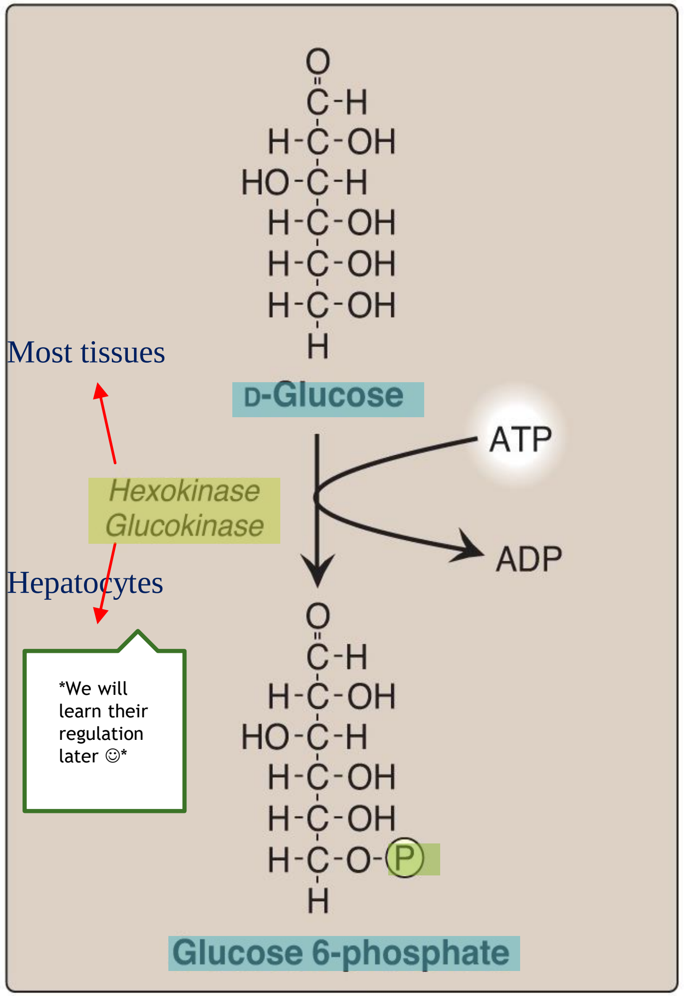
Glycolysis

**2- Anaerobic**
**(Absence of O2 or mitochondria or both of them)**

**The general concept:**
**Glycolysis** breaks down glucose and forms pyruvate with the production of two molecules of ATP. The pyruvate is the end product of **glycolysis**.

# Aerobic glycolysis reactions (1st and 2nd)

ايش الحكمة من إضافة مجموعة الفوسفات ؟ ببساطة عشان تسجن الجلكوز جوا الخلية ،كيف؟ لأن البروتينات اللي موجودة Cell membrane وتنقل الجلكوز من برا لجوا الخلية ما راح تتعرف على الجلكوز إذا كان معه بالتركيب أي شيء ثاني مثل الفوسفات



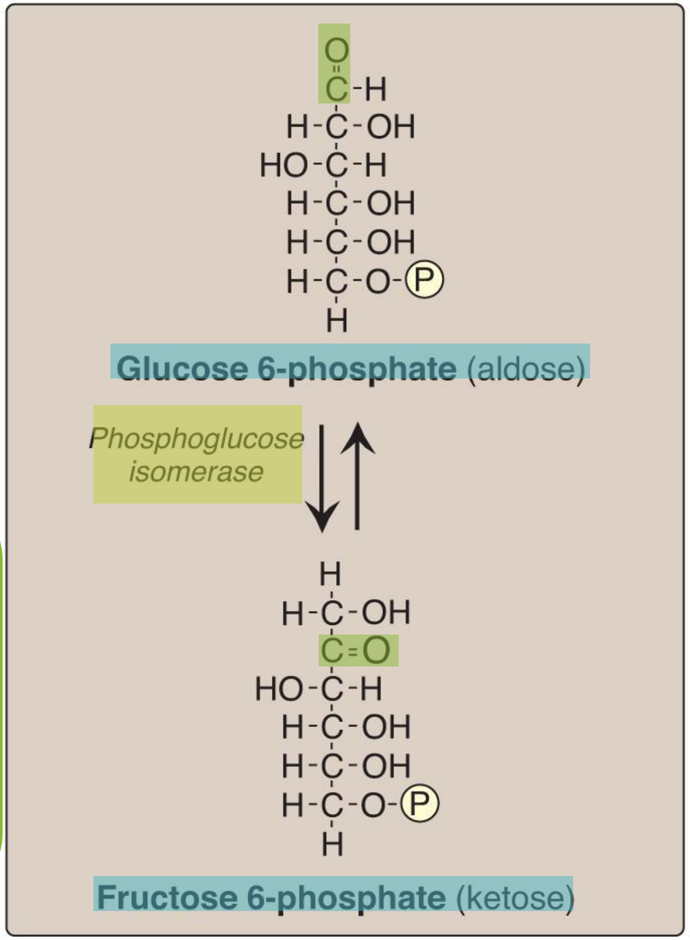



- **Reaction 1 :**
  From Glucose to Glucose 6-phosphate by hexokinase (in most tissues) or glucokinase (in liver), consuming 1 ATP.

Is irreversible and regulated step

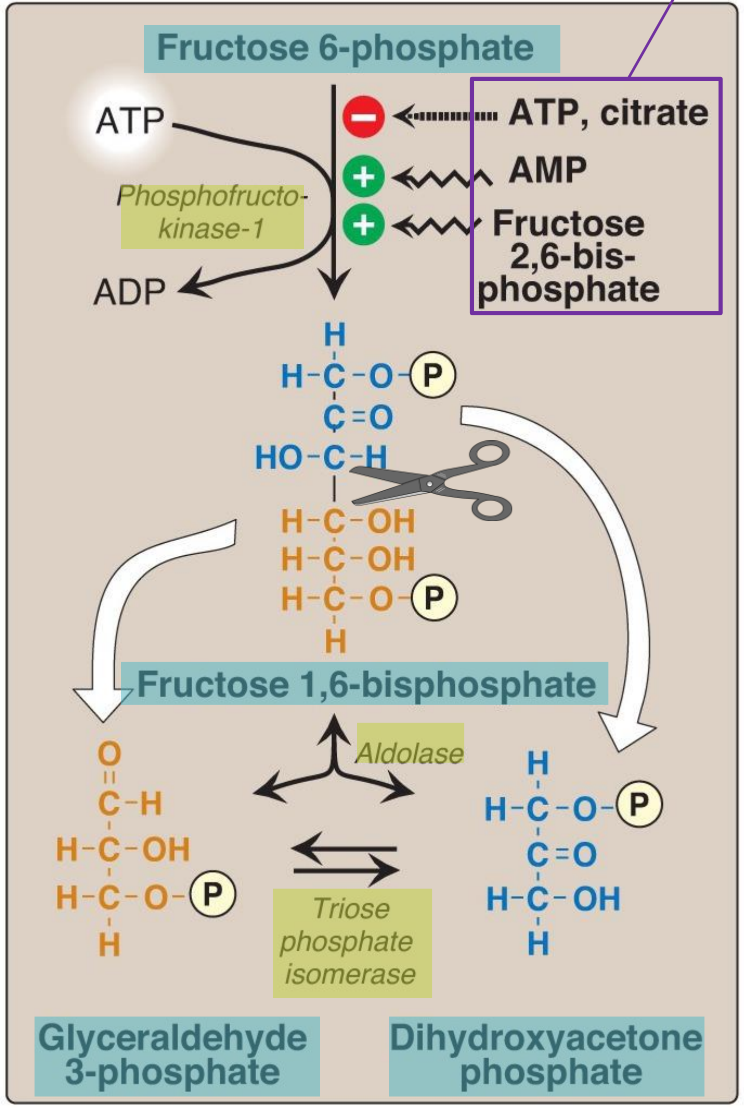
- **Reaction 2 :**
  From Glucose 6-phosphate to Fructose 6-phosphate by phosphoglucose isomerase. (isomerization)
  No consumption of ATP.

Is reversible and is not a rate-limiting or regulated step



# Aerobic glycolysis reactions (3rd - 5th)

*Will be explained later*






❖ **Reaction 3 :**
From Fructose 6-phosphate to Fructose 1,6-biphosphate by phosphofructo-kinase-1
consuming 1 ATP.

Is irreversible and regulated step

❖ **Reaction 4 :**
Fructose 1,6-biphosphate cleavage (split) into both Glyceraldehyde 3-phosphate and Dihydroxyacetone phosphate by aldolase
No consumption of ATP.

Is reversible and is not a rate-limiting or regulated step

❖ Reaction 5 :
From Dihydroxyacetone phosphate to Glyceraldehyde 3-phosphate
(total of 2 G3P in the reaction)
No consumption of ATP.
*reversible*

❖ Reactions 1-5 :
Energy consuming reactions

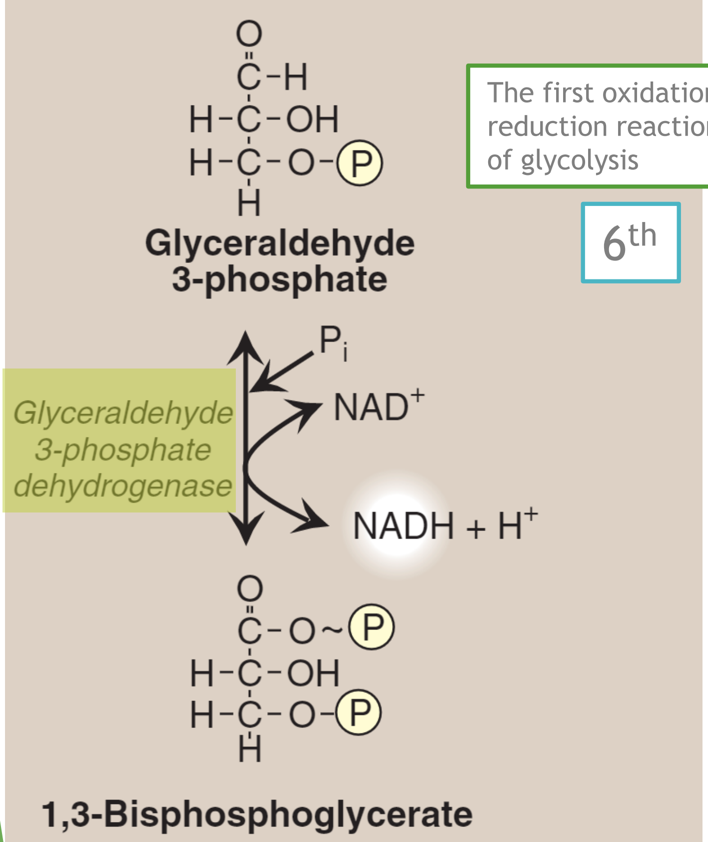
Notes ☺
The enzyme is called "aldolase" because it splits fructose 1,6-bisphosphate the "aldol" into two 3C phosphates:

- Di-hydroxy-acetone phosphate
- Glycer-aldehyde 3-phosphate.

(isomers of each other)

**Understand the molecules:**
Acetone= the simplest ketone (3C)
Glyceraldehyde= 3C monosaccharide, the simplest aldose.

# Aerobic glycolysis reaction (6th - 10th)




The first oxidation-reduction reaction of glycolysis

6th

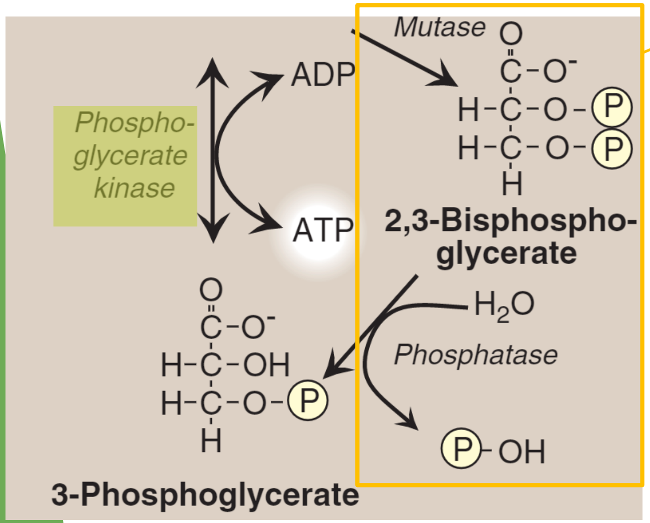
## 6th Reaction : (Oxidative level)

Oxidation to the molecule NAD+ →NADH
this reaction adds Phosphate group to the molecule by **Glyceraldehyde 3-Phosphate Dehydrogenase**

❖ Reactions 6-10 :
Every reaction is multiplied by 2, due to having 2 G3P **(glyceraldehyde 3-phosphate)**

*They're Energy **producing** reactions*

Understand the molecules involved:
Glyceraldehyde → when it's oxidized it becomes Glycerate (an acid)

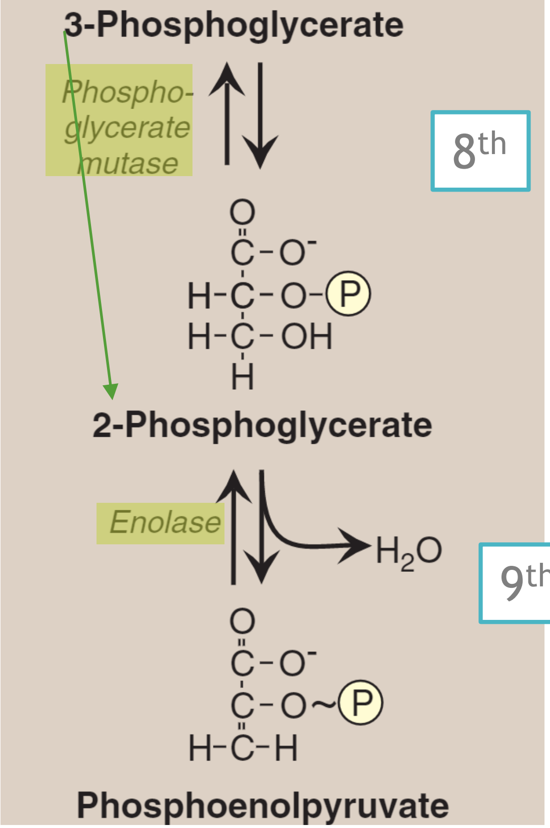



*We'll learn about this later in glycolysis in RBCs *

7th

## 7th Reaction : (Substrate-level)

Phosphate group add to ADP to become ATP.
By **Phosphoglycerate kinase**

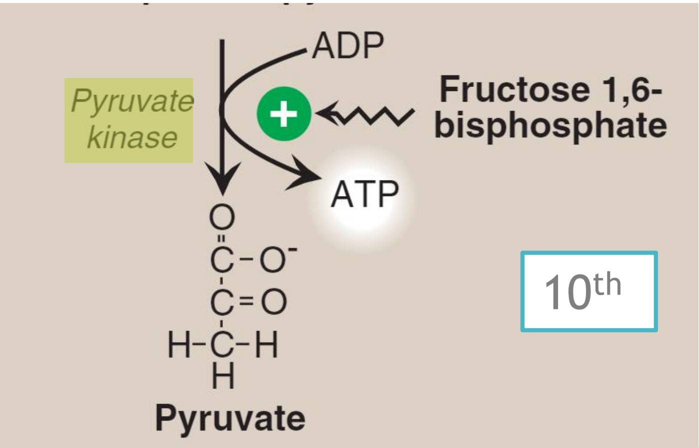



## $8^{th}$ Reaction :

It is isomer and what changes is : The P group change position from O in carbon-3 to O in carbon-2 by phosphoglycerate mutase

## $9^{th}$ Reaction :

Phosphoglycerate change to phosphoenolpyruvate by remove water by Enolase



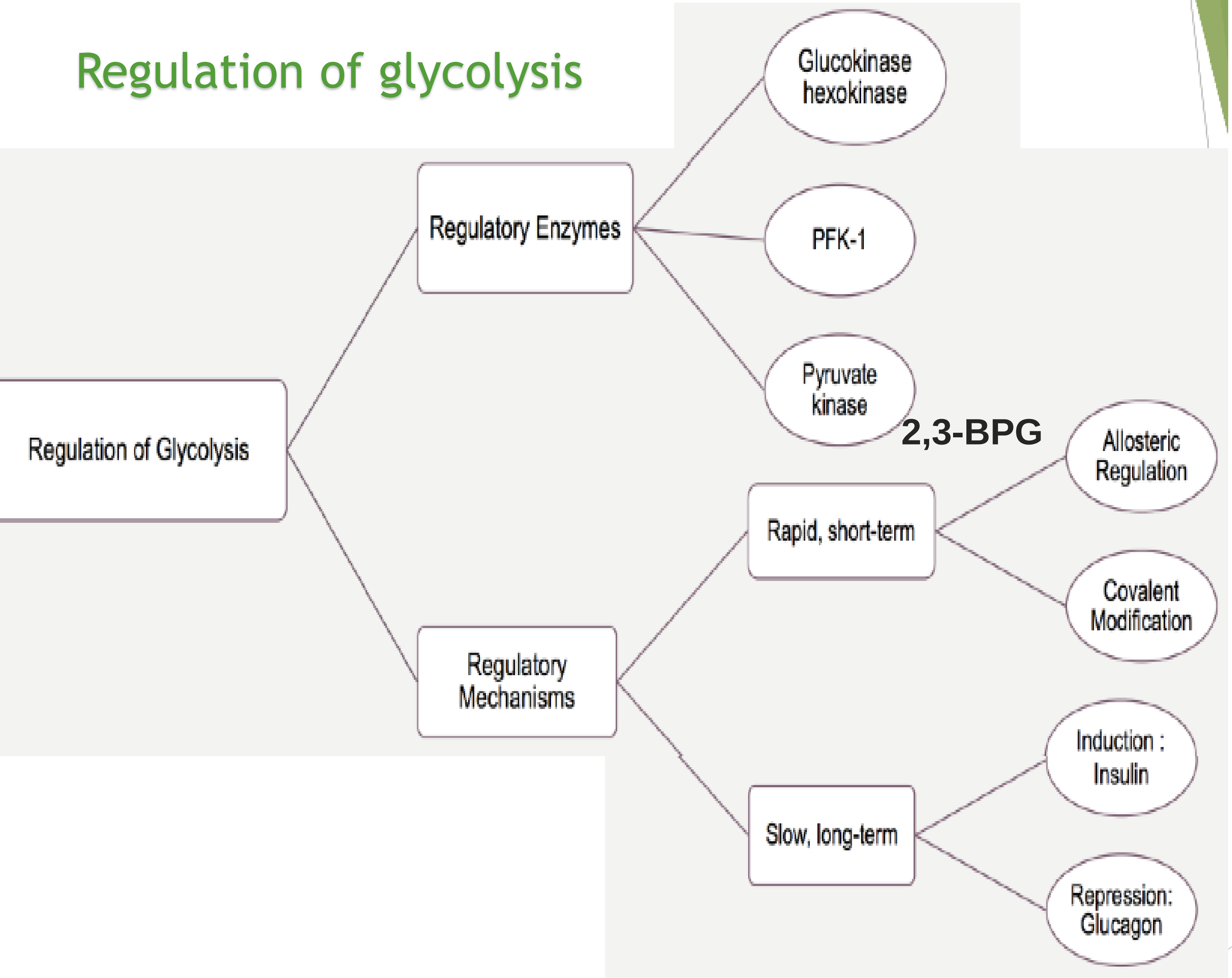

*End product of Glycolysis*

## $10^{th}$ Reaction : (Substrate-level)

Phosphoenolpyruvate → pyruvate by Pyruvate kinase .
Fructose 1,6-bisphosphate formed in 3rd step , it will go to the last step (it is Allosteric)

Is irreversible and regulated step

# Regulation of glycolysis




**Regulation of glycolysis:**

- **Regulatory enzymes**

The four regulatory enzymes are hexokinase, glucokinase, phosphofructokinase, and pyruvate kinase.

- **Mechanism of regulation:**
- Either short-term and rapid (we will learn this first- the next slides)
- Or long-term and slow (slide #13)

# Regulation of Hexokinase and Glucokinase

Regulation by: allosteric effectors.

Regulation (تنشيط او تثبيط) of hexokinase (in most cells) and glucokinase (in liver)

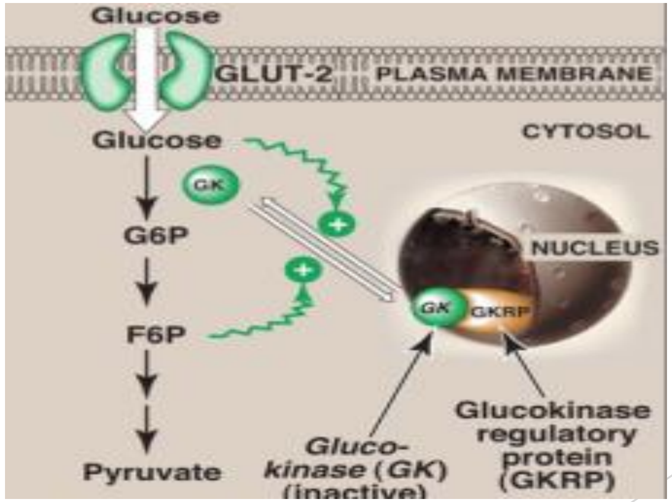
- **Hexokinase** : when Glucose 6-P (Fructose 6-phosphate is in equilibrium with it) is abundant it will indicate to the cell that it doesn't need hexokinase anymore and it will be inhibited directly.

**Glucokinase:** is an enzyme that facilitates **phosphorylation** of glucose to glucose-6-phosphate.
It is is an isozyme of hexokinase.

Glucokinase (hexokinase type 4)

Inhibited indirectly by Fructose 6-P

In the presence of high Fructose 6-P, Glucokinase will translocate and tightly bind to GKRP **(Glucokinase regulatory protein)** in the nucleus making it inactive.

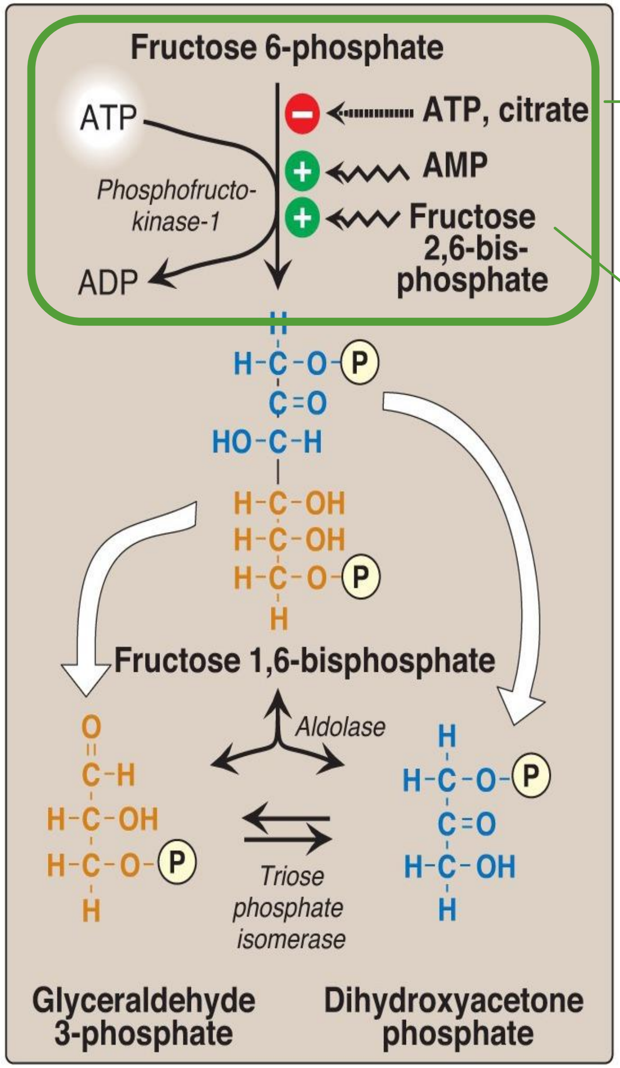



Stimulated indirectly by Glucose

when glucose levels is high in blood and hepatocytes (GLUT-2), glucokinase is released from GKRP to the cytosol.
GLUT-2 is glucose transporter in liver

# Regulation of PFK-1 (phosphofructokinase-1)

Regulation by: allosteric effectors.






When ATP and Citrate are abundant (more than enough) they inhibit the reaction

N.B they are not involved in the chemical reaction they have **allosteric** effect

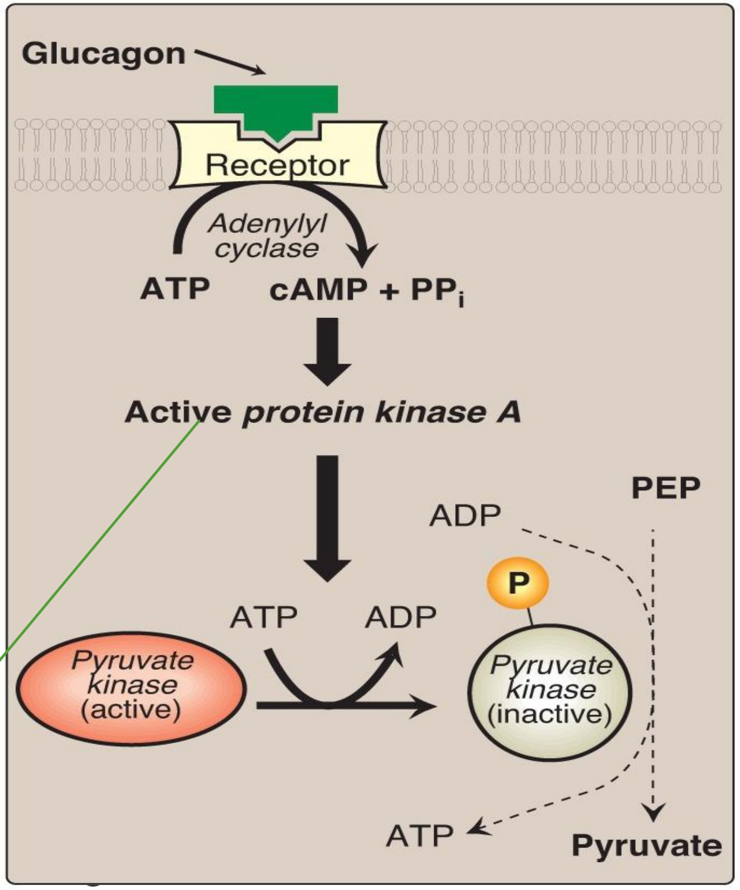
In contrast, AMP and Fructose 2,6-bisphosphate indicate low level of energy so when they're abundant they will activate the reaction

N.B they are not involved in the chemical reaction, they have an **allosteric** effect

PFK-1 (phosphofructokinase-1)
N.B it's a rate-limiting enzyme (because it can be inhibited which will stop the glycolysis)

# Pyruvate Kinase

## Regulation by: Covalent Modification




Covalent modification of hepatic *pyruvate kinase* results in inactivation of enzyme.

*Remember:
Converting PEP (Phosphoenolpyruvate) to Pyruvate is the last step in glycolysis

**Active Protein Kinase A** inhibits the transformation to pyruvate by phosphorylation of pyruvate kinase which leads to the inhibition of glycolysis.

**Pyruvate kinase** is the enzyme that catalyzes the final step of glycolysis. It catalyzes the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP), yielding one molecule of pyruvate and one molecule of ATP.

**Covalent modifiers** serve as regulators by controlling the phosphorylation and dephosphorylation of enzymes, resulting in the activation and inhibition of enzymatic activity.

**Example:** covalent modifiers activate protein kinase A which in turn phosphorylates, and deactivates pyruvate kinase.

# Pyruvate kinase deficiency: hemolytic anemia

- Pyruvate kinase deficiency is due to **genetic mutation**
- (it affects the survival of red blood cells)

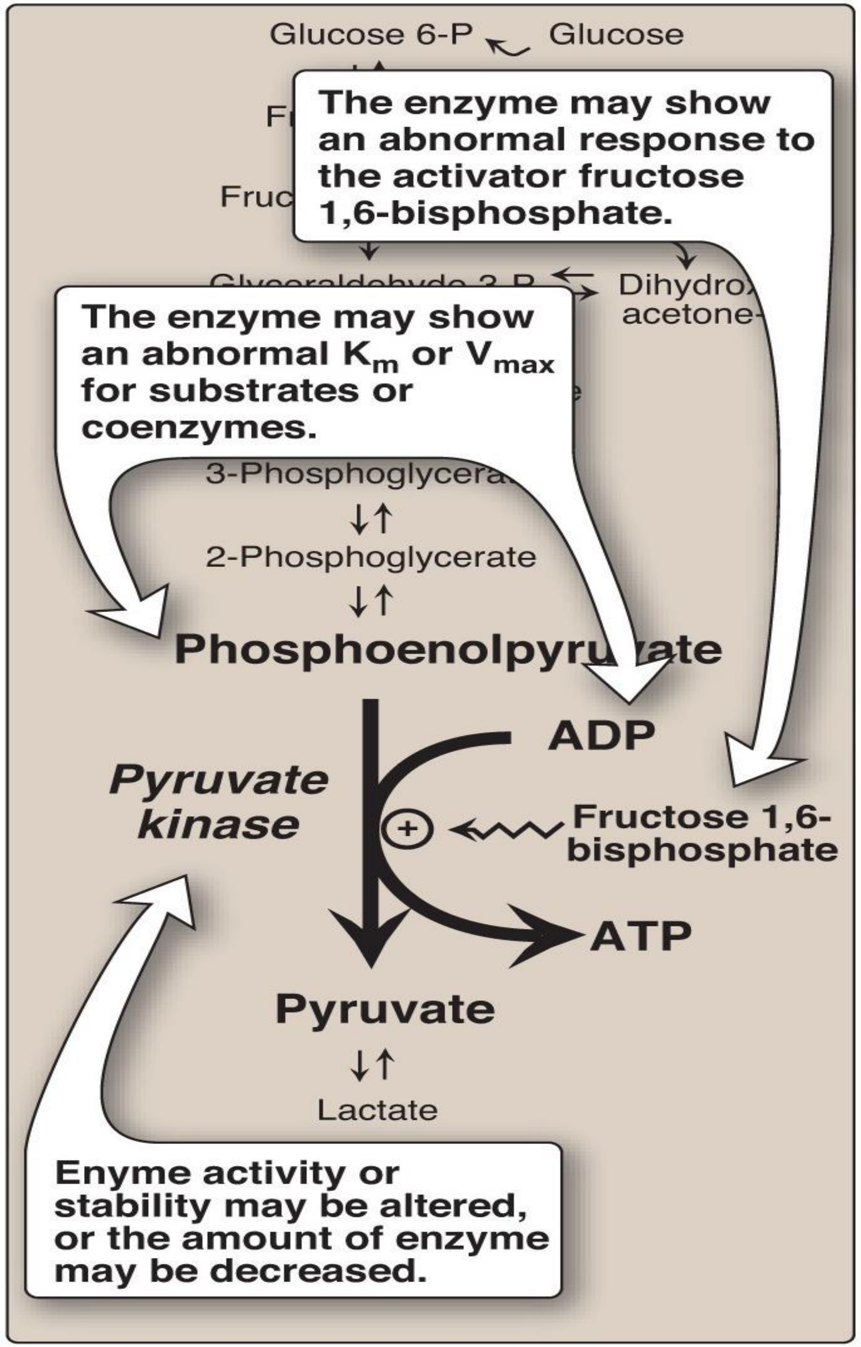
- **PK Mutation may lead to:**

  - Altered enzyme **Kinetics** .
  (inhibiting enzyme activity)
  (mutation in other than the active site)

  - Altered **response to activator.**
  (stopping enzyme activity)
  (mutation in the active site)

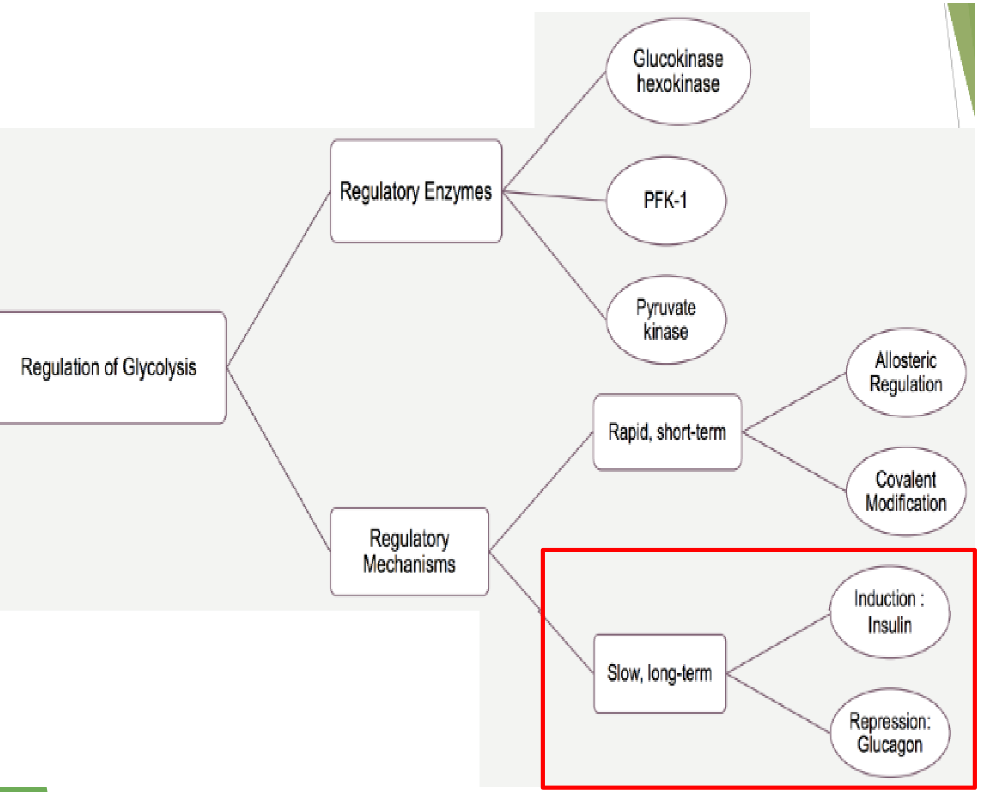
  - Altered enzyme **stability**.

  - Decreased **amount** of the enzyme



# Long term Regulation (Hormonal)

- It is slow



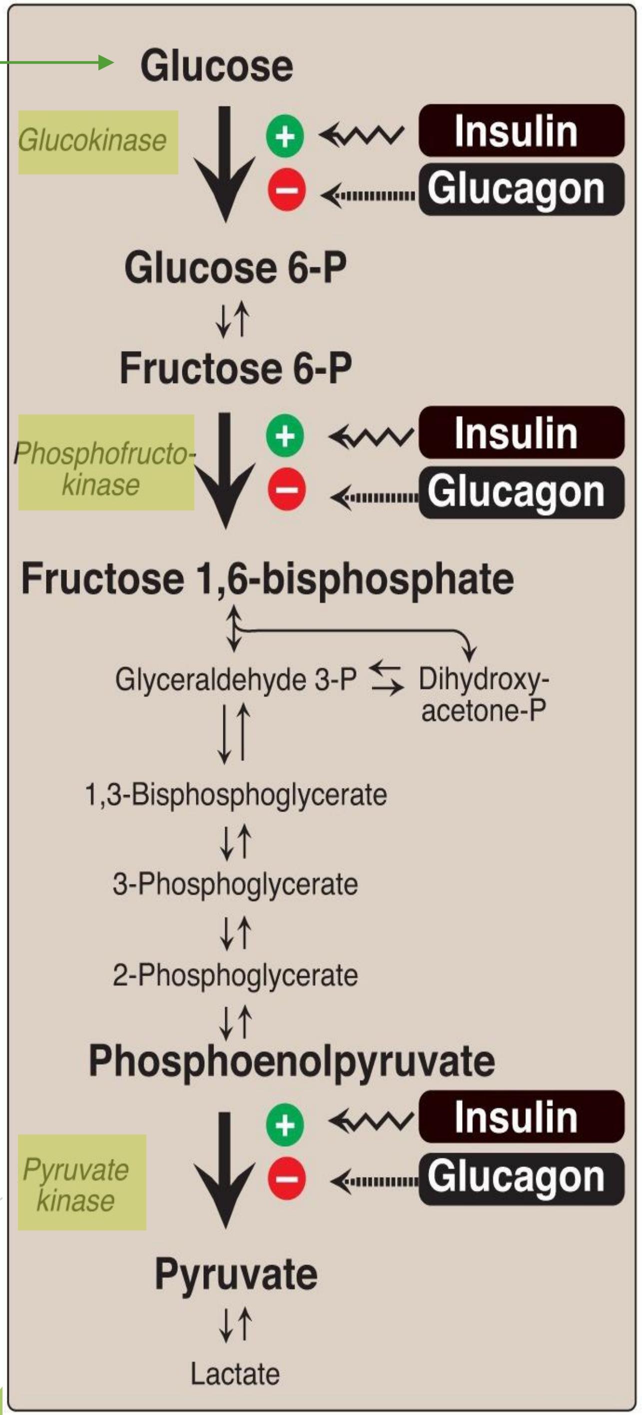

**Effect of insulin and glucagon on the synthesis of key enzymes of glycolysis in liver.**

**Insulin: (+)**

- Glucokinase
- Phosphofructokinase
- Pyruvate Kinase

**Glucagon: (-)**

- Glucokinase
- Phosphofructokinase
- Pyruvate Kinase



# Substrate-level phosphorylation Vs. Oxidative phosphorylation

❖ Phosphorylation is the metabolic reaction of introducing a phosphate group into an organic molecule.

| Oxidative phosphorylation | Substrate-level phosphorylation |
|---|---|
| The formation of high-energy phosphate bonds by phosphorylation of ADP to ATP | The formation of high-energy phosphate bonds by phosphorylation of ADP to ATP (or GDP to GTP) |
| the transfer of electrons from reduced coenzymes to molecular oxygen by ETC | cleavage of a high-energy metabolic intermediate (substrate). |
| mitochondria | cytosol or mitochondria |

Simple explanation ☺
Substrate-level: production of ATP molecules via transfer of a phosphate group from an intermediate high-energy substrate directly to ADP.

Oxidative: production of ATP molecules from the redox reactions of an electron transport chain

Gylcolysis and Krebs cycle use substrate-level phosphorylation. Electron transport chain uses oxidative phosphorylation.

**Substrate-level phosphorylation** is really important for:

- RBCs (they don’t have mitochondria)
- Muscle cells in oxygen-depleted condition.

# Aerobic Glycolysis: ATP Production

- ❖ Each NADH = 3 ATP will be produced by ETC in the mitochondria.
- ❖ ATP Consumed: 2 ATP
- ❖ ATP Produced:
  - Substrate-level: 2 X 2 = 4 ATP
  - Oxidative-level: 2 X 3 = 6 ATP
- ❖ Total: = 10 ATP

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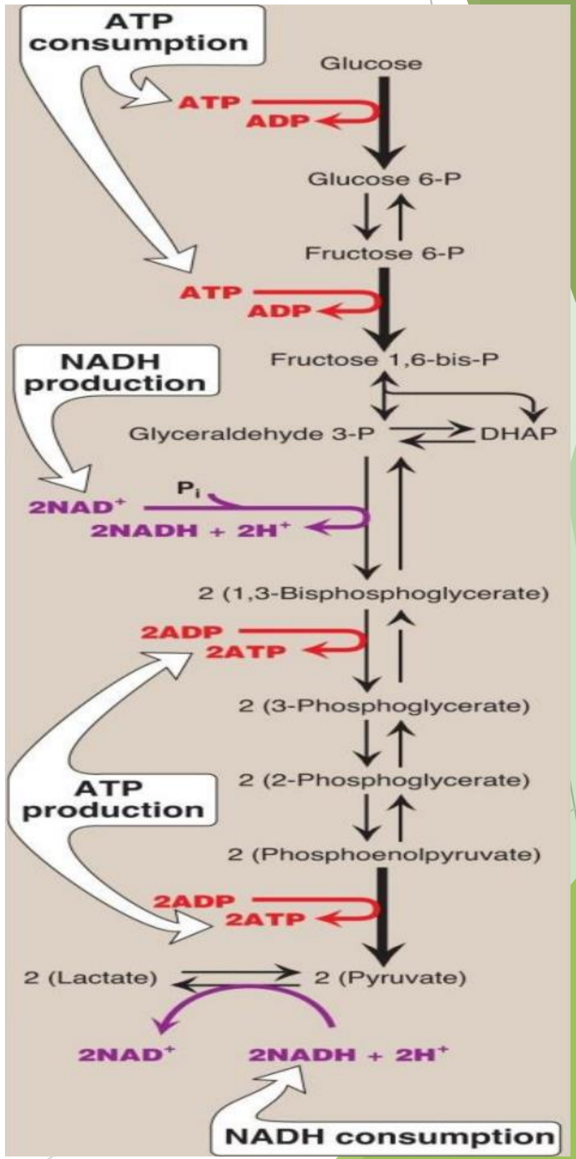
- ❖ Net: 10 – 2 = **8** ATP



# Anaerobic Glycolysis

Overview:
**Anaerobic glycolysis** is the transformation of glucose to lactate when limited amounts of oxygen ($O_2$) are available. **Anaerobic glycolysis** is only an effective means of energy production during short, intense exercise, providing energy for a period ranging from 10 seconds to 2 minutes.

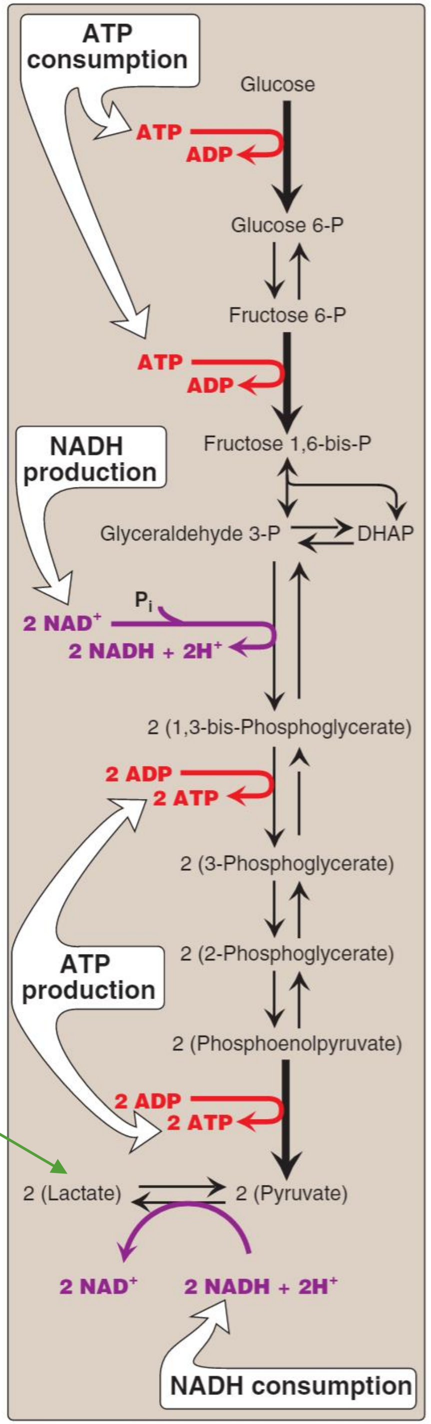
☺ Anaerobic Glycolysis is important in RBCs because they don't have mitochondria
*Will learn more in the next slides*

- **Anaerobic glycolysis less ATP production than aerobic.**

- The end product of anaerobic glycolysis is lactate "obligatory output". (why?)
Because if is not formed, All cellular NAD+ will be converted to NADH, with no means to replenish (fill again) the cellular NAD →Glycolysis stops →death of the cell

**From Lippincott**

Summary of anaerobic glycolysis. Reactions involving the production or consumption of ATP or NADH are indicated. The three irreversible reactions of glycolysis are shown with thick arrows. DHAP = dihydroxyacetone phosphate.

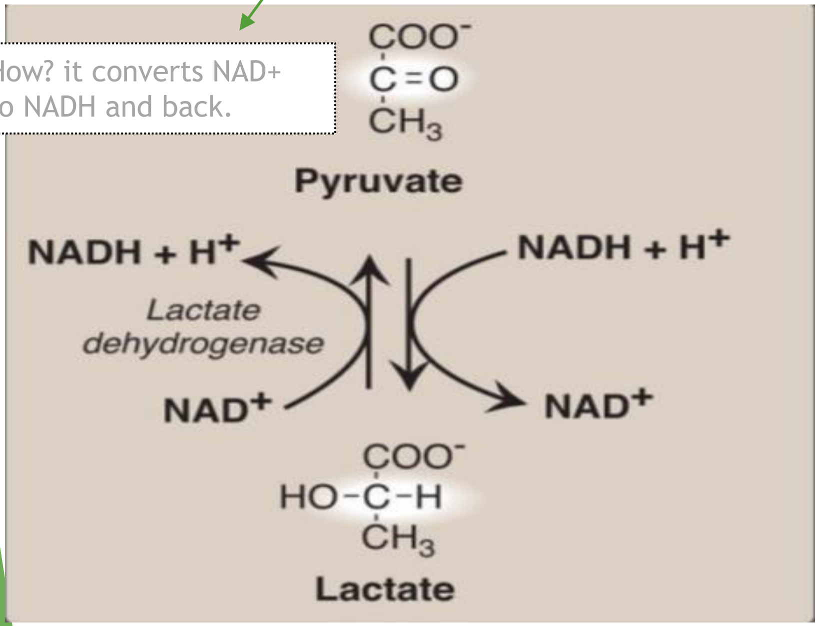


## Anaerobic Enzymes:

### Lactate Dehydrogenase

- An enzyme that catalyzes the conversion of lactate to pyruvic acid and back (reversible reaction)

How? it converts NAD+ to NADH and back.




# Anaerobic Glycolysis: ATP production

- ATP Consumed: 2 ATP
- ATP Produced:
  - Substrate-level: 2 X 2 = 4 ATP
  - ~~Oxidative-level: 2 X 3 = 6 ATP~~
- Total: = 4 ATP
- Net: 4 - 2 = 2 ATP

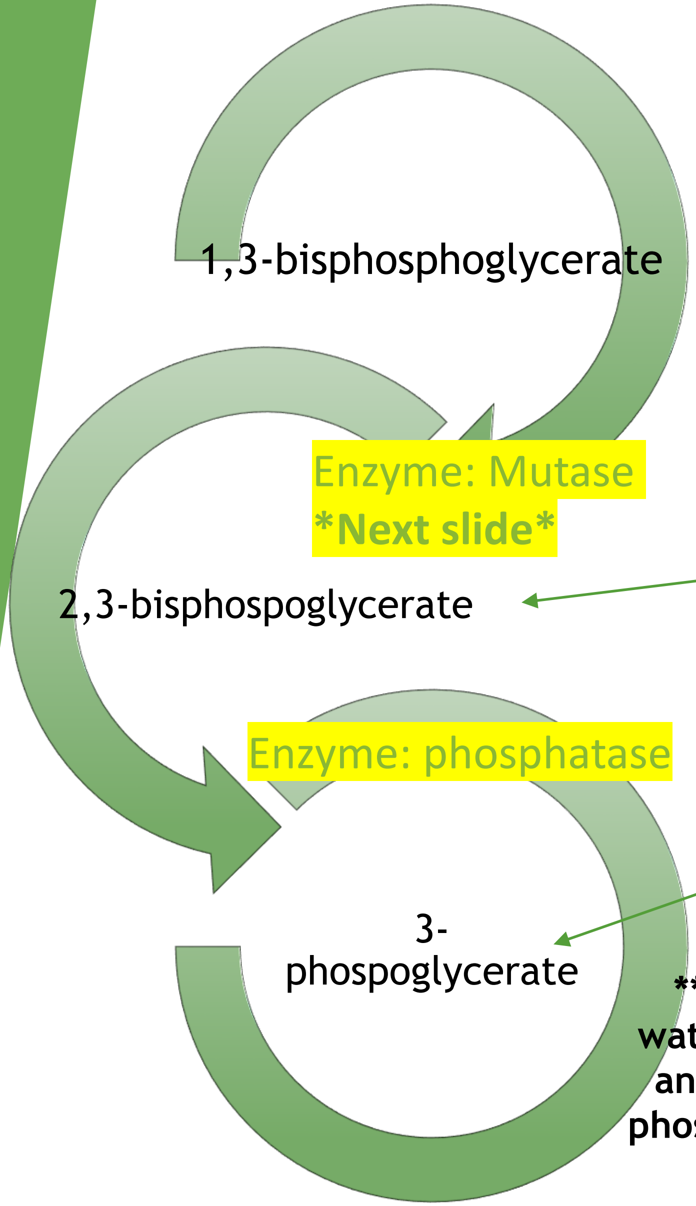
**In Anaerobic glycolysis:** Oxidative phosphorylation is cancelled, because the NADH molecules don't reach the ETC to produce ATP in anaerobic glycolysis.

**NADH** can NOT be used by ETC (oxidative-level) because:

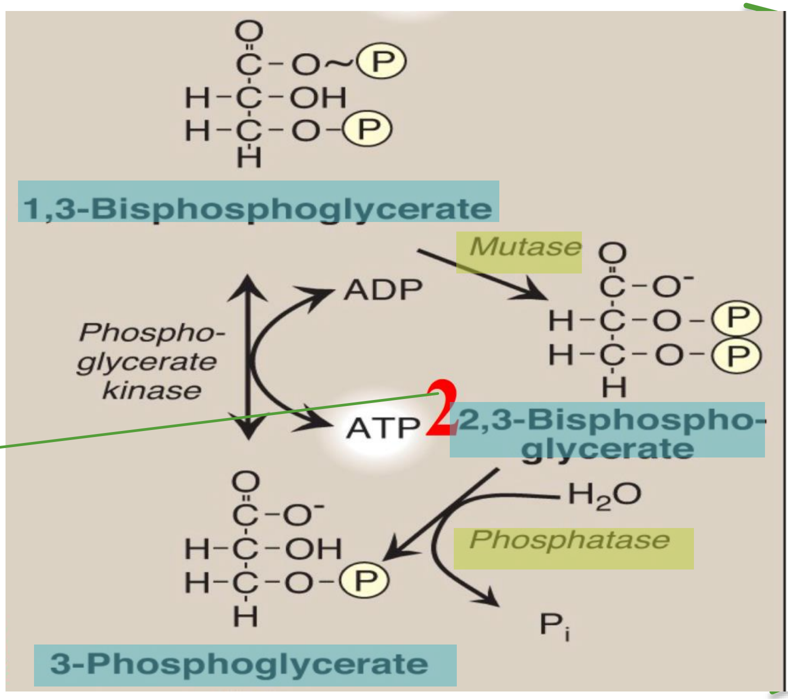
- there is no O2 and/or no mitochondria.

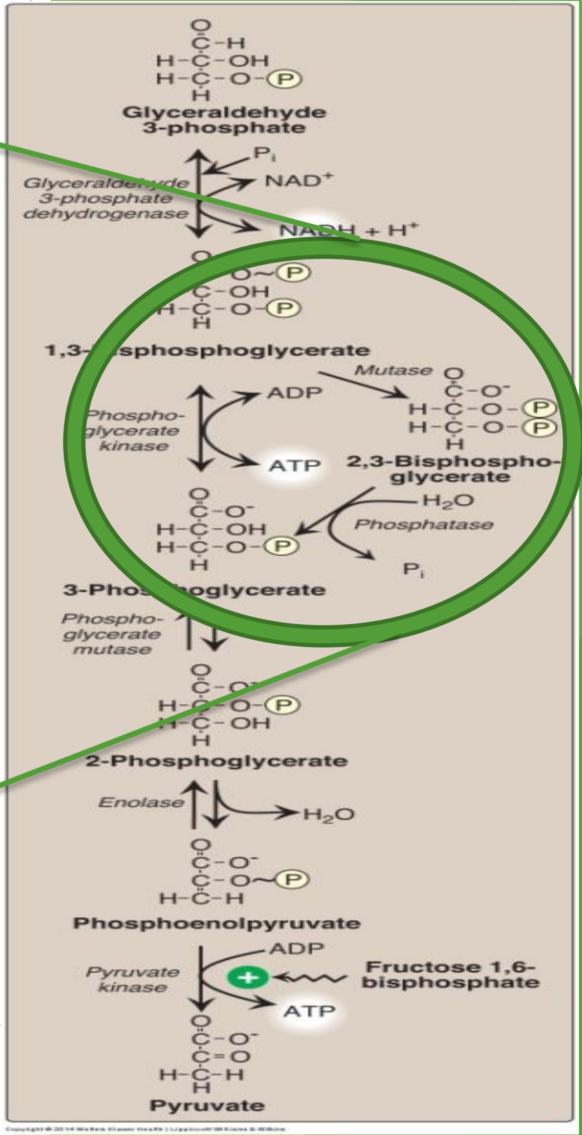
However, **NAHD** help in lactate production.

# Anaerobic Glycolysis in RBCs (2,3-BPG Shunt)




**by adding water molecule and removing phosphate group




- All the steps are the same with other anaerobic glycolysis except **2,3-BPG** Shunt→ only present in RBCs.
- 2,3-BPG: 2,3bisphospoglycerate

# Anaerobic Glycolysis in RBCs (2,3-BPG Shunt) Continued

**Mutase enzyme :**

- It is important for association and dissociation between $O_2$ and hemoglobin.
- Increase in "2,3-BPG" will help with loss of association between $O_2$ and hemoglobin and will release more $O_2$.
- It usually occurs with people who live in high altitude.

Remember:
1- NO production of ATP in formation of "2,3-BPG".
2- "2,3-BPG" comes back to "3-Phosphoglycerate" by Phosphatase enzyme

Glycolysis in RBC : ATP Production

| | |
|---|---|
| ATP consumed : | 2 ATP |
| ATP produced : | |
| Substrate-level | 2X2= 4 ATP Without Shunt |
| | Or 1X2= 2 ATP With Shunt |
| Total | 4 ATP |
| Net : | 4 – 2 = 2 ATP Without Shunt |
| | Or 2 – 2 = 0 ATP With Shunt |

# Summary: Aerobic glycolysis

Phosphorylation of glucose

Isomerization of glucose 6- phosphate

Phosphorylation of fructose 6- phosphate

Cleavage of fructose 1,6- bisphosphate

Isomerization of dihydroxyacetone phosphate

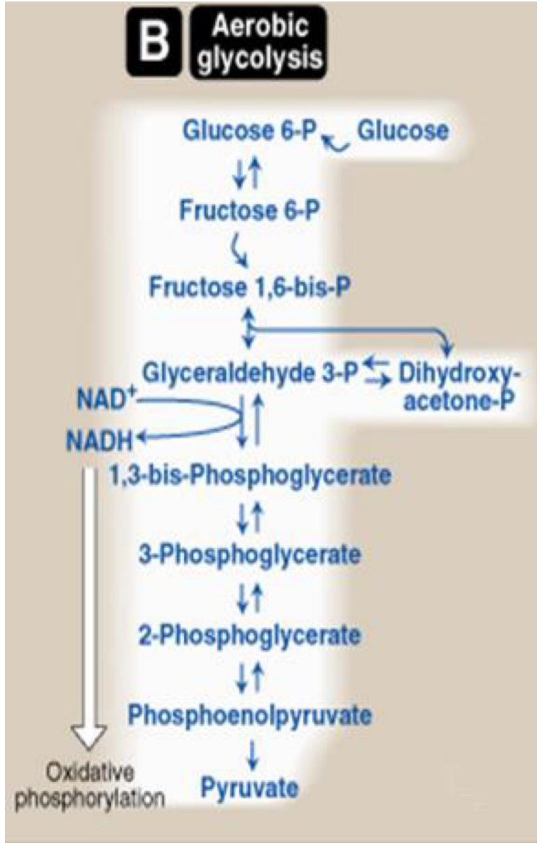
Oxidation of glyceraldehyde 3- phosphate

Synthesis of 3- phosphoglycerate , producing ATP

Shift of the phosphate group

Dehydration of 2- phosphoglycerate

Formation of pyruvate, producing ATP



# Glycolysis in RBC : Summary

- End product: Lactate
  No net production or consumption of NADH

- Energy yield: If no 2,3-BPG shunnt is formed: 2 ATP
  If 2,3-BPG shunt occurs: 0 ATP

- PK Deficiency hemolytic anemia depends on:
  Degree of PK Deficiency
  Compensation by 2,3-BPG

# MCQs

1-Where does glycolysis occur ?

A-inner of mitochondria B-matrix of mitochondria C- cytosol

2-What is the final molecule product of glycolysis ?

A-lactate B-phosphoenolpyruvate C-pyruvate

3-Which of the following is the 3rd molecule in the glycolysis pathway ?

A- fructose-1,6-biphosphate B- 3-phosphoglycerate

C- fructose-6-phosphate

4-How many ATP are used up in glycolysis per glucose(the net) ?

A- 4 B- 2 C- 6 D-0

5-How many NADH are produced by glycolysis per glucose ?

A- 4 B-6 C-0 D-2

- https://www.youtube.com/watch?v=hDq1rhUkV-g
- https://www.youtube.com/watch?v=FE2jfTXAJHg

1-c

2-c

3-a

4-b

5-d

- Girls team members:

1- روان سعد

2- نوره الشبيب

- Boys team members:

1- محمد المهوس

2- طلال الطخيم

3- خالد القحطاني

4- فهد العتيبي

5- عبدالعزيز الصومالي

6- هشام القوسي

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نوره السهلي.