بِسْمِ الله الرحمن الرحيم

Glycolysis

Biochemistry Team 437

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Highlights

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: An overview

- Glycolysis, the major pathway for glucose <u>oxidation</u>, occurs in the cytosol of all cells.
- It is unique, in that it can function either:

  Aerobically **<u>Or</u>** Anaerobically

  Depending on:

  Availability of oxygen **<u>And</u>** Intact mitochondria

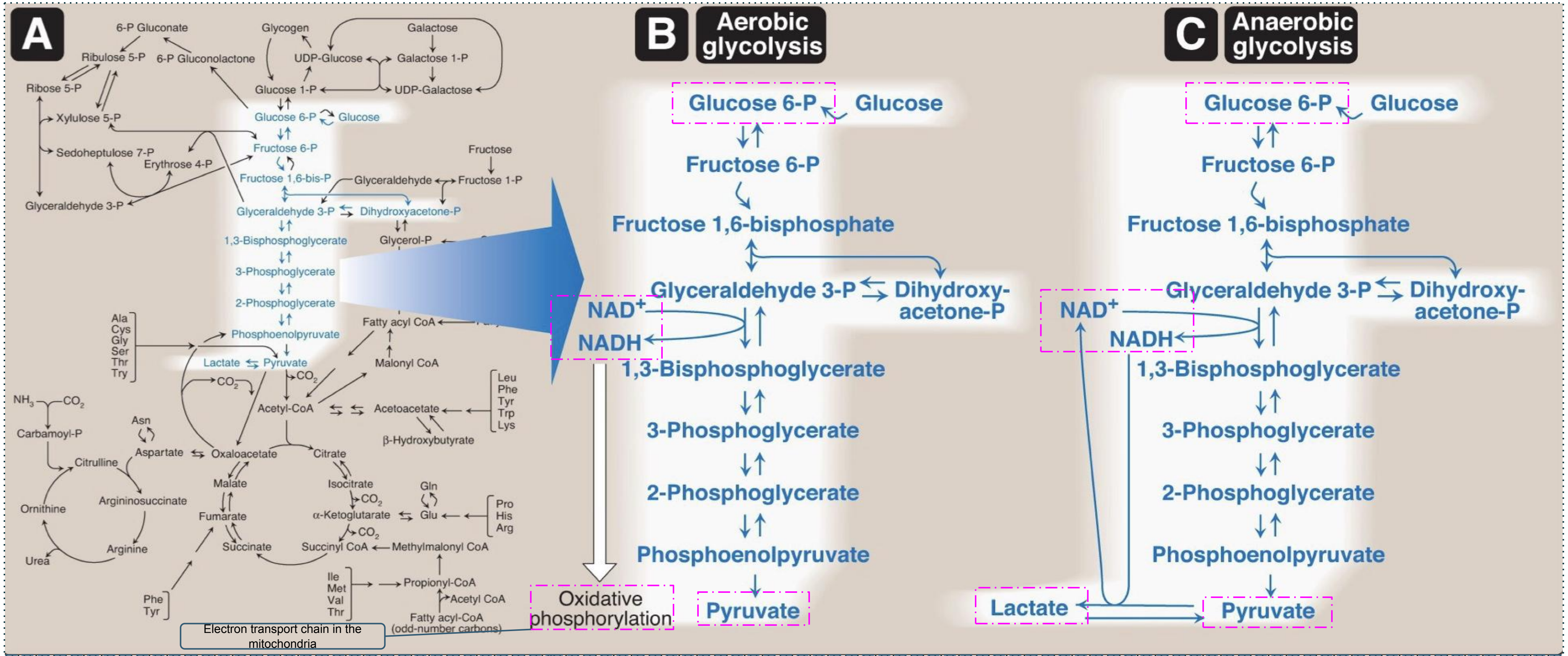
- It allows tissues to survive in presence or absence of oxygen, e.g., <u>skeletal muscle</u>.

  (When exercising and not able to provide enough oxygen to the muscle tissue, the muscle tissue will use anaerobic glycolysis in the absence of oxygen to provide enough energy for survival.)

- **<u>RBCs</u>**, which lack mitochondria, are completely reliant on glucose as their metabolic fuel, and metabolizes it by anaerobic glycolysis. (it's their only source of ATP)

Glycolysis: breaking down **glucose** (<u>initial substrate</u>) and forming **pyruvate** (<u>end product</u>) producing <u>2 molecules of ATP</u>.

Aerobic VS Anaerobic



Aerobic glycolysis:

- Starts with glucose.
- Ends up with pyruvate.
- Uses one NAD+ to make NADH.
- NADH goes into electron transport chain (in the mitochondria) and produces ATP.
- Then NAD+ will be regenerated.

Anaerobic glycolysis:

- Starts with glucose but does not end with pyruvate.
- Pyruvate will be converted to lactate because this pathway doesn't have a way of regenerating NAD+ (Remember: cells use anaerobic pathway when they don't have a mitochondria so NADH can not go into the electron transport chain. Now notice in the picture, NADH is converted to NAD+ when pyruvate is converted to lactate.)
- If NAD+ isn't available glycolysis will stop.
- So the purpose of converting pyruvate to lactate is regenerating NAD+ so glycolysis goes on.

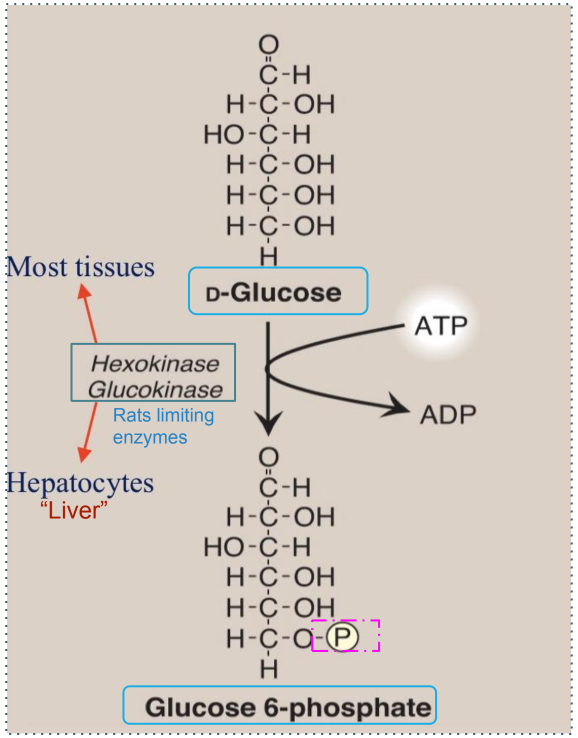
Aerobic glycolysis

There are 10 steps in aerobic glycolysis:

- 3 irreversible steps (regulatory points) ***important***
- 7 reversible steps (can go in either direction)

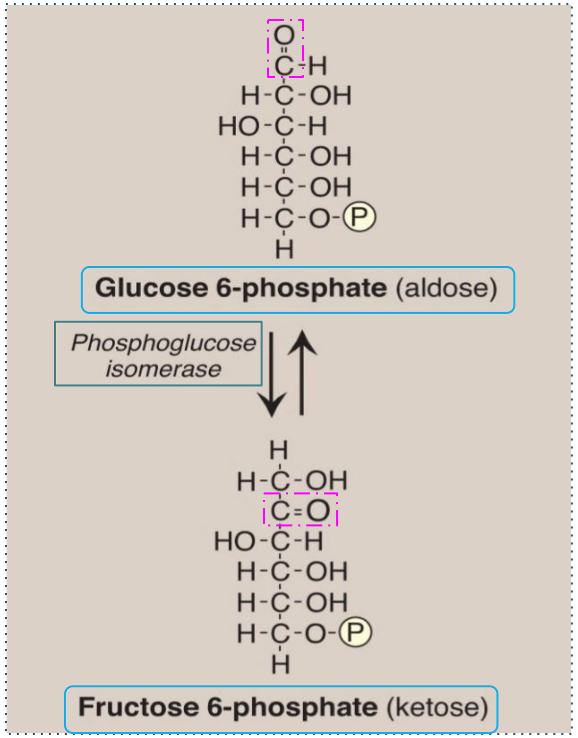
First reaction:

- Irreversible step (regulated step)
- Consumes 1 ATP
- Adding a phosphate group locks glucose inside the cell *since transporters won't recognize it in this form.*



Second reaction:

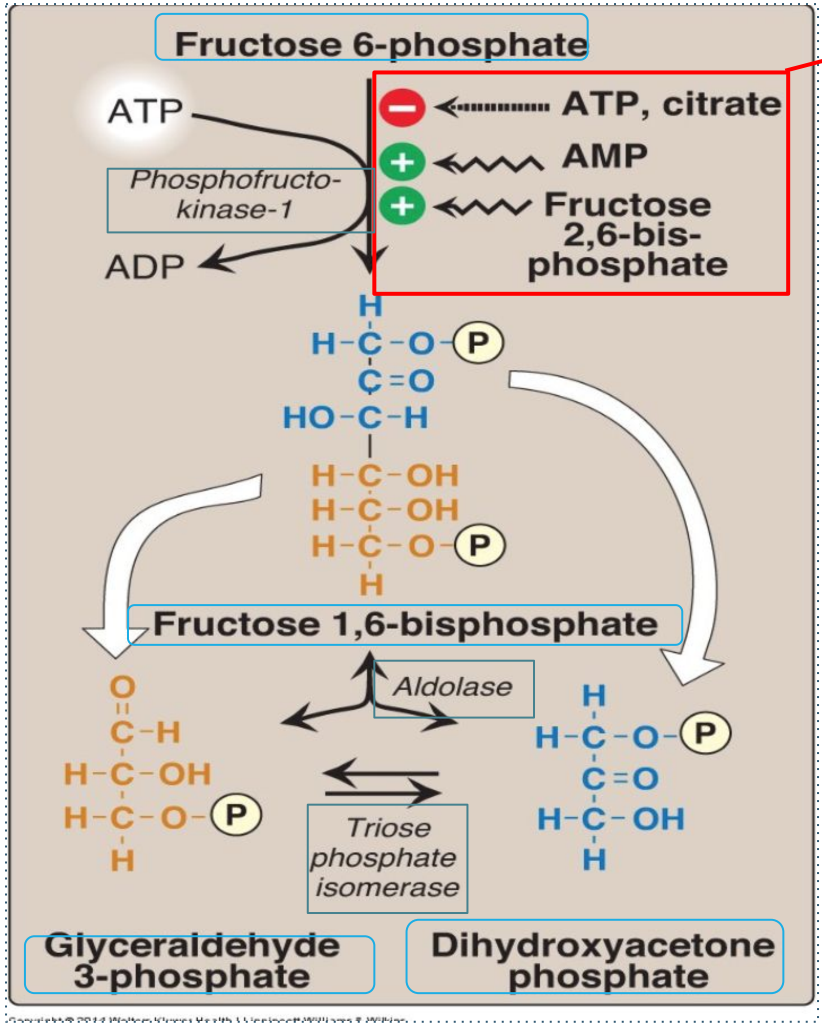
- Reversible step
- Just making an isomer (rearrangement)



Reminder: it is important to know all the enzymes, but mostly stress on the enzymes that can be regulated.

Aerobic glycolysis

Reactions :3rd - 5th



- When **ATP** and **Citrate** are abundant they will inhibit the reaction.
- When **AMP** and **Fructose 2,6-bisphosphate** (switch) are abundant they will activate the reaction.

3rd reaction:

- From Fructose 6-phosphate to Fructose 1,6-bisphosphate. By an enzyme called Phosphofructokinase-1
- This is an irreversible reaction.

4th reaction:

- **Fructose 1,6-bisphosphate** split into both **Glyceraldehyde 3-phosphate** and **Dihydroxyacetone phosphate**. By an enzyme called Aldolase.
- This is a reversible reaction

5th reaction:

- From **Dihydroxyacetone phosphate** to **Glyceraldehyde 3-phosphate**. By an enzyme called Triose phosphate isomerase.
- This a reversible reaction

Aerobic glycolysis
(Reactions: 6-10)

Reactions (6-10):
The conversion of **2 moles of glyceraldehyde 3-phosphate** (we got the **2** G3P from reaction 4 and 5) into **2 moles of pyruvate**.
Pay attention that the number of moles of all metabolites in these steps (6 - 10) is multiplied by 2!

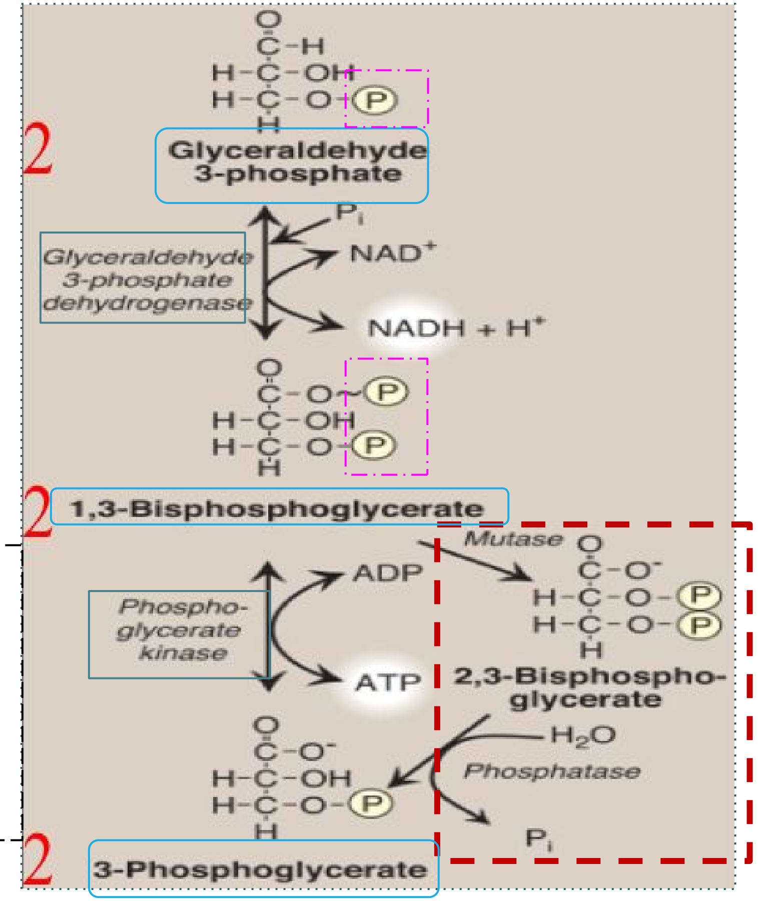
6th reaction:

- 2 Glyceraldehyde 3-Phosphate → 2 1,3-Bisphosphoglycerate
- By an enzyme called Glyceraldehyde 3-phosphate dehydrogenase (adds phosphate group).
- NAD^+ → (2) NADH

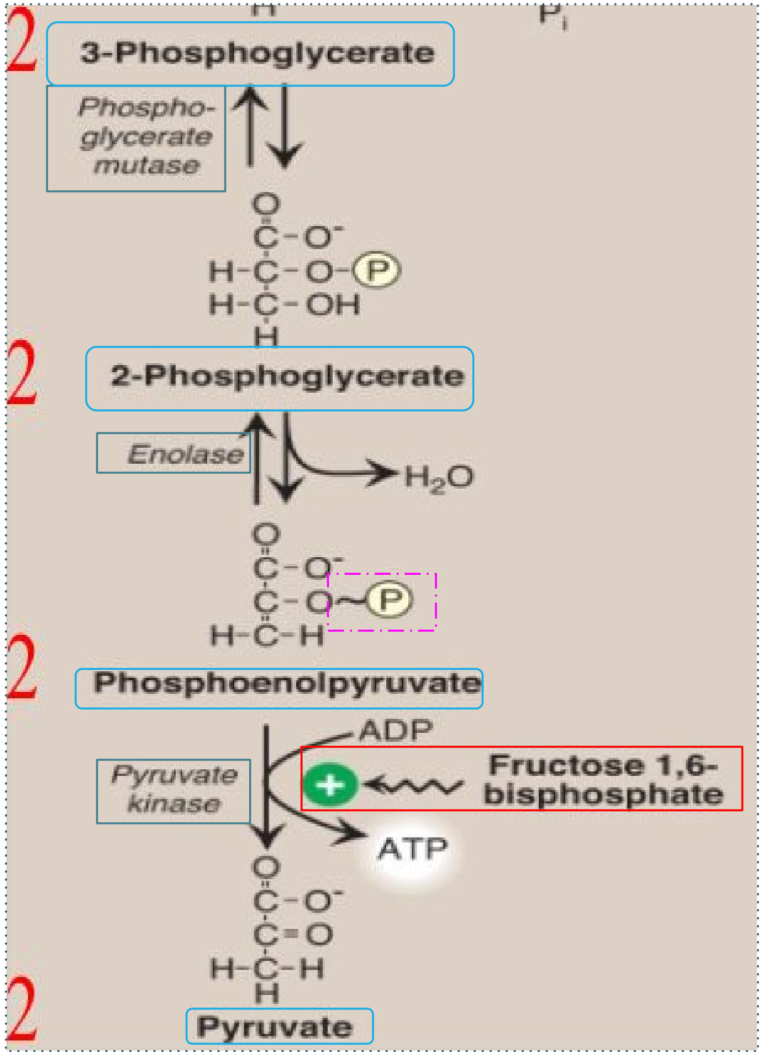
Understand the molecules involved: **(Team 436)**
Glyceraldehyde when it's oxidized it becomes Glycerate (an acid)

7th reaction:

- 2 1,3-Bisphosphoglycerate → 2 3-Phosphoglycerate
- By an enzyme called Phosphoglycerate kinase (adds phosphate group).
- ADP → (2) ATP



(2,3- BPG Shunt) Will be discussed later



8th reaction:

- 2 3-Phosphoglycerate → 2 2-Phosphoglycerate
- Structural rearrangement by enzyme Phosphoglycerate mutase.

9th reaction:

- 2 2-Phosphoglycerate → 2 Phosphoenolpyruvate
- Dehydrated (Remove H2O) by enzyme Enolase.

10th reaction:

- 2 Phosphoenolpyruvate → 2 Pyruvate
- By an enzyme called Pyruvate Kinase, which is activated by Fructose 1,6-bisphosphate.
- ADP → (2) ATP
- This step is irreversible and regulated.

Reminder for easier memorization
Kinase: always works with phosphate group
Dehydrogenase: always results is NADH formation

Regulation: Glucokinase/ Hexokinase

Hexokinase: It is inhibited by the reaction product, glucose-6-P which accumulates when further metabolism of this hexose is reduced.

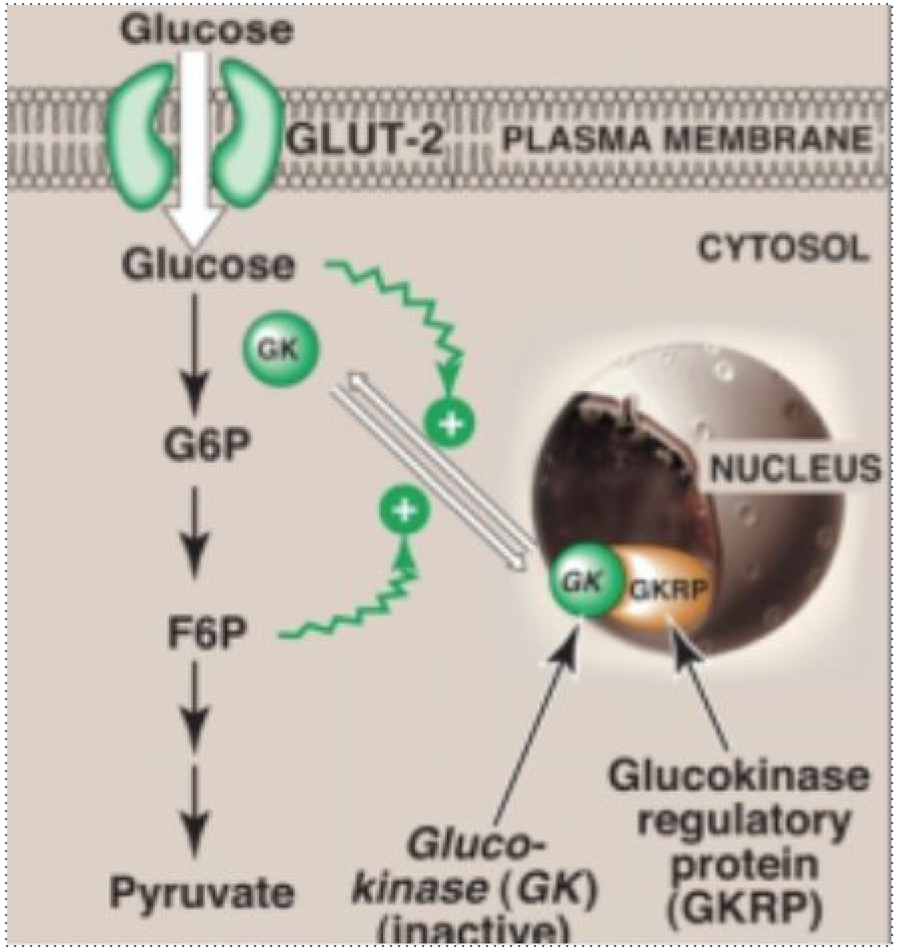
Glucokinase: It is inhibited indirectly by Fructose-6-P and is indirectly stimulated by glucose.

Glucokinase (GK) Regulation:

- In the presence of high fructose-6-phosphate, GK translocates and binds tightly to GKRP (glucokinase regulatory protein) in the nucleus, making it inactive.

Glucokinase is scared of fructose-6-phosphate, when it is present in high conc., the enzymes runs and hides in the nucleus and holds tightly onto glucokinase regulatory protein.

- When glucose levels are high in blood and hepatocytes (GLUT2), GK is released from GKRP and enters the cytosol.



- High amount of Fructose-6-phosphate will inhibit the reaction
- High amount of glucose will release the inhibition and that's why it's called an indirect activation

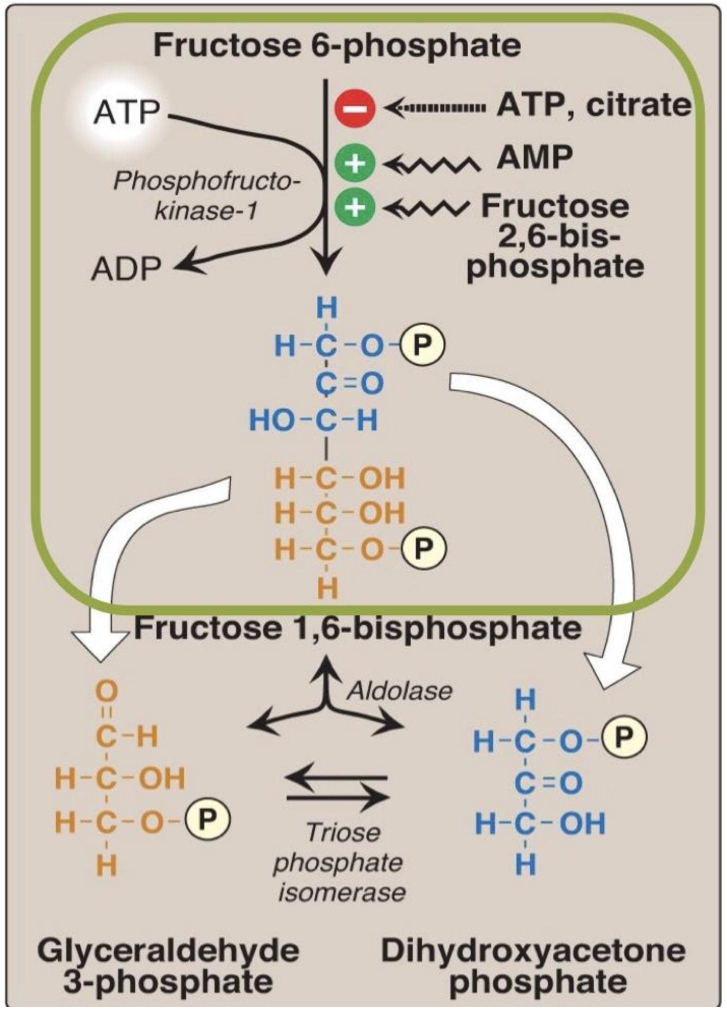
Regulation: PFK-1(phosphofructokinase-1)

Inhibited by:

- **ATP** (cell have plenty of energy (ATP) —>stop glycolysis[by inhibiting the enzymes of glycolysis pathway])
- **citrate** (formed in the Krebs cycle)

Activated by:

- **AMP** (high levels of AMP means the cell needs energy so glycolysis will be activated)
- **Fructose 2,6-bisphosphate** (switch between glycolysis and gluconeogenesis) (when you eat you will have lost of sugar and carbohydrates in your blood so insulin will be secreted and the insulin will produce fructose 2,6-bisphosphate , fructose 2,6-bisphosphate will inhibit gluconeogenesis and activates glycolysis)

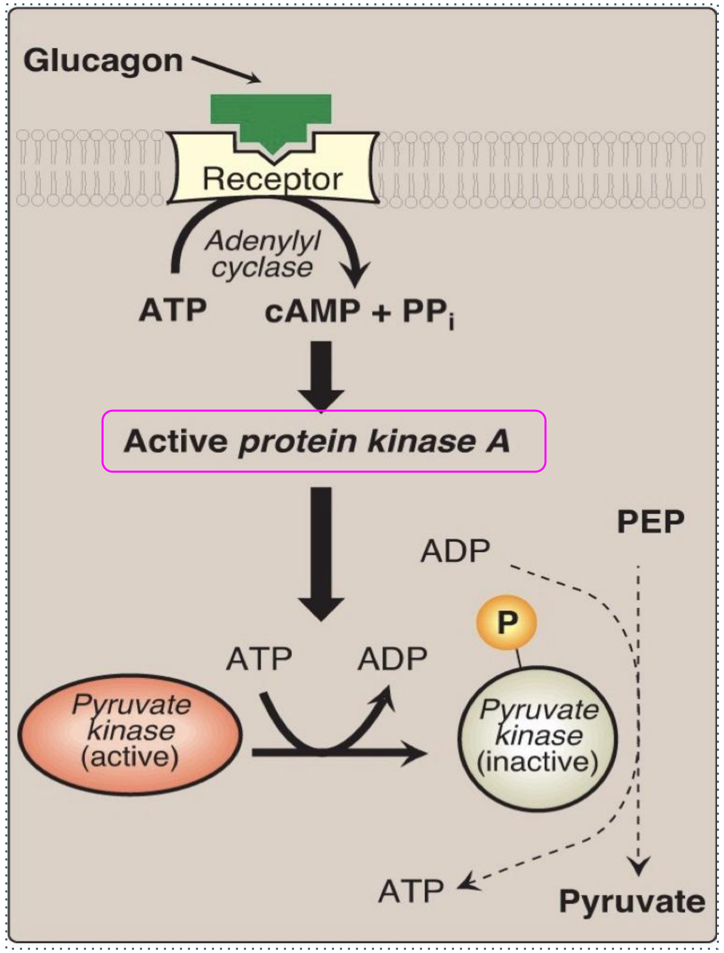


Pyruvate kinase covalent modification

- Glucagon is released when the body is starving (low ATP).
- Glucagon activate adenylyl cyclase.
- Adenylyl cyclase increases the production of cAMP.
- cAMP **activates** protein kinase A.
- Protein kinase A **phosphorylates** pyruvate kinase.
- Pyruvate becomes inactive.
- Phosphoenolpyruvate can not be converted to pyruvate.

Phosphoenolpyruvate will be used in the production of glucose (gluconeogenesis).

We have low ATP and we shut down the ATP production, why? Because the body is starving it needs glucose (even if the body doesn't have enough ATP it needs to have glucose because the brain can only use glucose)

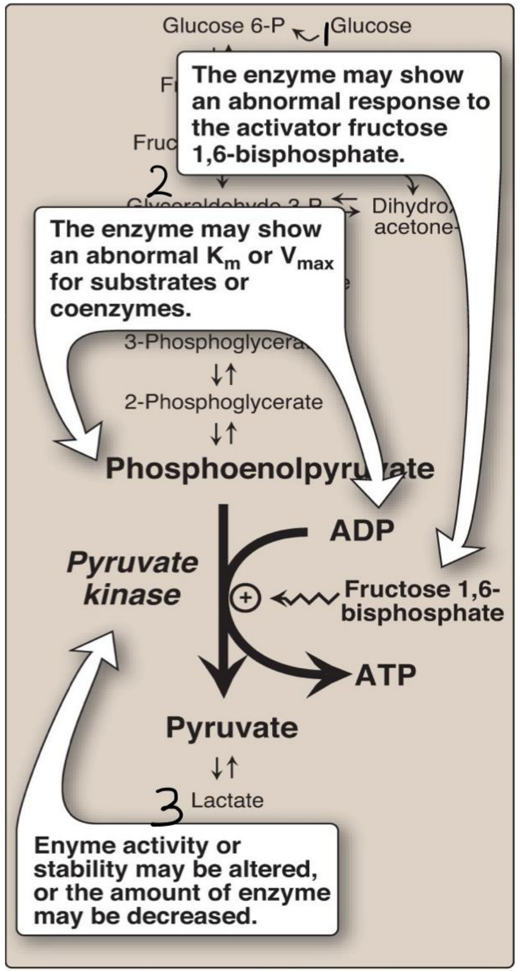


Pyruvate kinase Deficiency Hemolytic Anemia

Pyruvate kinase mutation may lead to:

1. Altered responses to activator.
2. Altered enzyme kinetics.
3. Decreased the amount of the enzyme or its stability.

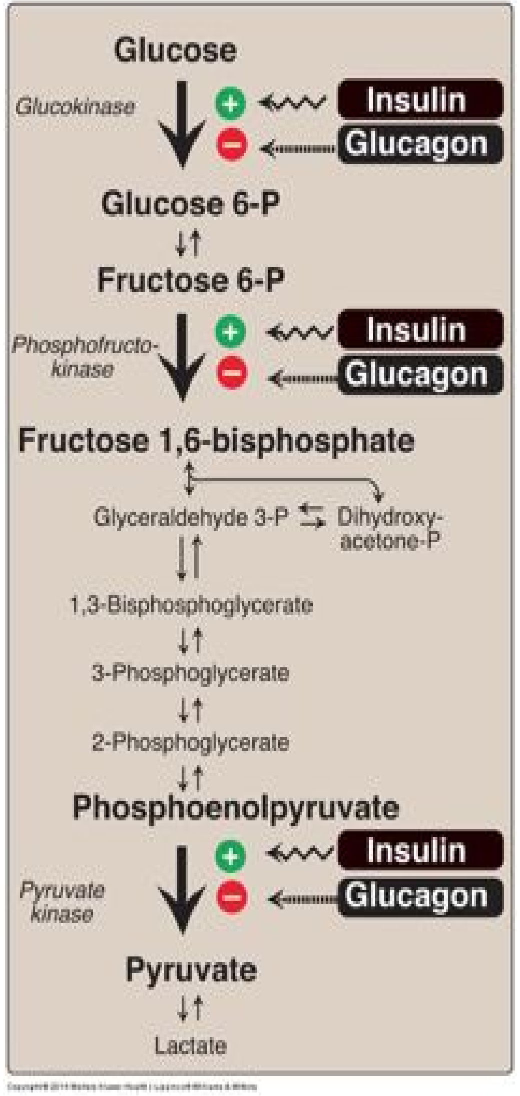
RBC can only produce ATP by anaerobic glycolysis
No energy (ATP) production, membrane shape will not maintain, phagocytes and macrophages, will come and eat it ⇒ Hemolytic anemia



Long-Term Regulation of Glycolysis

- **Insulin (Induction +)**
  - Glucokinase
  - Phosphofructokinase
  - Pyruvate Kinase

- **Glucagon(Repression -)**
  - Glucokinase
  - Phosphofructokinase
  - Pyruvate Kinase

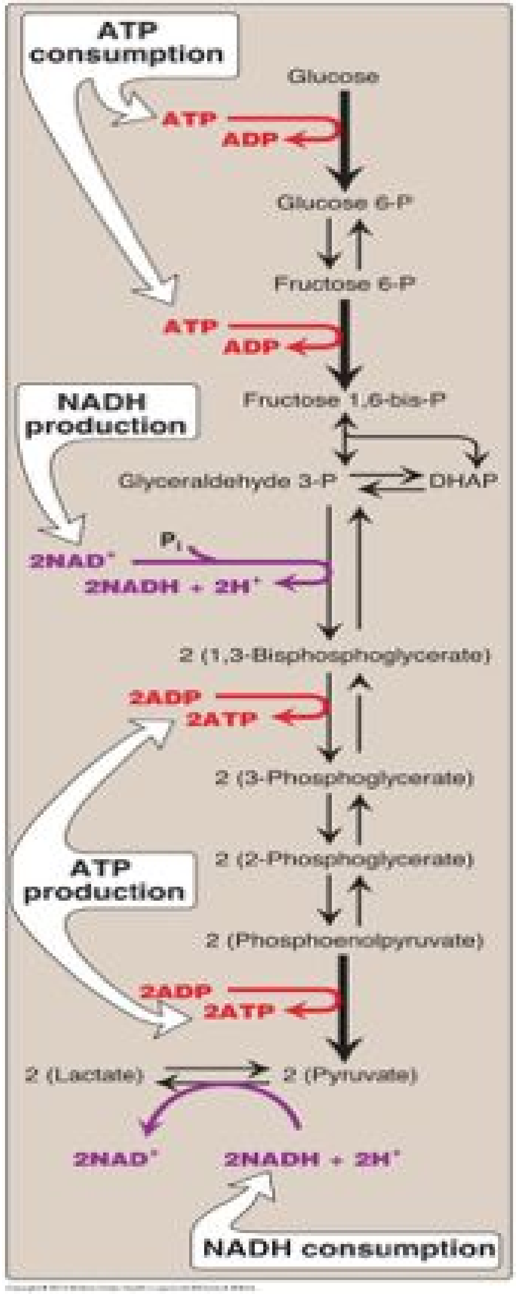


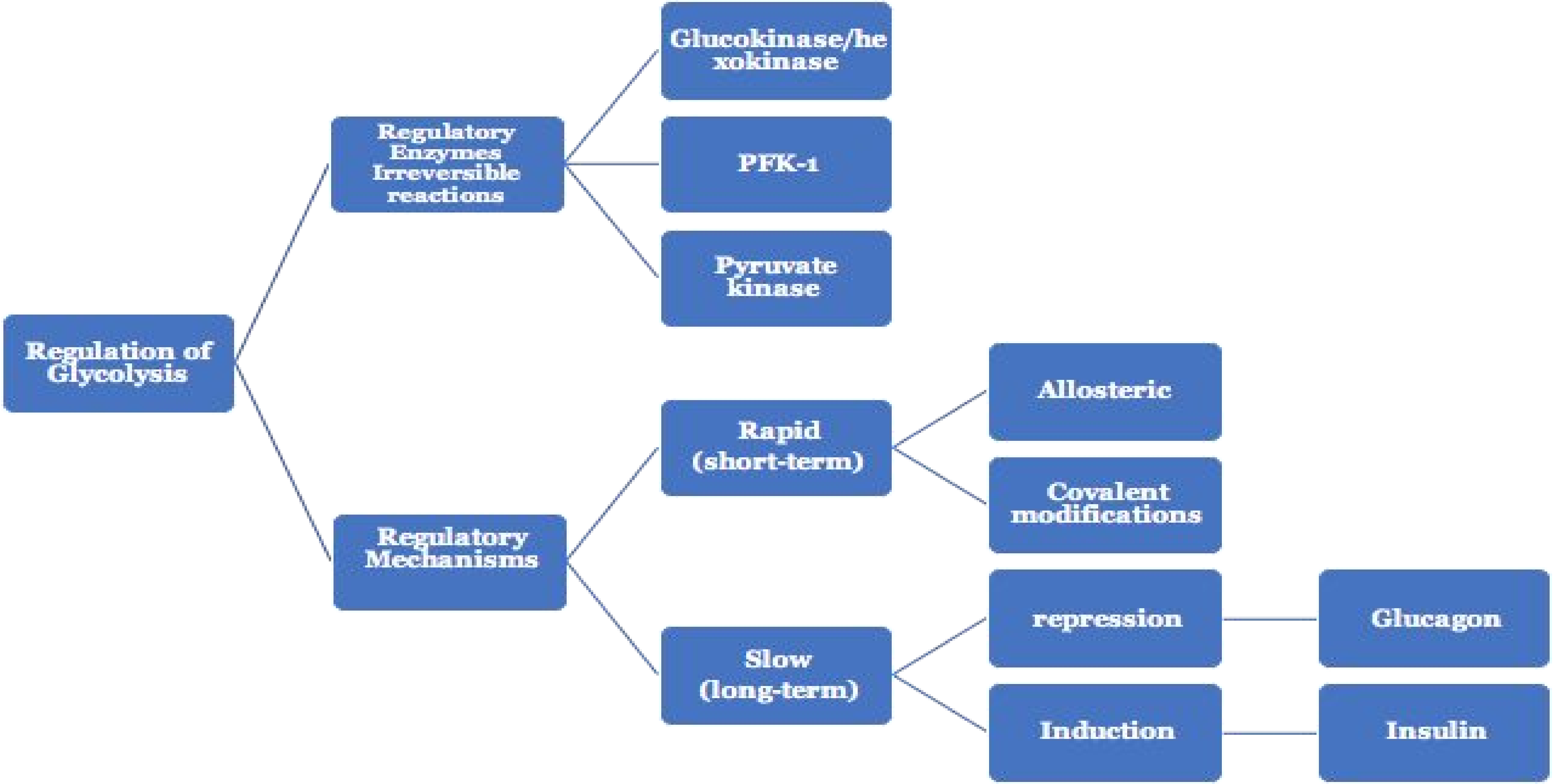
Effect of insulin and glucagon on the synthesis of key enzymes of glycolysis in liver.
team 436*

Glycolysis

For each NADH, **3 ATP** will be produced by **ETC in the mitochondria**

1 NADH = 3 ATP





Apply the above mechanisms for each enzyme where applicable

Substrate-level phosphorylation Vs. Oxidative phosphorylation

- **Phosphorylation** is the metabolic reaction of **introducing a phosphate group** into an **organic molecule.** (add phospate)

Oxidative phosphorylation	Substrate-level phosphorylation
formation of high-energy phosphate bonds by phosphorylation of **ADP** to **ATP**	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** via the **electron transport chain (ETC)**	**cleavage** of a high-energy metabolic intermediate (substrate)
occurs in the **mitochondria**	occur in **cytosol** or **mitochondria**

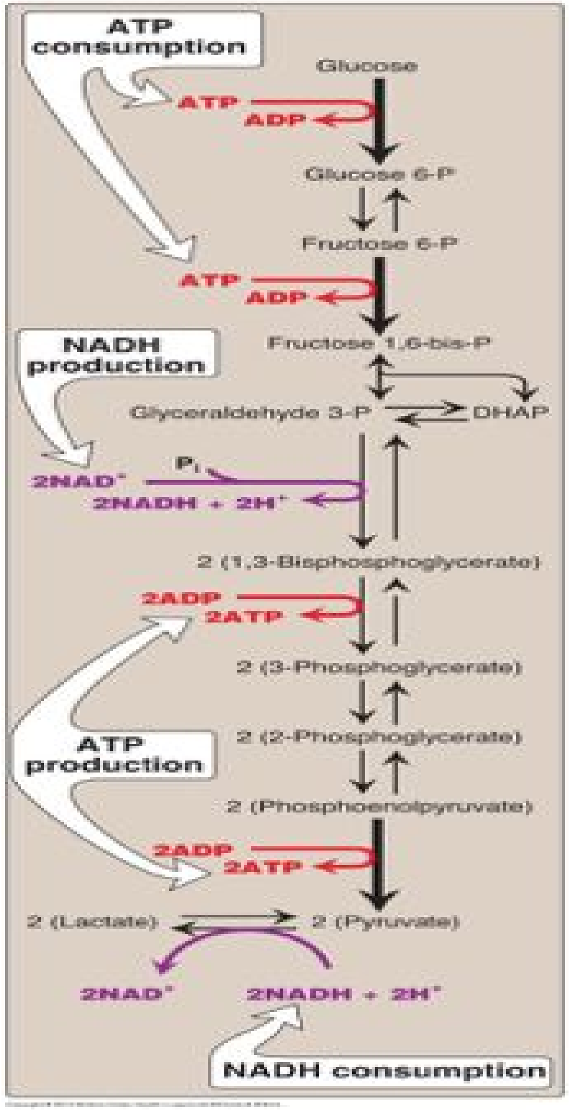
substrate-level phosphorylation video

Aerobic Glycolysis (Net ATP produced)

- ➢ **ATP Consumed:** **2** ATP

- ➢ **ATP Produced:**
  - Substrate-level: 2 X 2 = **4** ATP
  - Oxidative-level: 2 X 3 = **6** ATP
  - Total: **10** ATP

- ➢ **Net:** 10−2= **8** ATP

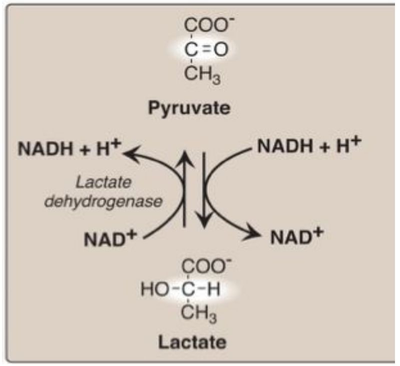


Anaerobic Glycolysis

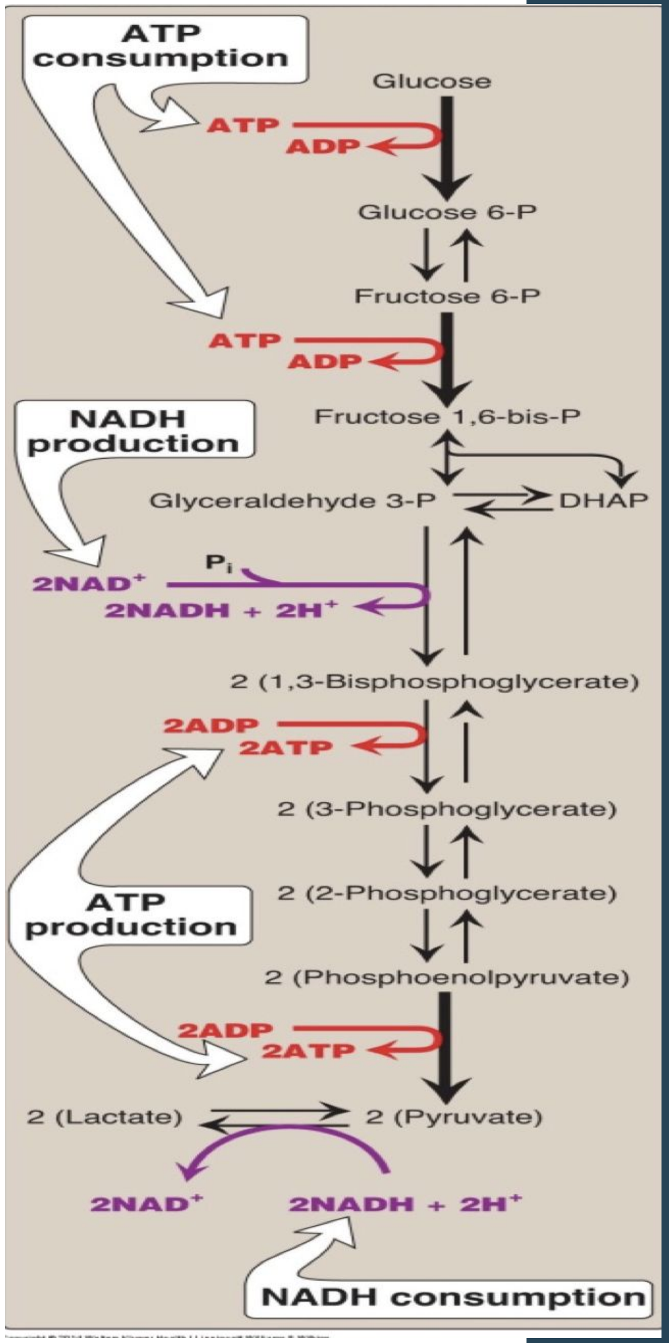
- ***NADH produced cannot be used by ETC for ATP production.***
  (No O2 and/or No mitochondria).

- ***Anaerobic glycolysis produce less ATP than the aerobic glycolysis.***

- 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



- The enzyme used to convert pyruvate to lactate is **lactate dehydrogenase**,NADH is produced from this reaction (one for each pyruvate), this reaction is reversible



Anaerobic Glycolysis (Net ATP produced)

- **ATP Consumed:** **2** ATP

- **ATP Produced:**
  - Substrate-level: 2 X 2 = **4** ATP
  - Total: 4 ATP

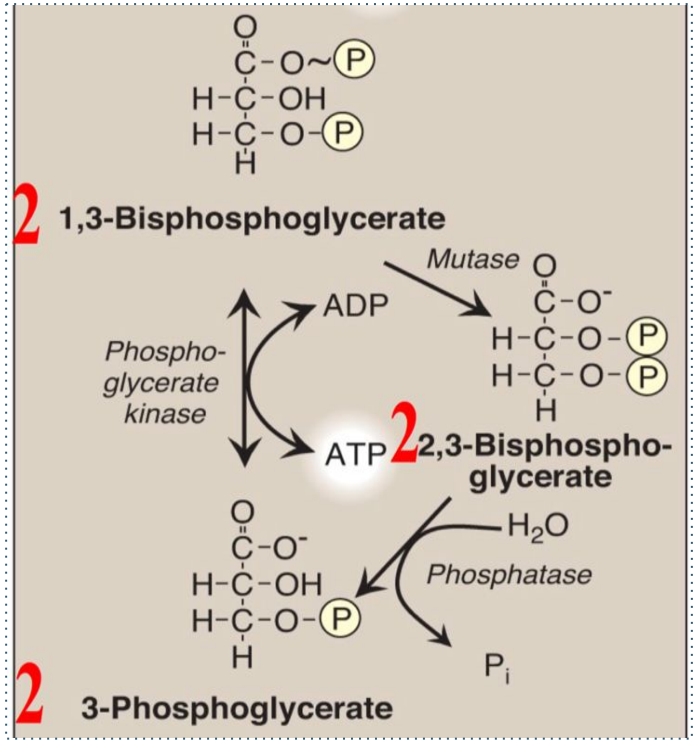
- **Net:** 4-2 = 2 ATP

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

1,3-Bisphosphoglycerate → **Mutase** → 2,3-Bisphosphoglycerate → **Phosphatase** → 3-Phosphoglycerate

Mutase: (Team 436)
- ➔ 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.

Phosphatase: remove phosphate group



- 2,3- BPG can be produced by taking an **alternate pathway** in glycolysis.
- By taking this path we skipped the generation of 2 ATP molecules, as a result the ATP produced here is **zero**.
- **Importance of 2,3-Bisphosphoglycerate:**
  2,3- BPG is produced in red blood cells because it helps hemoglobin **deliver oxygen**.
  Each hemoglobin binds **4 molecules of oxygen** (**8 oxygen atoms**).

Glycolysis in RBCs

End product:

Lactate

No net production or consumption of NADH

Energy yield:

If **no 2,3-BPG** is formed: **2 ATP**

If **2,3-BPG** shunt occurs: **0 ATP**

PK (Pyruvate Kinase) Deficiency hemolytic anemia depends on:

Degree of PK Deficiency

Compensation by 2,3-BPG

ATP Consumed:

2 ATP

ATP Produced:

Substrate-level 2 X 2 = 4 ATP (Without shunt)

or 1 X 2 = 2 ATP (With shunt)

Total 4 or 2 ATP

Net:

4 – 2 = 2 ATP (Without shunt)

2 – 2 = 0 ATP (With shunt)

Even if there is no ATP produced in the 2,3-BPG shunt it will deliver oxygen and other tissues will utilize it in the TCA cycle and oxidative phosphorylation and make ATP so the person can survive.
However if no 2,3-BPG is there the person will die of hypoxia.

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.

- **Net energy produced in:**
  Aerobic glycolysis: 8 ATP
  Anaerobic glycolysis: 2 ATP
- **Net energy produced in glycolysis in RBCs:**
  Without 2,3 BPG synthesis: 2 ATP
  With 2,3 BPG synthesis: 0 ATP

MCQs

1)The end product of anaerobic glycolysis is :

a) pyruvate b) lactate. c) phosphoenolpyruvate.

2) There are ……….steps in aerobic glycolysis

a)13. b)20. c)10

3)in glycolysis the first 3 steps are :

a) irreversible b) reversible. c) could be irreversible and reversible

4) in the 5th reaction **Dihydroxyacetone phosphate** converted to **Glyceraldehyde 3-phosphate**. By an enzyme called

a)Triose phosphate isomerase. b)Enolase. c) Aldolase

1) B
2) C
3) A
4) A

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