Glycolysis

Editing File

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Objectives

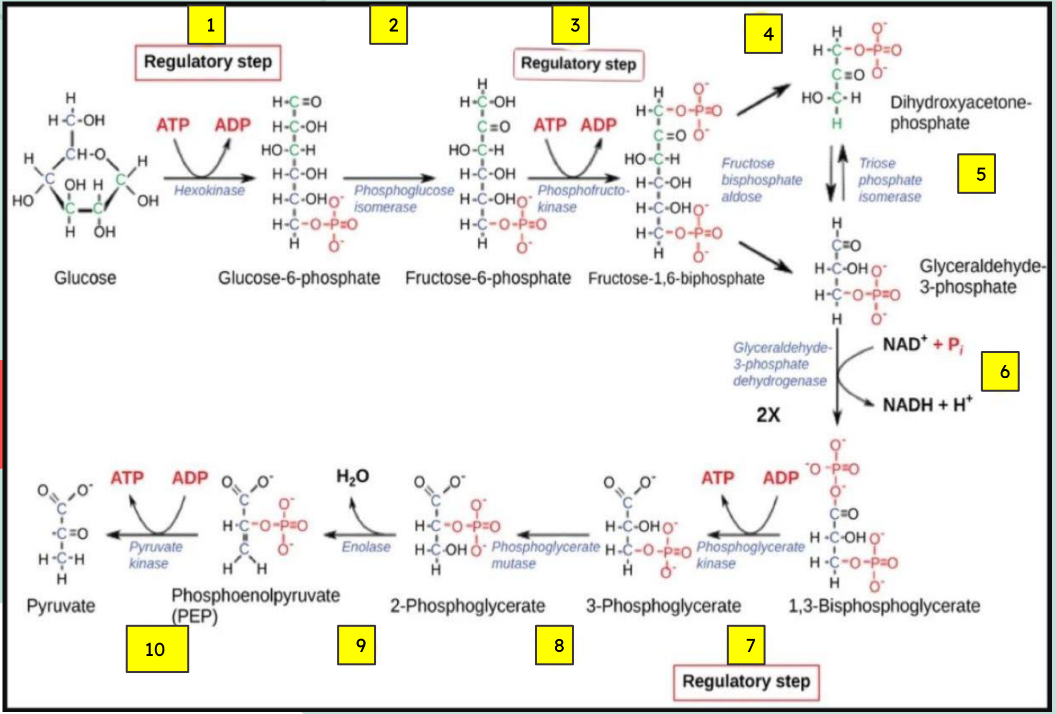
- Recognize glycolysis as the major oxidative pathway of glucose.
- List the main reactions of glycolytic pathway.
- Discuss the rate-limiting enzymes/Regulation.
- Assess the ATP production (aerobic/anaerobic).
- Define pyruvate kinase deficiency hemolytic anemia.
- Discuss the unique nature of glycolysis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

GLYCOLYSIS

- Glycolysis, the major pathway for glucose oxidation, occurs in the cytosol of all cells.
  (oxidative phosphorylation is in the mitochondria)
- It is unique, in that it can function either 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, which lack mitochondria, are completely reliant (depending) on glucose as their metabolic fuel, and metabolizes it by anaerobic glycolysis.

Aerobic Glycolysis Overview

Click on the number of the step:



Glycolysis consists of 10 steps

3 — **7**

Regulatory steps
- Irreversible
- Most important steps
- Steps where the enzymes are regulated

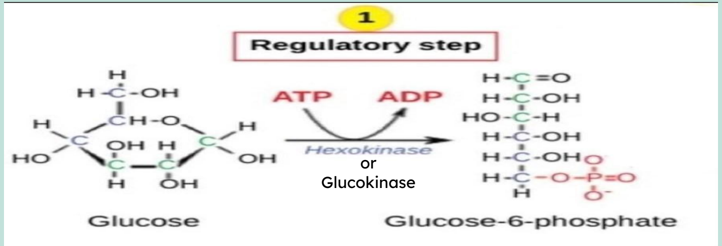
-Reversible

You need to know the products and the substrate and the enzymes for reaction 1,2,3,4,5,10
Will be explained next slides.

For the rest of the reactions you only need to know the amount of ATP and NADH produced from it except for reaction 9 you have to know the enzyme name

Aerobic glycolysis (1)

01



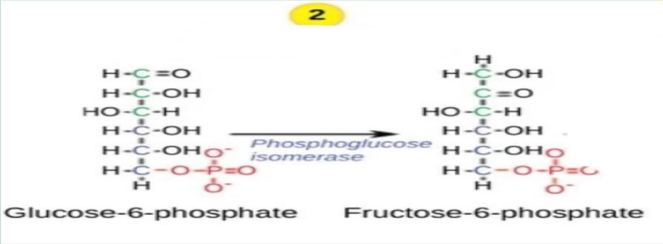
ATP in this step: -1 ATP
Net from first step till now: -1 ATP

Click on enzyme names:

- Kinase means phosphorylation enzyme " an enzyme that adds a phosphate group "
- Glucokinase is a hexokinase isozymes (isoforms) they both have the same function (adding phosphate group to the glucose molecule at the 6th C)
- ***Irreversible***
- ATP in (energy consuming) requires ATP
- Regulation of hexokinase and glucokinase

isoforms: enzymes doing similar functions but are different in structure

02

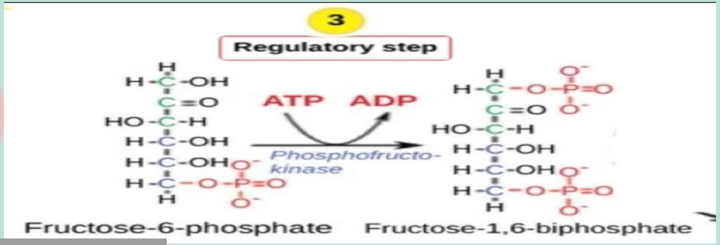


ATP in this step: ZERO
Net from first step till now: -1 ATP

- isomerase means it changes the configuration and no energy lost
- Reversible
- Isomerization from aldose form to ketos form

Aerobic glycolysis (2)

03

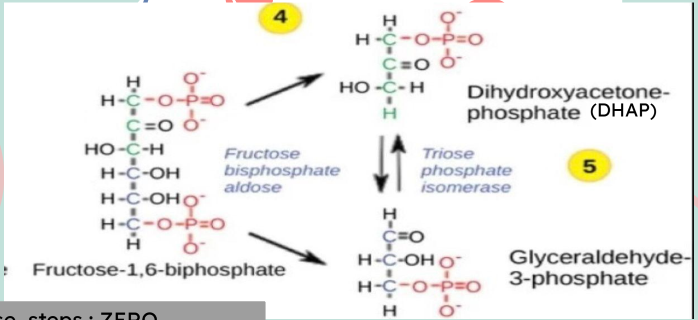


ATP in this step: -1 ATP
Net from first step till now: -2 ATP

- **Irreversible**
- ATP in (energy consuming)
  requires ATP
- ADP out
- Can be regulated :
  - Activated + AMP (a signal of low energy state) and Fructose 2,6-bisphosphate.
  - Inhibited - ATP and citrate. (they are signals of high energy state)
- Enzyme: Phosphofructokinase-1 (PFK-1)

Click on enzyme name:

4 & 5



ATP in these steps : ZERO
Net from first step till now: -2 ATP

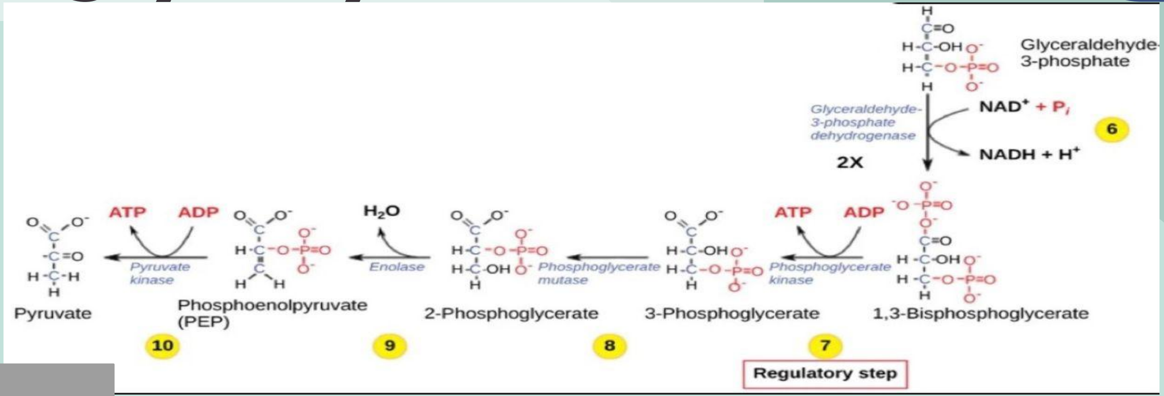
- Aldolase breaks down carbon-carbon double bond.
- Enzyme: Aldolase

- Isomerization from ketos form (DHAP) to aldose from (Glyceraldehyde 3-phosphate).
  - Enzyme: Triose phosphate isomerase

Aerobic glycolysis (3)

1 NADH -> 3 ATP
1 FADH2 -> 2 ATP

06 to 10



06

ATP in this step : +6 ATP
Net from first step till now: +4 ATP

2x (Pi and NAD+) in.

- 2x (NADH and H+) out(NADH is going to enter the ETC and Oxidative phosphorylation "in mitochondria" and produces 3 ATP)
- Enzyme: Glyceraldehyde 3-phosphate dehydrogenase

07

ATP in this step : +2 ATP
Net from first step till now: +6 ATP

- 2x ADP in.
- 2x ATP out. produces ATP
- Enzyme: Phosphoglycerate kinase

8 and 9

ATP in these steps : ZERO
Net from first step till now: +6 ATP

Enzyme: Phosphoglycerate mutase

Enzyme: Enolase (removes water molecule)

10

ATP in this step : +2 ATP
Net from first step till now: +8 ATP

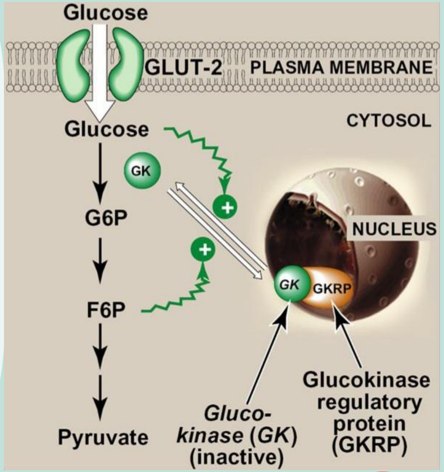
Click on enzyme name:

- **Irreversible**
- 2x ATP out. produces ATP
- Can be regulated (activated) : Fructose 1,6-Bisphosphate .
- Regulation of pyruvate kinase A
- Enzyme: Pyruvate kinase

Regulation: Glucokinase/Hexokinase

Regulation of : hexo<u>kinase</u> (in most cells) and gluco<u>kinase</u> (in liver or we can say hepatocyte).

- **Hexo<u>kinase</u>**: it is **<u>inhibited</u>** by the reaction product, glucose-6-P <u>(**1st** reaction)</u> which accumulates when further metabolism of this hexose is reduced.
- **Glucokinase** : (GK): It is inhibited <u>indirectly</u> by Fructose-6-P <u>(2nd reaction)</u> and is <u>indirectly</u> stimulated by glucose.
  - In the presence of high fructose-6-phosphate, Glucokinase (GK) translocates and binds tightly to GKRP (glucokinase regulatory protein) in the **<u>nucleus</u>**, making it **inactive** (by translocation into the nucleus).
  - When glucose levels are high in blood and hepatocytes (GLUT-2), GK is released from GKRP and enters the **<u>cytosol</u>**.



Regulation: Glucokinase/Hexokinase cont

	Hexokinase	Glucokinase
Site	All tissues	hepatocytes
Inhibited	glucose-6-Phosphate	Fructose-6-Phosphate
Stimulated by	-	Glucose (indirectly)

Regulation of enzymes: PFK-1 & Pyruvate Kinase

Phosphofructokinase-1 (PFK-1) enzyme:

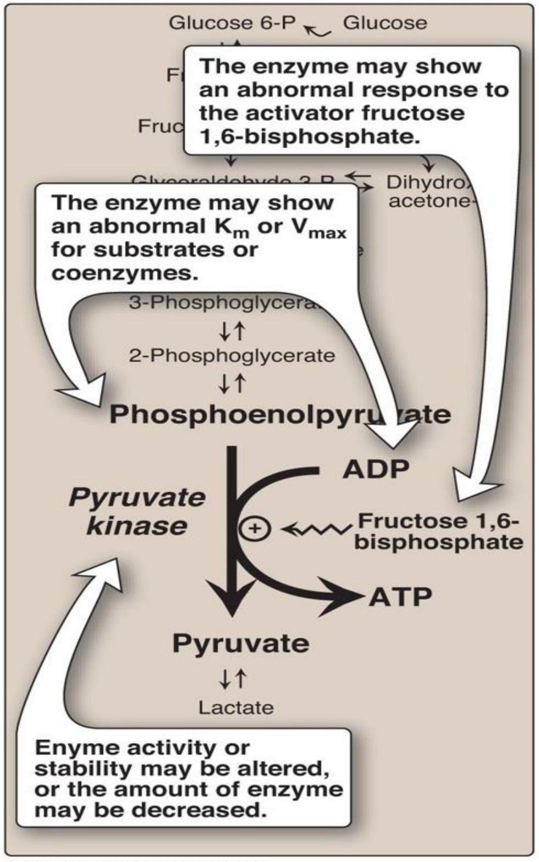
- Rate limiting enzyme
- Reaction number 3 in glycolysis , is Irreversible reaction
- Can regulate glycolysis through allosteric regulation.
- \+ Activated by AMP and Fructose 2,6-bisphosphate.
- \- Inhibited by ATP and citrate.
- regulatory mechanism : rabid , short term.

Pyruvate Kinase covalent modification

- Reaction number 10 in glycolysis , Irreversible reaction.
- Once glucagon (hormone) bind to the receptor it will activate the adenylyl cyclase that will produce cAMP which will activate protein kinase A. This protein will inhibit pyruvate kinase by adding P group to it (phosphorylation).
- \+ Activation of enzyme can be done by phosphatase (removes a phosphate group)
- Protein kinase A is cAMP dependent
- When it’s in the active form 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.
  - regulatory mechanism : rabid , short term

Pyruvate Kinase Deficiency Hemolytic Anemia

- Degree of deficiency is determined by mutation type (complete or mild/partial)
- Pyruvate kinase mutation may lead to :
  1. Altered enzyme Kinetics . (mutation in the allosteric binding site and its goal to inhibit enzyme activity) partial deficiency
  2. Altered response to activator . (mutation in the active site and its goal to stop enzyme activity) complete deficiency
  3. Decreased amount of the enzyme or its stability.



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Long- Term Regulation of Glycolysis

in reactions 1 , 3 and 10 :
(irreversible , Rate limiting enzymes)

Insulin: Induction

Glucagon: Repression

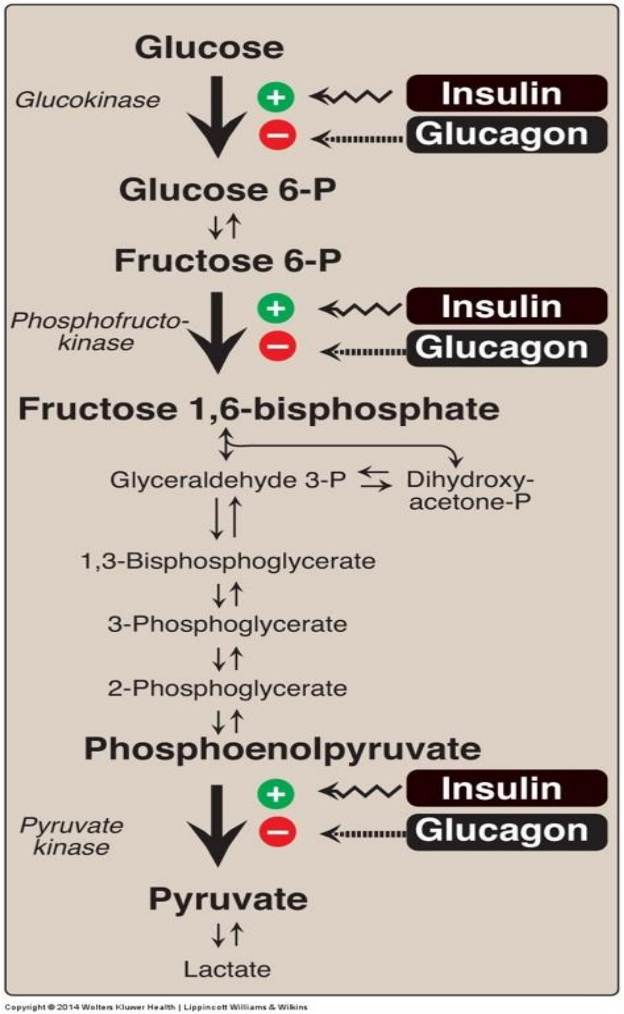
Summary

Regulatory Enzymes (Irreversible reactions):

- Glucokinase/hexokinase
- PFK-1
- Pyruvate kinase

Regulatory Mechanisms:

- Rapid, short-term: Allosteric(Glucokinase/Hexokinase ,PFK-1), Covalent modifications (Pyruvate kinase)
- Slow, long-term: Induction/repression (insulin & Glucagon)



Phosphorylation

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

*It's important in cellular process such as protein synthesis, cell division, signal transduction, cell growth.

Oxidative phosphorylation

- The formation of high-energy phosphate bonds by phosphorylation of ADP to ATP coupled to the transfer of electrons from reduced coenzymes(NADH & FADH) to molecular oxygen via (ETC).
- Indirect ATP formation through redox reactions involving O2 as a final electron acceptor.
- In mitochondria

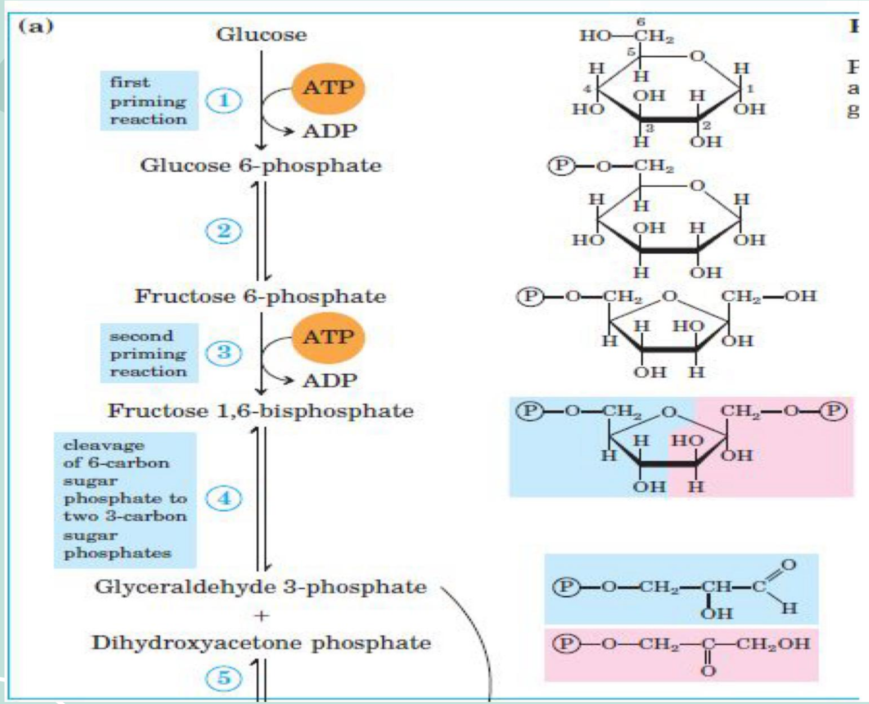
(in electron transport chain ETC)

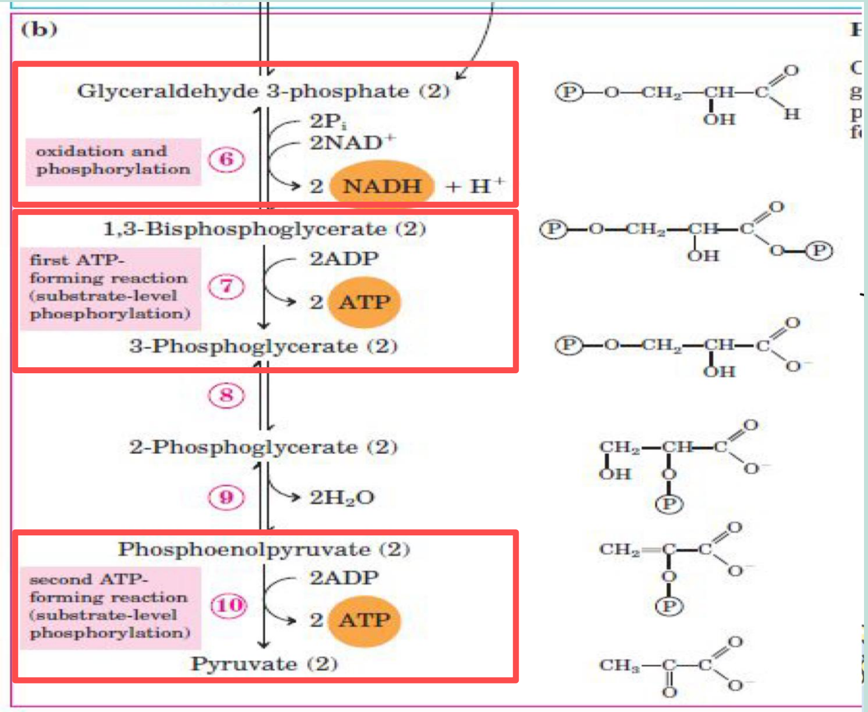
Substrate-level phosphorylation

- The formation of high-energy phosphate bonds by phosphorylation of ADP to ATP (or GDP to GTP) coupled to cleavage of a high-energy metabolic intermediate (substrate).
- Direct ATP formation through phosphate transfer from substrate to ADP.
- It may occur in cytosol or mitochondria

(It occurs in glycolysis & Kreb cycle)

Phosphorylation





Aerobic Glycolysis
(Net ATP produced)

ATP Consumed	2 ATP consumed in reaction 1 and 3	
ATP Produced	Substrate-level (Directly)	Oxidative-level
	2 ATP x 2 = 4 ATP Reaction 7 and 9	2 NADH x 3 = 6 ATP Reaction 6 For each NADH, 3 ATP will be produced by ETC in the mitochondria
Total	10 ATP	
Net	10 - 2 = **8 ATP**	

Anaerobic Glycolysis

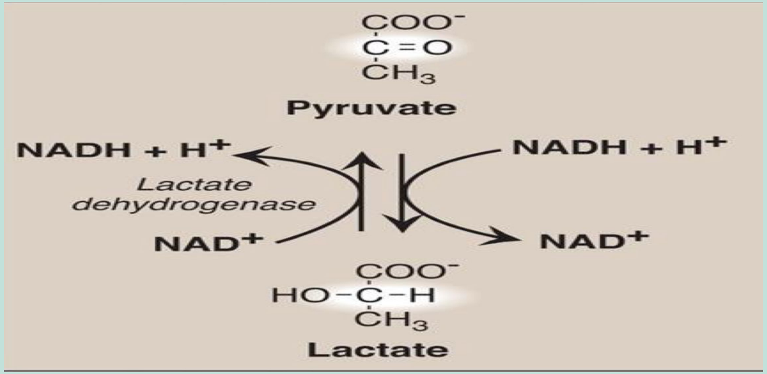
- Anaerobic glycolysis is the process by which the normal pathway of glycolysis is routed to produce lactate.

- It occurs at times when energy is required in the absence of oxygen.

- NADH produced **cannot** be used ETC for ATP production, due to the lack of (O2 or/and no mitochondria).

- **Less** ATP production, as compared to aerobic glycolysis.

- Lactate is an obligatory end product **why?**
  Because if it 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.



- Reversible reaction.
- Lactate dehydrogenase enzyme is used for both direction .
- **2x** NADH + H^+ in .
- **2x** NAD^+ out .

Anaerobic Glycolysis
ATP Production

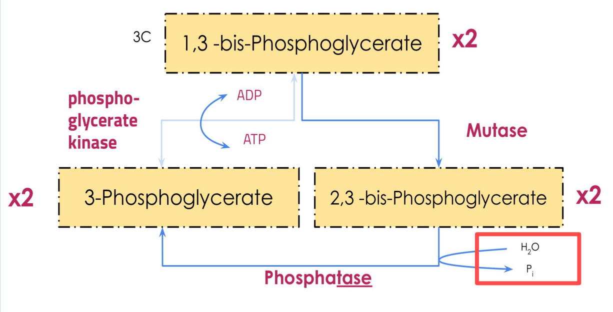
ATP Consumed	2 ATP	
ATP Produced	Substrate-level (Directly)	Oxidative-level
	2 ATP x 2 = 4 ATP	2 NADH x 3 = 6 ATP the hole energy will be consumed to produce lactic acid **(0 ATP)**
Total	4 ATP	
Net	4 -2 = **2 ATP**	

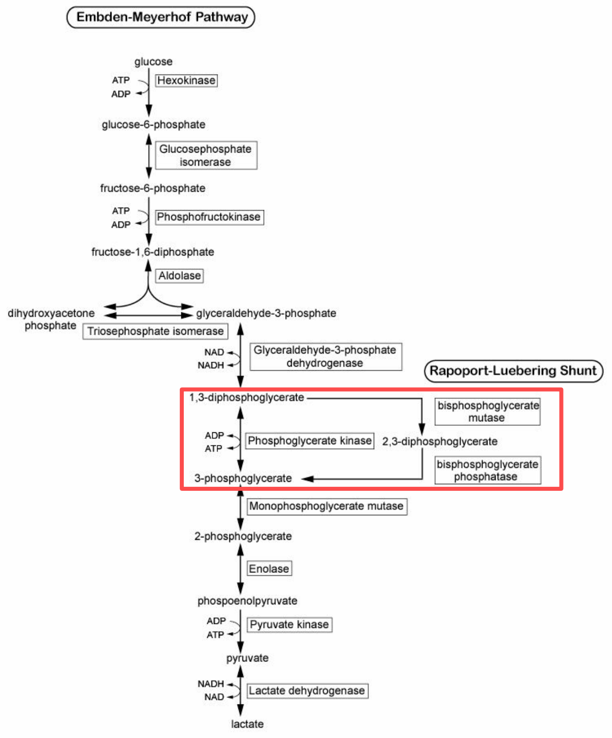
Anaerobic Glycolysis in RBCs (2,3-BPG Shunt)

- Shunt means diversion(تحويلة)
- 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

2,3-BPG shunt help us in loading and unloading of oxygen from hemoglobin.

No ATP production.





Glycolysis in RBCs
(Net ATP produced)

ATP Consumed	2 ATP	
ATP Produced	Substrate-level (Directly)	Oxidative-level
	2 ATP x 2 = 4 ATP **(without shunt)** 1 ATP x 2 = 2 ATP **(with shunt)**	2 NADH x 3 = 6 ATP the hole energy will be consumed to produce lactic acid **(0 ATP)**
Total	4 ATP (without shunt) **OR** 2 ATP (With shunt)	
Net	4 - 2 = **2 ATP (without shunt)** 2 - 2 = **0 ATP (with shunt)**	

Glycolysis in RBCs
(Summary)

End product	Energy yield	PK Deficiency hemolytic anemia Depends on
- Lactate - No net production or consumption of NADH	If NO 2,3-BPG is formed: 2 ATP If 2,3-BPG shunt occurs: 0 ATP	Degree of PK Deficiency (mutation) Compensation by 2,3-BPG

Take Home Messages

- Glycolysis is the major oxidative pathway for glucose.
- Glycolysis is employed by all tissues.
- Glycolysis is a tightly-regulated pathway.
- PFK-1 is the rate-limiting regulatory enzyme.
- Glycolysis is mainly a catabolic pathway for ATP production, but it has some anabolic features (amphibolic).
- Pyruvate kinase deficiency in RBCs results in hemolytic anemia.

MCQs

1. The net ATP production in Aerobic Glycolysis is :			
A) 8 ATP	B) 4 ATP	C) 2 ATP	D) 10 ATP
2. The final product of Anaerobic Glycolysis is :			
A) Pyruvate	B) Citrate	C) Lactate	D) A-Co
3. There are (—) steps reaction in aerobic glycolysis :			
A) 3	B) 7	C) 10	D) 11
4. Any of these enzymes converts Fructose 6-phosphate into Fructose 1,6-bis-phosphate?			
A) enoloose	B) lactase	C) phosphofructoKinase-1	D) glucase
5. During glycolysis, Glucose will be converted into glucose 6-phosphate in the liver by which enzymes?			
A)Glucokinase	B) Glucose-6-p dehydrogenase	C) Phosphoglucose isomerase	D)Hexokinase

1)A — 2)C — 3)C — 4)C — 5)A

SAQs Question 1

What is the end product of anaerobic glycolysis ?

Lactate

SAQ Question 2

How many NADH are produced by glycolysis per glucose?

2 NADH

SAQ Question 3

what are PFK-1 inhibitors and activator ?

The activators are **AMP** and **Fructose 2,6-bisphosphate**

the inhibitors are **ATP** and **citrate**

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