Glycolysis

Glucose transport through the cell membrane

Glucose and Sugars are polar molecules, so they can NOT cross the lipid bilayer of the membrane → Require specific carrier

2 Types of Glucose carriers

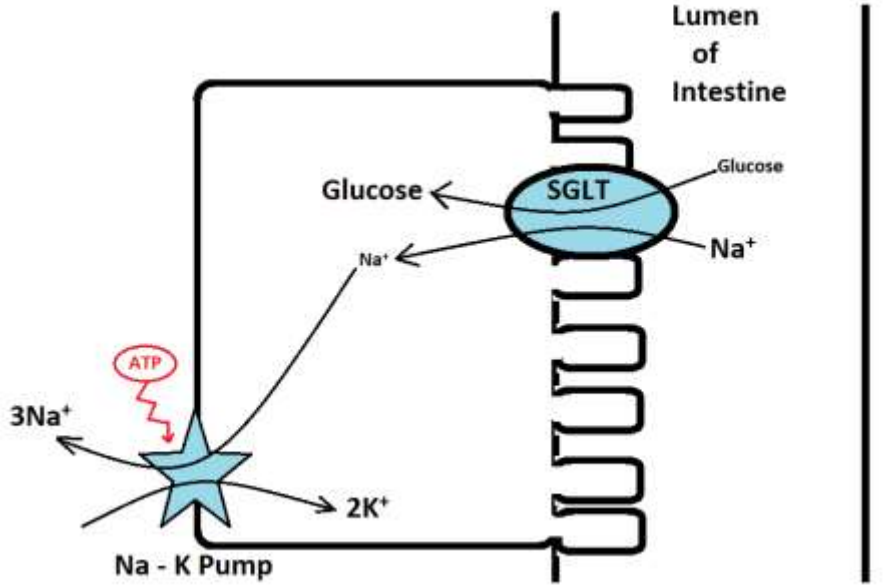
1. **Sodium-dependent glucose cotransporter (SGLT).**

Its Na^+ and ATP-dependent cotransport system.

Found only in epithelial cells of the intestine, renal tubules.

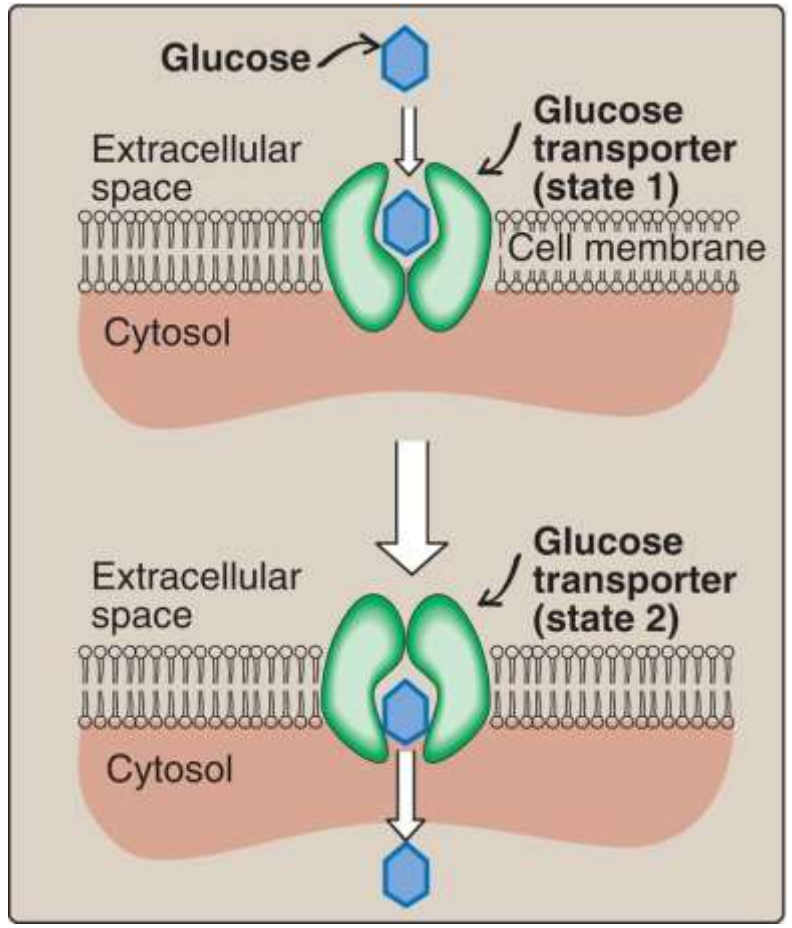
transports glucose **against** its concentration gradient in company with Na^+ which is transported down its electrochemical gradient. (**symport)** ينقل مادتين بنفس الاتجاه

Na^+ gradient is maintained by Na-K ATPase pump which consume ATP



B. In other tissues, Glucose is transported from high concentration to low concentration (No Na^+ or ATP required) through **Na^+ - Independent Facilitated Diffusion Transporter (GLUT)**

- It's passive transport system "facilitated Diffusion"
-14 types "Isoform" of GLUT 1 – 14
-They are monomeric protein transporters
-Has two conformations
- Extracellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane via facilitated diffusion.
-They are **uniporters** because they transport one molecule at a time



In most body tissues GLUT transport Glucose from plasma to the inside of the cell

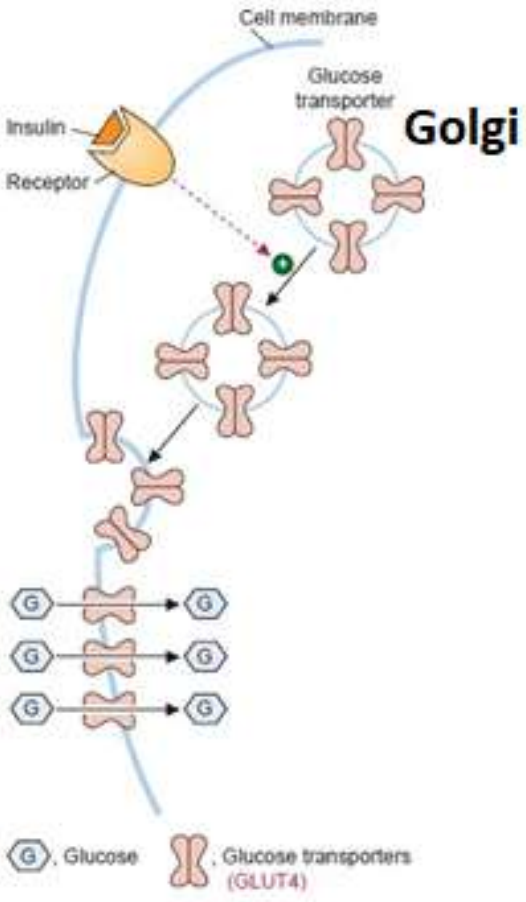
انسجة قادرة على تصنيع جلوكوز

In ***liver and Kidney cells*** (Glucogenic tissues) they have GLUT 2:

- After meal when plasma glucose level is high GLUT 2 transport Glucose from plasma to the inside of these cells
- During starvation GLUT 2 transport Glucose from inside of these cells to plasma in order to maintain normal plasma Glucose level

GLUT isoform	Tissue distribution	Specialized function
GLUT2 يعمل عندما يكون فرق تركيز الجلوكوز بين خارج وداخل الخلية عالي جدا	Liver, kidneys, small intestine, and pancreatic β cells. **Insulin - independent**	**In small Intestine:** absorption of Glucose and galactose **In liver:** transport Glucose from blood to the liver cell after meal for storage as glycogen; and from liver cell to blood when glucose level is low **In kidneys:** transport Glucose from blood the kidney cell after meal and from kidney cells to blood when glucose level is low since kidney cells can synthesize glucose during starvation **In Pancreatic β-cells:** GLUT 2 is **Glucose sensor**: it transport Glucose from blood into pancreatic β-cells only when glucose level in blood is high this stimulate pancreatic β-cells to release Insulin.
GLUT4	Cardiac and skeletal muscles and adipose tissue	Their number increase in these tissues by Insulin (**Insulin Stimulated**), glucose uptake from the blood
GLUT 7	ER of Liver and Kidneys	Important for **Gluconeogenesis**

GLUT 1: BBB and RBCs
GLUT 3: Neurons
GLUT 5: Fructose transporter in small intestine and spermatozoa



Glycolysis: it's the first stage of Glucose metabolism and it's a **universal pathway** that occurs in all cells

General notes:

Some tissues in your body such as ***Brain, RBCs***, Cornea, lens, Retina, Kidney Medulla, Testis, Leukocytes, and white muscle fibers have an absolute requirement (مطلقة) of Glucose (حاجة), so your blood should has glucose all the time.

Glucose sources:

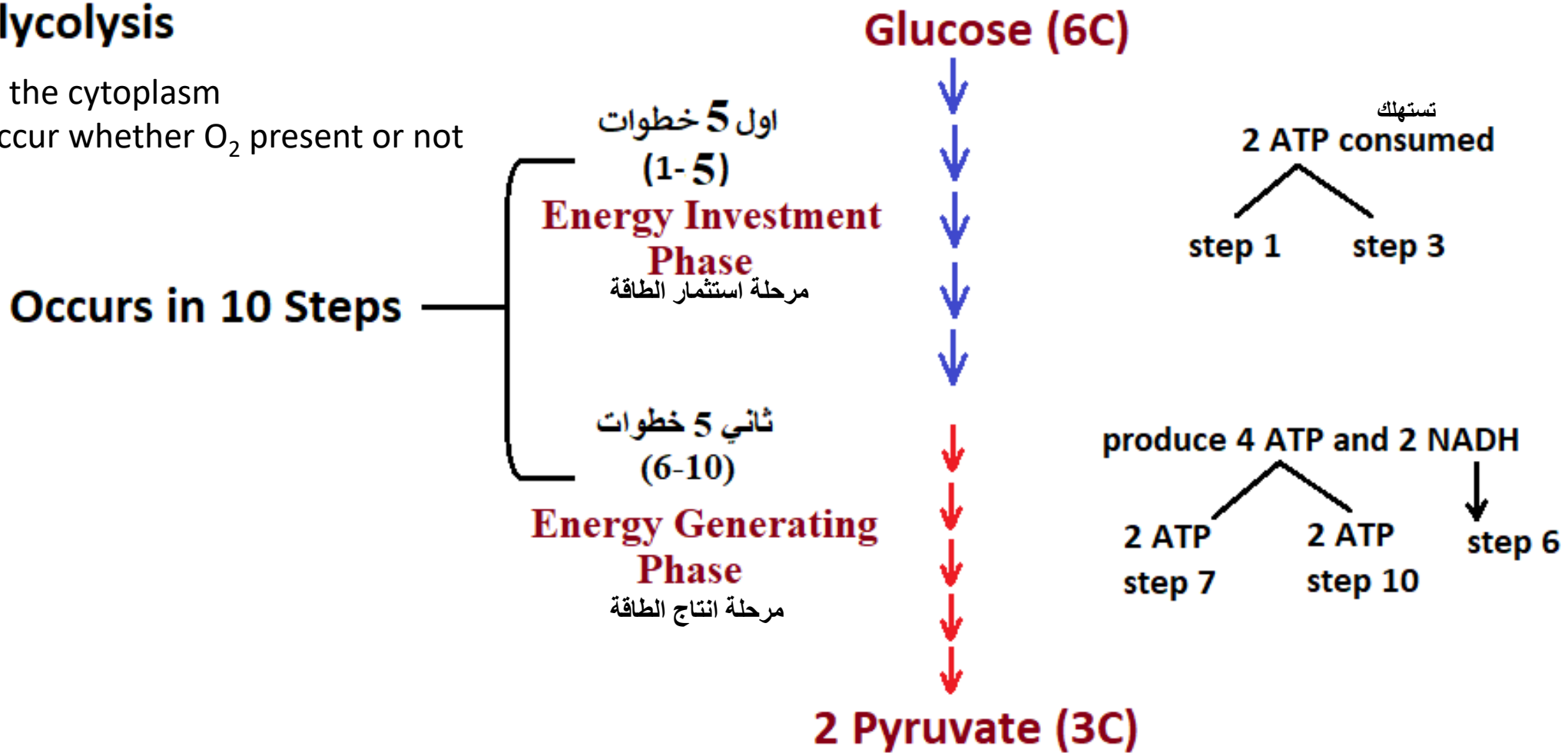
1. Diet (sporadic) متقطع
2. 6-8 hours after meal (2hours post (بعد الامتصاص) absorptive), Dietary glucose will be depleted (ينفذ) (Fasting (صيام)) you depends on Liver glycogen for 16-18 hours
3. After liver glycogen is finished (prolonged (المستمر) fasting or Starvation); Gluconeogenesis in Liver and Kidneys will be your sustained source (المصدر) of Glucose

Gluconeogenesis will be you sustained source of glucose until you eat again or Die

تذكر انو وقت المجاعة انتا ما عندك نقص بالطاقة لانو جسمك بحتوي على دهون تعطيك طاقة لغاية شهر على الاقل المشكلة عندك بتكون بنقص الجلوكوز

Glycolysis

- In the cytoplasm
- Occur whether O_2 present or not

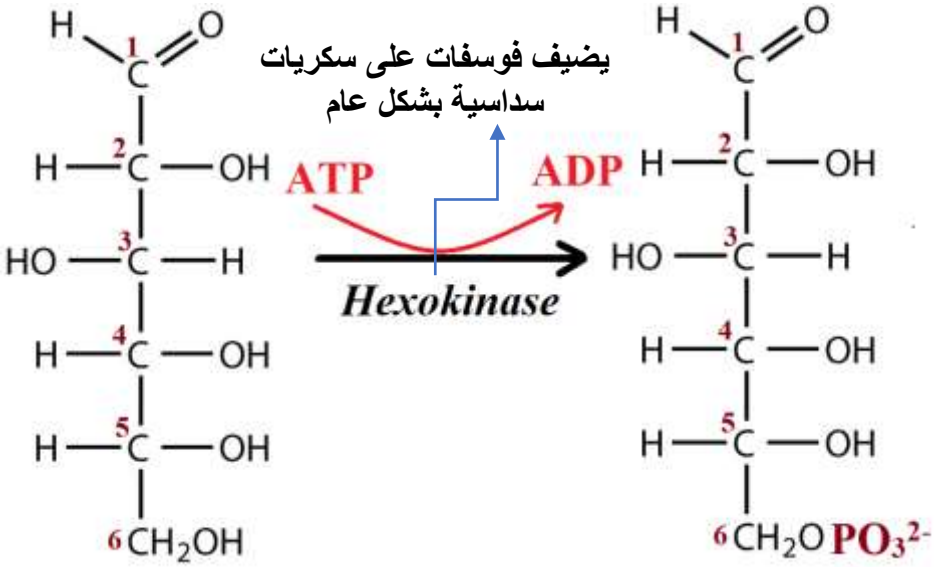


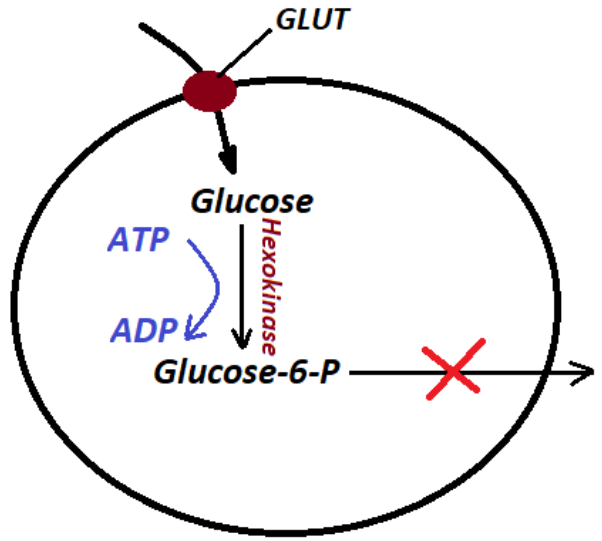
- Steps **1, 3, 10** are **Irreversible** steps, and are the **control** steps
  (غير عكسية — خطوات التحكم)
- **The rate limiting Step (Most important control step, Committed step) is Step 3**
  (الخطوة المحددة لسرعة ال Glycolysis)
- **Irreversible Steps have highly negative ΔG**

Glycolysis

Step 1

"Phosphorylation"





- ATP: is a source of Phosphate and Energy
- Glucose-6-P can NOT get out of the cell (no carriers for phosphorylated sugars in the cell membrane), so this step **trap** (تحتجز) glucose inside the cell

This step is Catalyzed by ***Hexokinase***

4 Isozymes of Hexokinases I,II,III, and IV

Hexokinases I, II, and III found in body tissues (example skeletal muscles)

Hexokinase IV also called ***Glucokinase (GK)*** found in Liver and Pancreatic β-cell

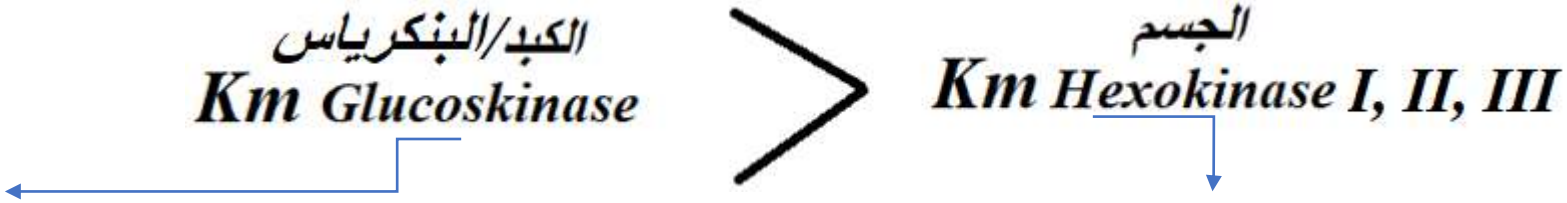
تخصص واسع

All have broad specificity; add phosphate to different Hexoses

بالتالي بتقدر تحكي انو وظيفة ال Hexokinase انو يحتجز الجلوكوز داخل الخلية حتى يصيرله Metabolism

Basics:

- ***Body tissues*** تحتجز trap glucose for energy production, so they must be able to trap glucose even if it's concentration in the blood is low. Also body tissues must not trap glucose more than they need because they cannot store it, so when blood glucose is very high body tissues must not be able to trap glucose more than they need.
- ***Liver cells:*** store glucose as glycogen after meals; when blood glucose level is high, so by logic liver must be able to trap glucose **only when it's concentration is high**.
- ***Pancreatic β-cells*** secret Insulin **only** when blood glucose level gets high, so these cells must trap glucose **only if it's blood concertation is high** in order to recognize يعرف it's time to secret insulin.



High km → Low affinity for glucose
→ It works only when the blood glucose level is high
→ Liver and pancreatic β-cells trap glucose only if it's concentration is high (Hyperglycemia)
Liver→ for storage as glycogen
pancreatic β-cells → its **glucose sensor** for insulin secretion

low km → high affinity for glucose
→ It works even if Glucose level in blood is low
→ Body tissue can trap glucose even if its blood level is low because it's the preferred source of energy

Fasting blood glucose level = 4-5mM

Hexokinases I, II, and III has lower Km and Vmax

Glucokinase has higher Km and Vmax

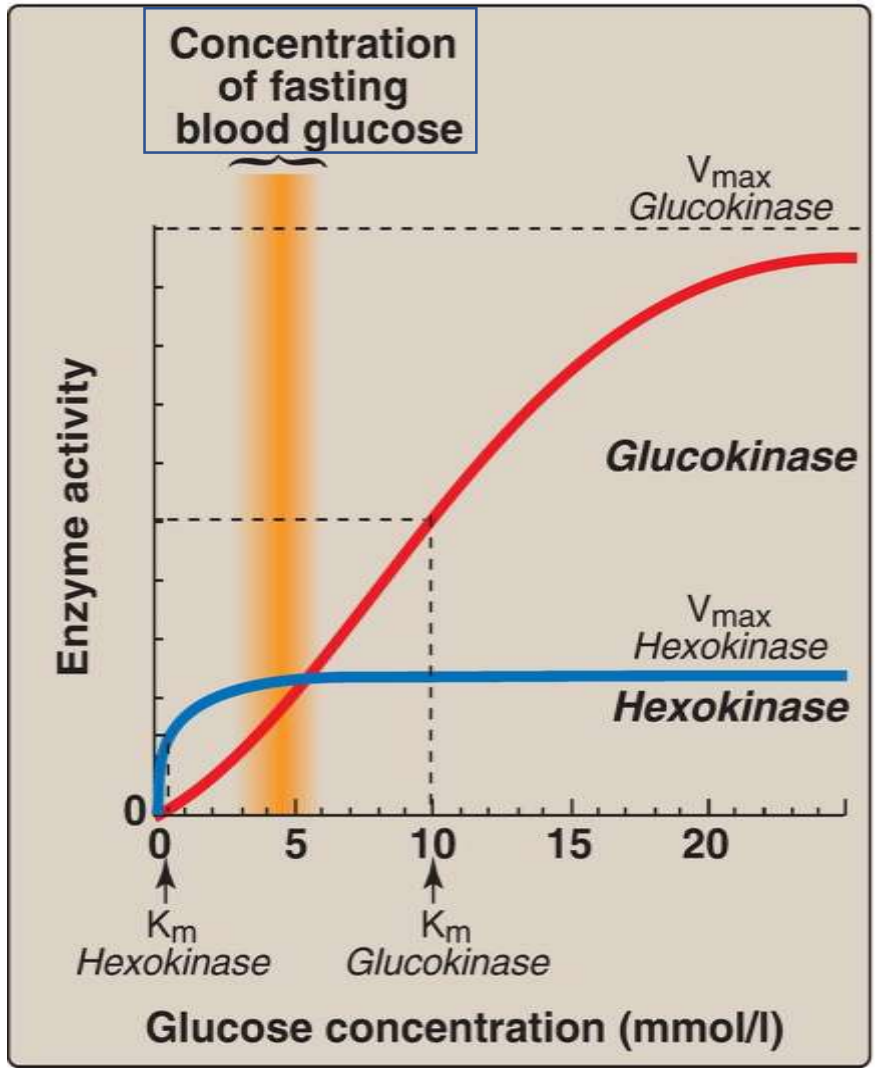
What does that suppose to mean?

Hexokinases I, II, and III work whether glucose concentration is low or high so body tissues can trap glucose anytime.

have low Vmax so that at very high glucose concentration body tissues cannot trap (sequester) تحتجز glucose more than they need

Glucokinase work only when glucose level is high so liver trap glucose when it's blood level is high to be stored as glycogen

Has high Vmax so when blood glucose gets high liver is able to trap more glucose and remove excess glucose in the portal vein after high carbohydrate meal



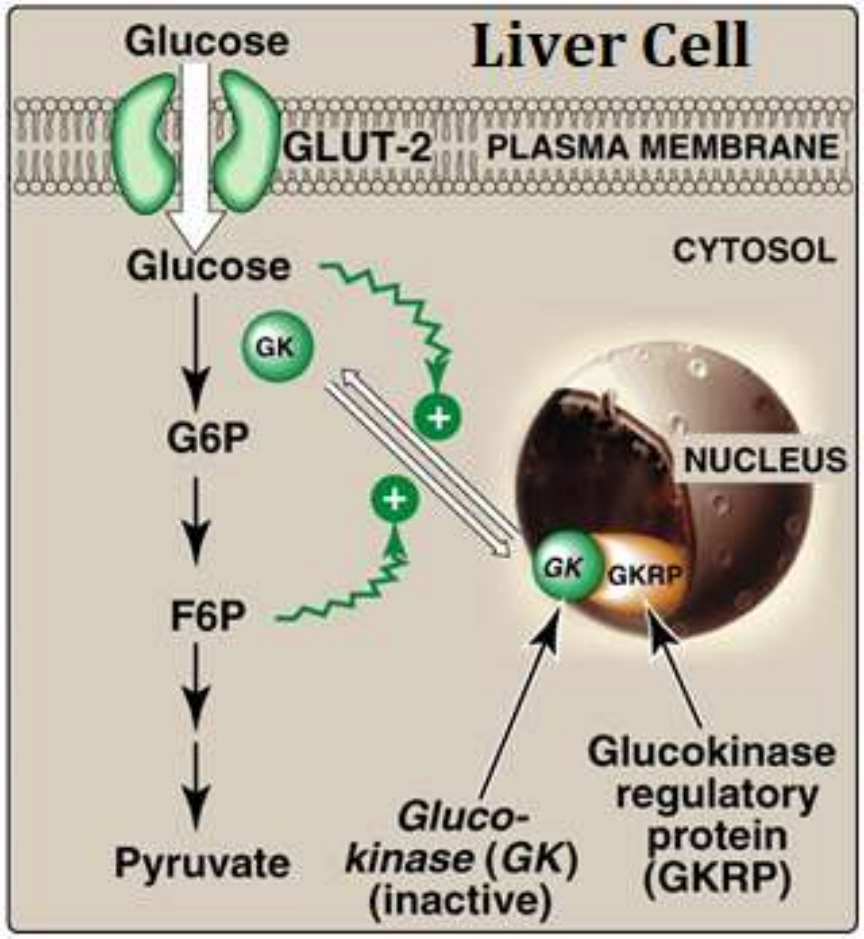
Hexokinases are simple not Allosteric enzymes

Control of Hexokinases

Hexokinases I, II, and III: are inhibited directly by ***Glucose-6-P*** (Product inhibition) so when G-6-P increase there is no need to trap more glucose

Glucokinase Control

- ***Fructose-6-P*** stimulate binding of GK tightly to GKRP; this make GK inactive and transported to the nucleus
- If ***Glucose*** is high, Glucose stimulate the dissociation (انفصال) of GK from GKRP this will activate GK and get out of nucleus



- **Note: GKRP is a competitive inhibitor for glucokinase**

↑ Insulin ↓ Glucagon → High Glucose

High $\frac{Insulin}{Glucagon}$ ratio (well Fed)

→ activate transcription of GK gene in the liver

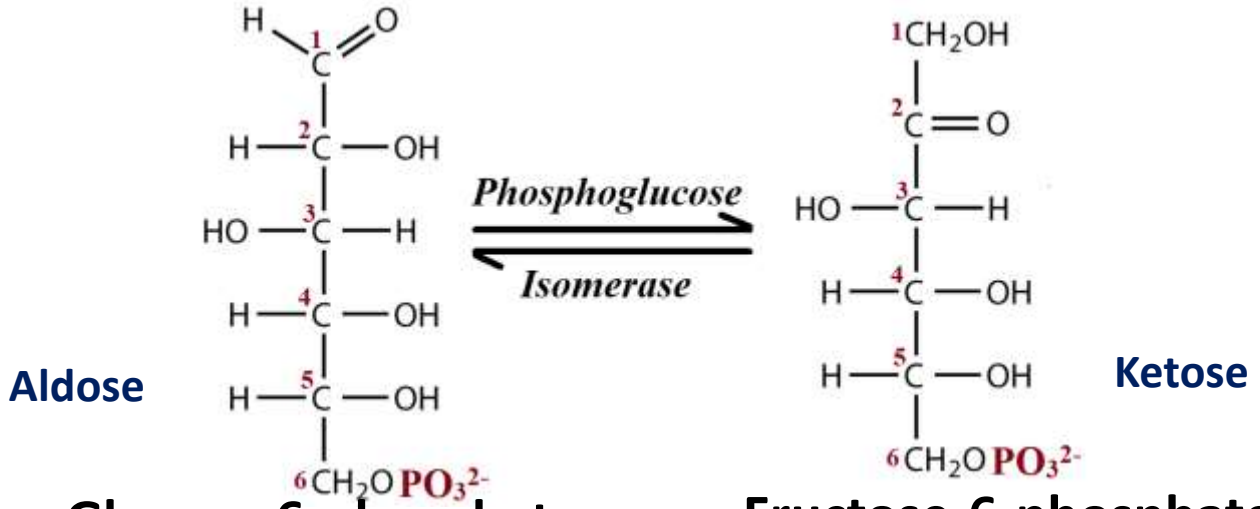
→ Increase the amount of GK

Glucokinase is induced by insulin; while hexokinases I,II,III is not

Step 2

“Isomeration”

اعادة ترتيب ذرات



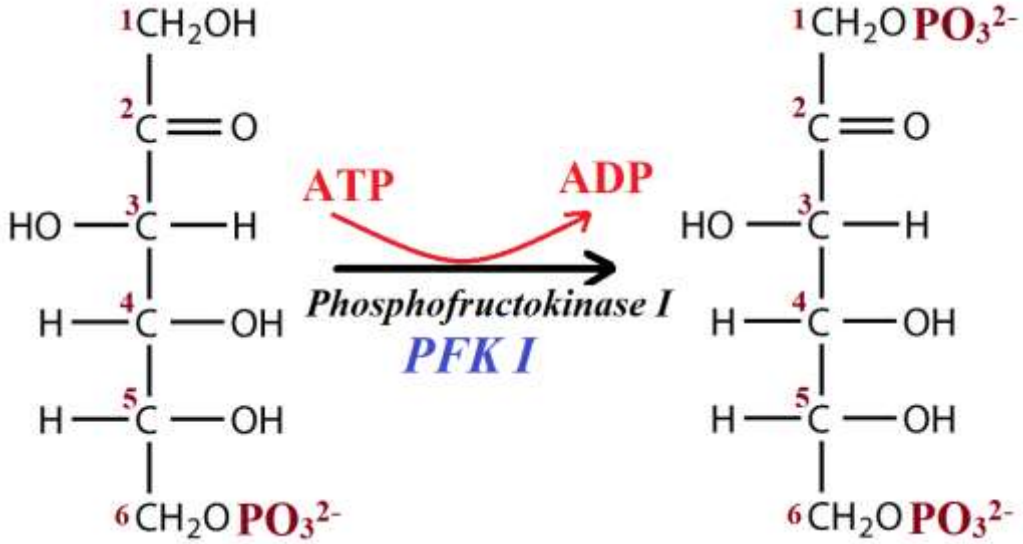
Glucose-6-phosphate

Fructose-6-phosphate

Step 3

“Phosphorylation”

- **this is the most important step “Rate Limiting Step, committed step” in Glycolysis**



Fructose-6-phosphate

Fructose-1,6-bis-phosphate

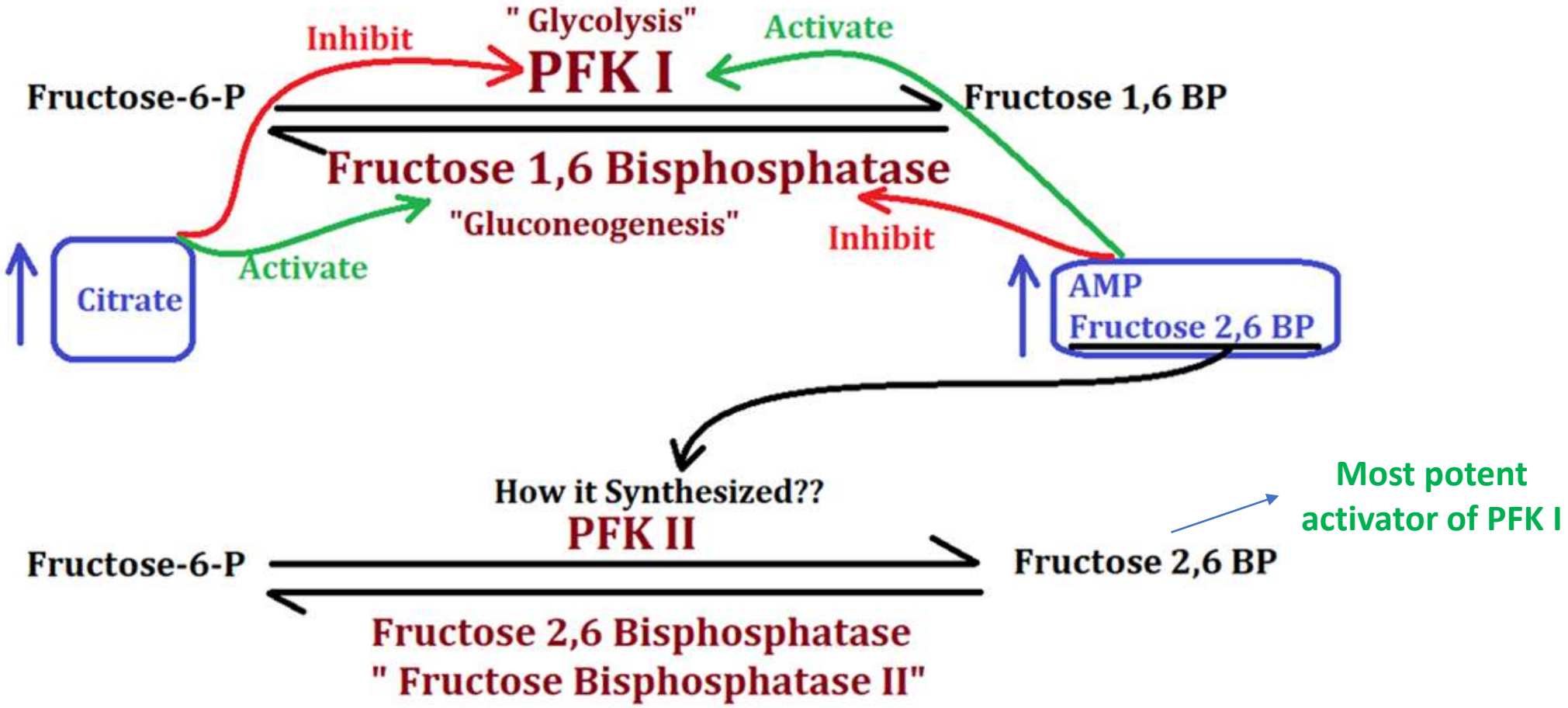
This intermediate
Commit to glycolysis

Allosteric Control:

↑ ATP, Citrate (both indicate high energy level of the cell) → Inhibit PFK I

↑ AMP, Fructose 2,6 Bisphosphate → activate PFK I

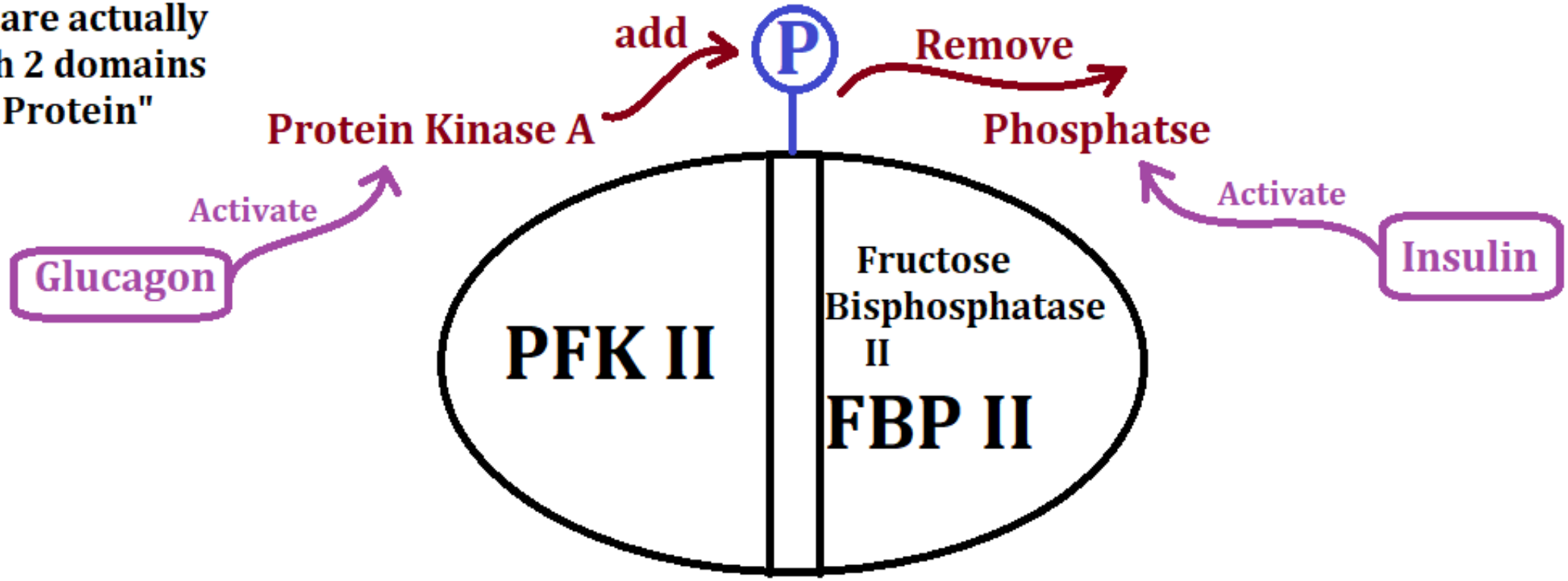
Step 3 in Glycolysis and Gluconeogenesis



- If PFK II is active → ↑ Fructose 2,6 BP → PFK I is activated "Glycolysis"
- If Fructose Bisphosphatase II (FBP II) is active → ↓ Fructose 2,6 BP → Fructose 1,6 bisphosphatase is activated "Gluconeogenesis

In the Liver

these 2 Enzyme are actually one protein with 2 domains "Bifunctional Protein"



* If this complex is phosphorylated →→ FBP II is active →→ Gluconeogenesis is active

* If this complex is dephosphorylated →→ PFK II is active →→ Glycolysis is active

In Words:

When Glucose is available → high Insulin/Glucagon ratio, Insulin bind to its receptor activating ***phosphatases*** which Remove phosphate from PFKII/FBPII bifunctional protein

PFKII is activated

FBFII is inactivated

Now the level of Fructose 2,6 Bisphosphate is increased which is the most potent activator for PFK I

PFKI is activated (Glycolysis is active)

Fructose 1,6 Bisphosphatase is inhibited (Gluconeogenesis is inactive)

When Glucose is low → low insulin/Glucagon ratio, Glucagon bind to its receptor activating an enzyme called ***adenylate cyclase*** which convert ATP to cAMP; now cAMP activate ***Protein Kinase A (PKA)*** which add phosphate to PFKII/FBPII bifunctional protein

PFKII is inactivated

FBFII is activated

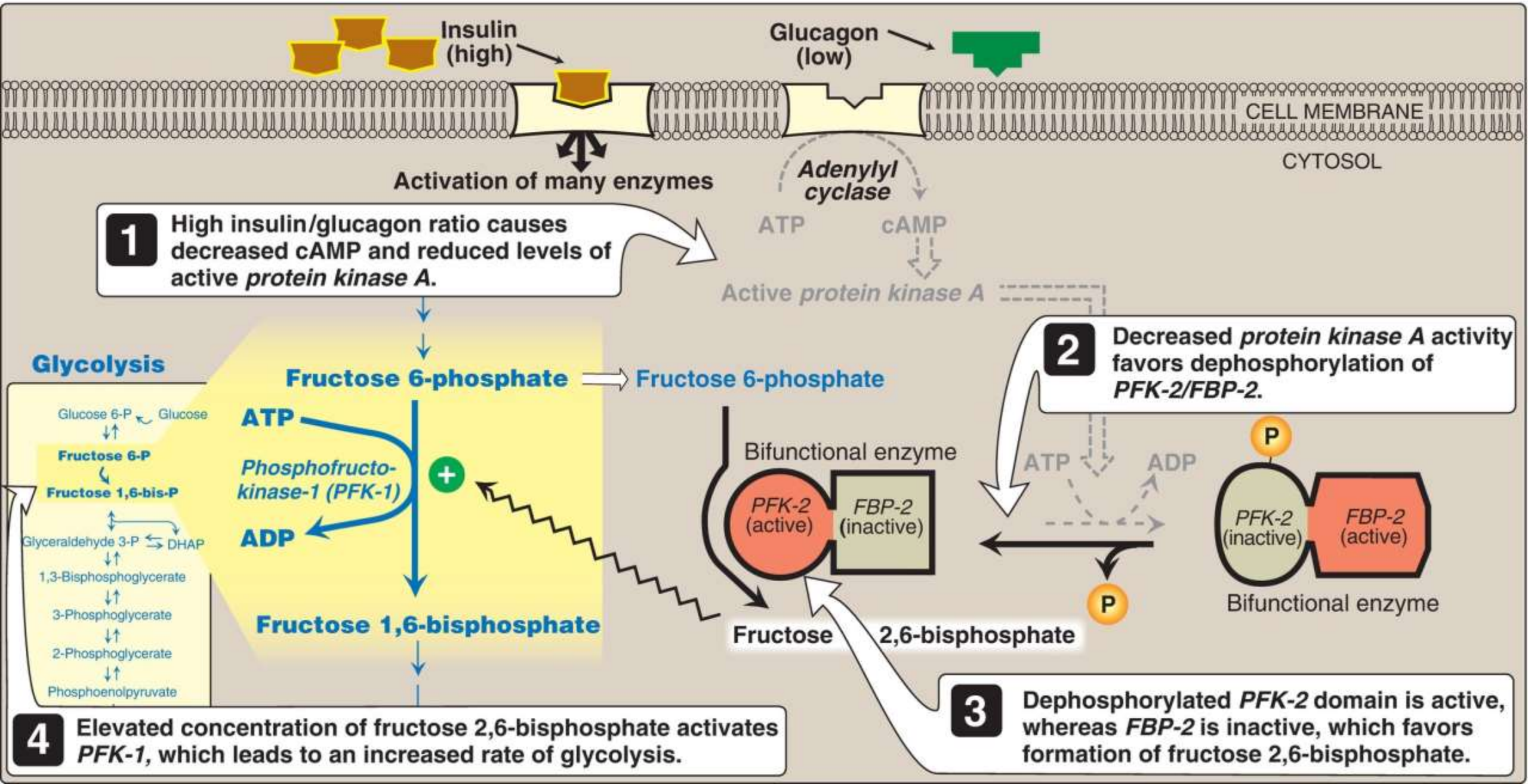
Now the level of Fructose 2,6 Bisphosphate is decreased

Fructose 1,6 Bisphosphatase is not inihibited (Activated)

Gluconeogenesis is active/Glycolysis is inactive

لا جديد فقط رسمة الكتاب لشرح الموضوع

اقرأ الخطوات من 1 الى 4 ثم اشرح تأثير ال Glucagon للمراجعة



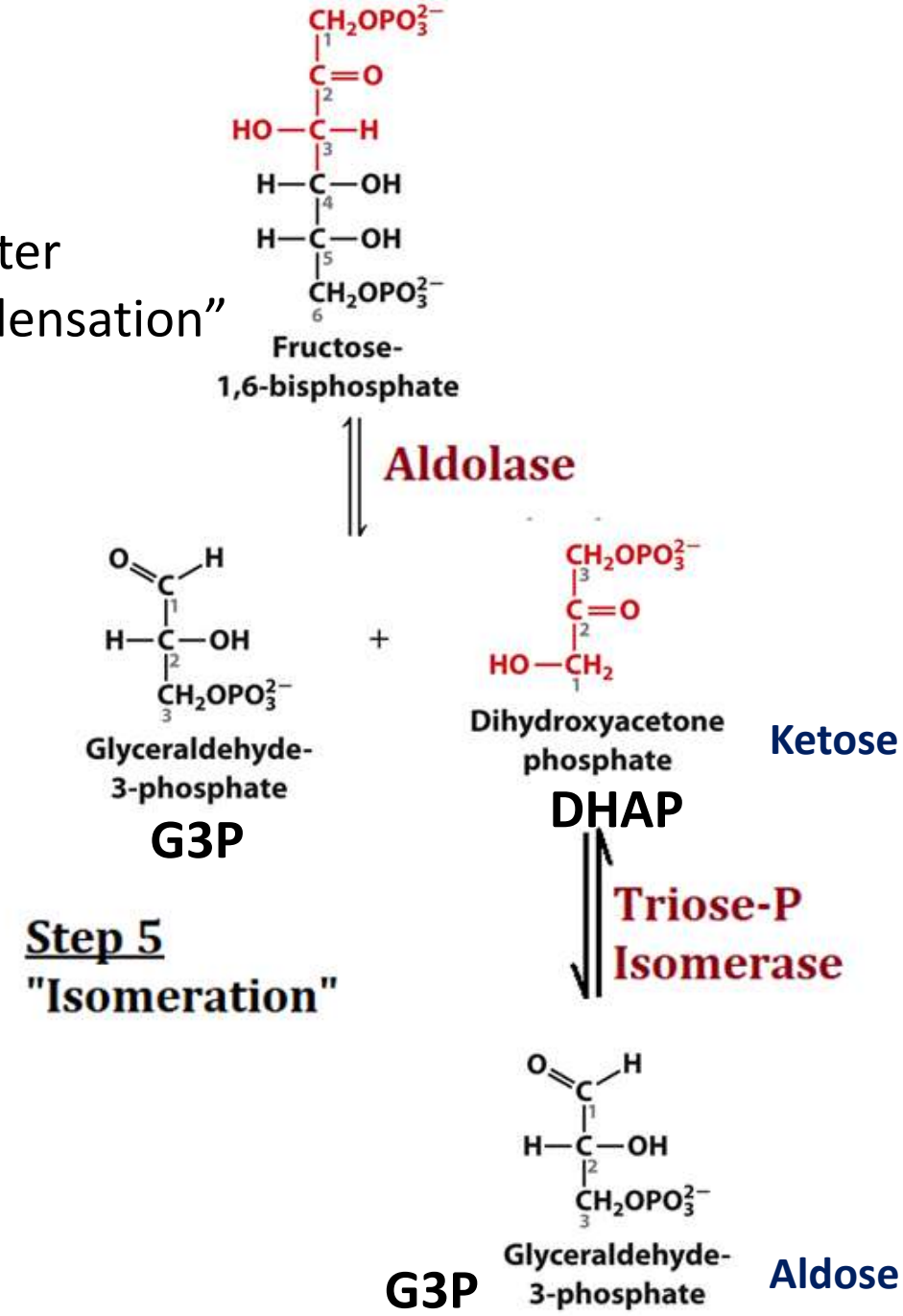
Step 4

“Cleavage”

Not Hydrolysis

The Enzyme ***Aldolase*** is Lyase, degrade without using water

This reaction is called Aldol Cleavage “Reverse Aldol condensation”



Up to this step

Glucose → 2 Glyceraldehyde-3-P

And 2 ATP are consumed in steps 1,3

From now on you should remember that we have 2 Glyceraldehyde-3-P **2 x لذلك نضرب النواتج**

ΔG -ve ΔG +ve

Step 6 "Oxidation + phosphorylation"

Contain high energy phosphate

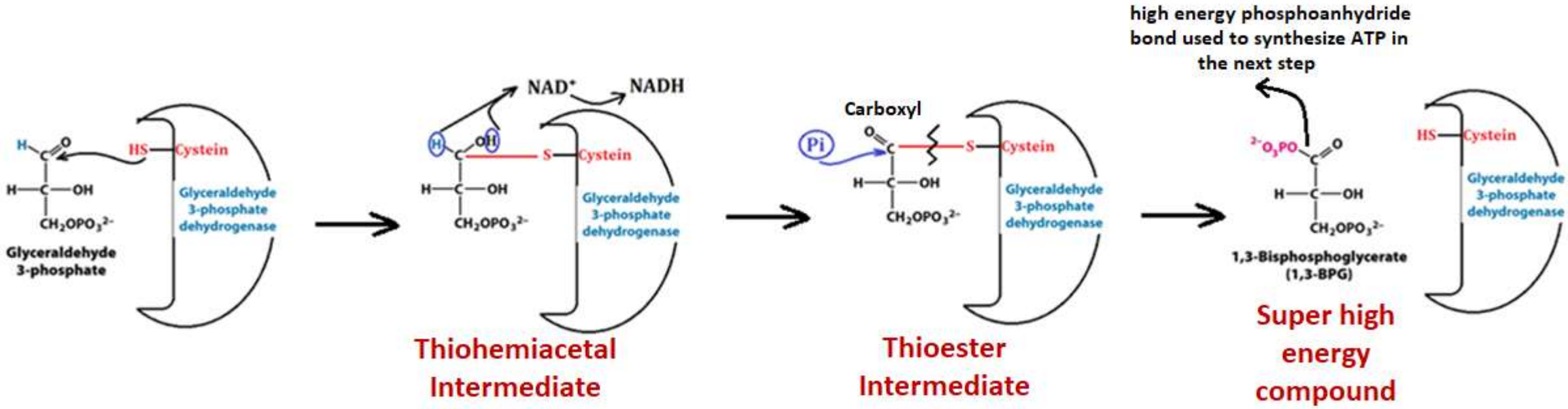
So, in this step there are

-Oxidation

-Phosphorylation, but Phosphate here is not from ATP, its just a Phosphate ion Pi

- aldehyde group in glyceraldehyde 3-phosphate is oxidized to carboxyl group that is bind to the Pi forming **1,3- bisphosphoglycerate (1,3-BPG)**
- It is the first and only oxidation-reduction reaction of glycolysis
- Pentavalent arsenic poisoning (Arsenate "AsO_4^{-3}") compete with Pi to bind in this step

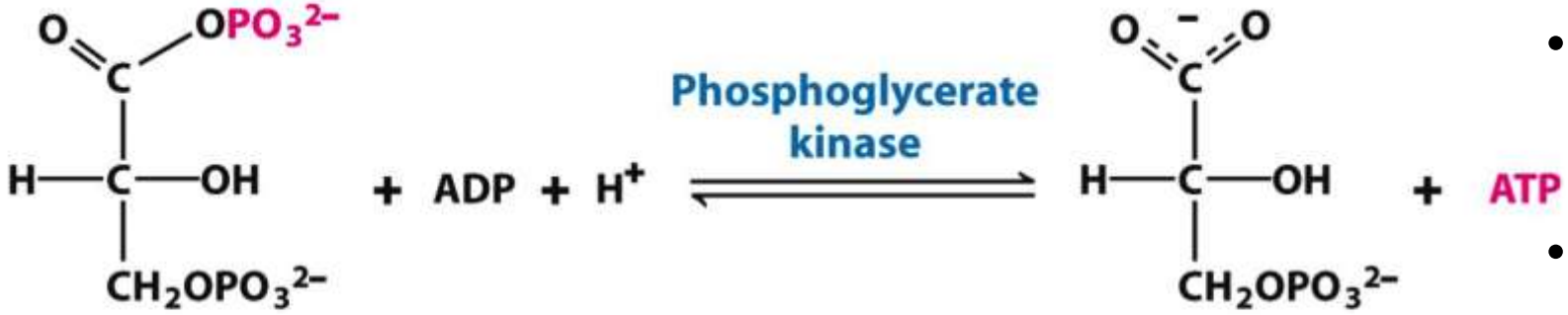
Mechanism of Glyceraldehyde-3-P dehydrogenase



1. The enzyme has Cysteine in its active site. The SH groups of cysteine attack the aldehyde carbonyl carbon of the Substrate forming a covalent intermediate called ***Thiohemiacetal intermediate***
2. The S is oxidized to ***Thioester intermediate*** while NAD^+ is reduced to NADH
3. Pi attack C1 carboxyl of the S forming 1,3 bisphosphoglycerate and regenerating the original form of the enzyme

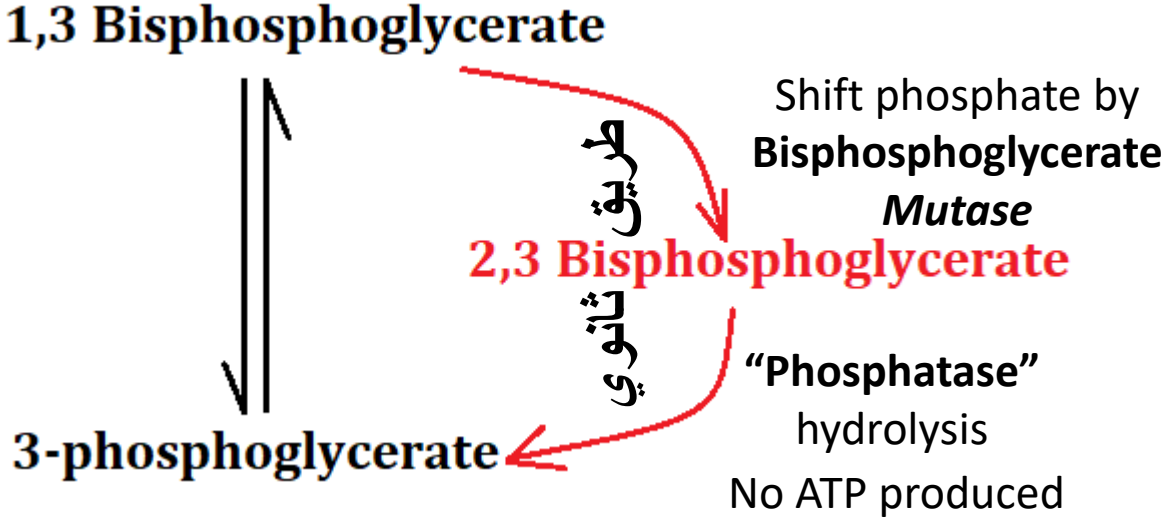
Step 7

"Transfer of Phosphate group to ADP"



- In this step we produce ATP by substrate level phosphorylation
- Up to this step Net ATP = Zero
- unlike most other kinases, phosphoglycerate Kinase is ***physiologically reversible***.
- Arsenate prevent ATP synthesis in this step

Note: In RBCs some 1,3 Bisphosphoglycerate can be converted to 2,3 Bisphosphoglycerate

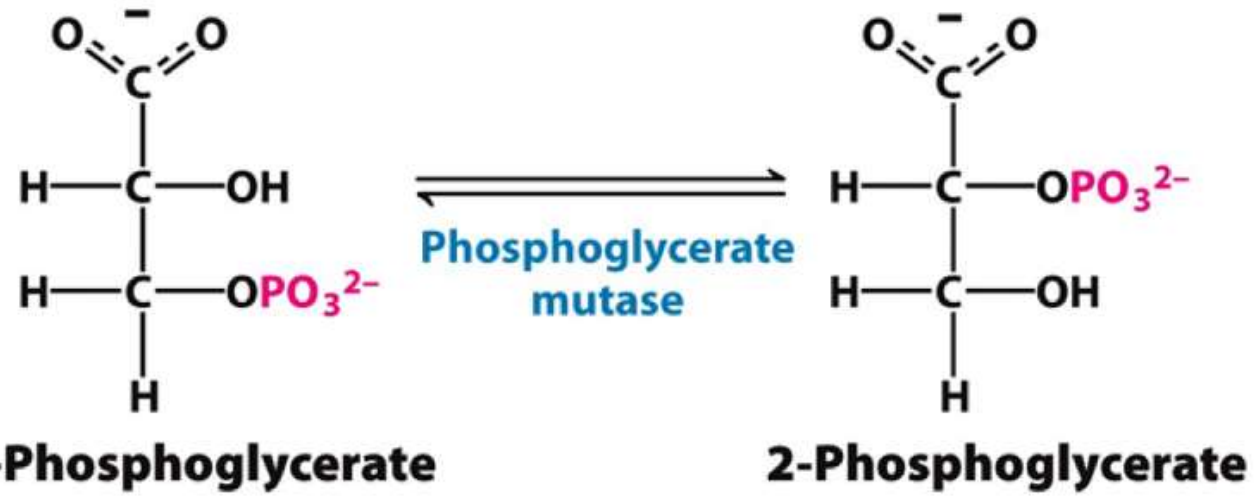


- In RBCs 2,3 Bisphosphoglycerate ↓ affinity between O_2 and Hemoglobin this will ↑ O_2 release

Step 8

“Isomeration”

Shift P group

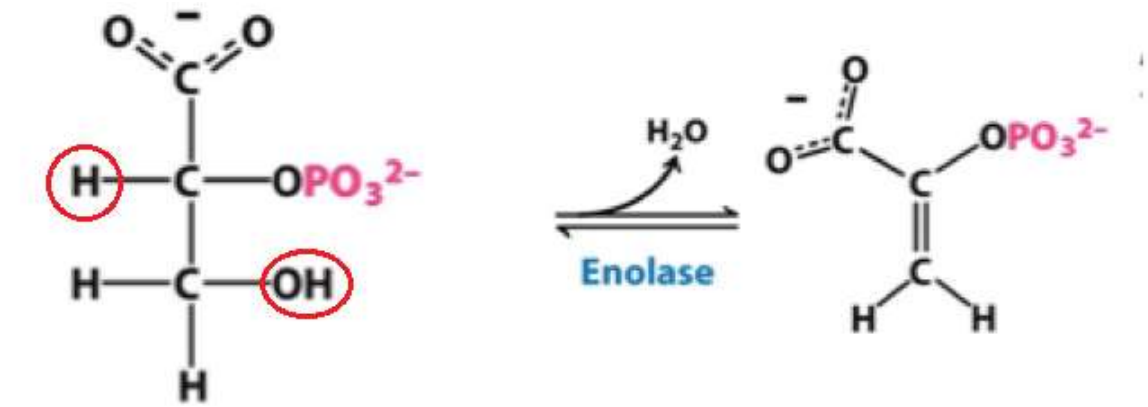


3-Phosphoglycerate

2-Phosphoglycerate

Step 9

Dehydration



2-Phosphoglycerate

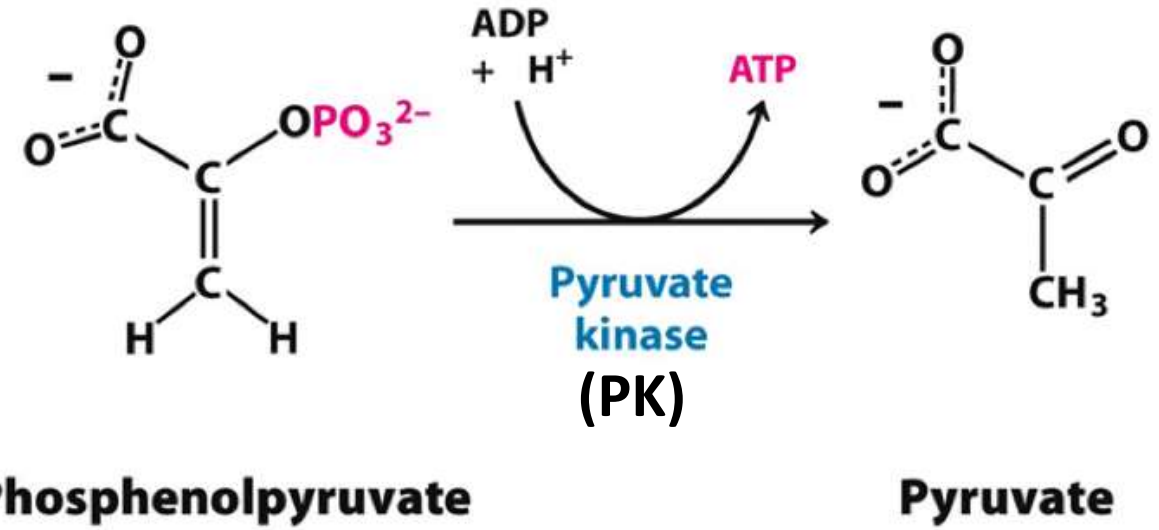
Phosphenolpyruvate

Contain high energy phosphate

مهم

Fluoride inhibit ***Enolase***, So Fluoridated water, and toothpastes prevent dental caries by inhibiting bacterial Enolase.

Step 10



" Transfer of Phosphate group to ADP"

Substrate Level phosphorylation

This Enzyme has an allosteric Control:

↑ ATP → Inhibit Pyruvate Kinase

↑ Fructose 1,6 Bisphosphate → activate pyruvate kinase "feed forward activation"

Liver pyruvate Kinase also has a covalent control:

- **↓Glucose → Glucagon → phosphorylation of liver Pyruvate kinase→ inhibition**
- **↑Glucose → Insulin → Dephosphorylation of liver Pyruvate kinase → Activation**

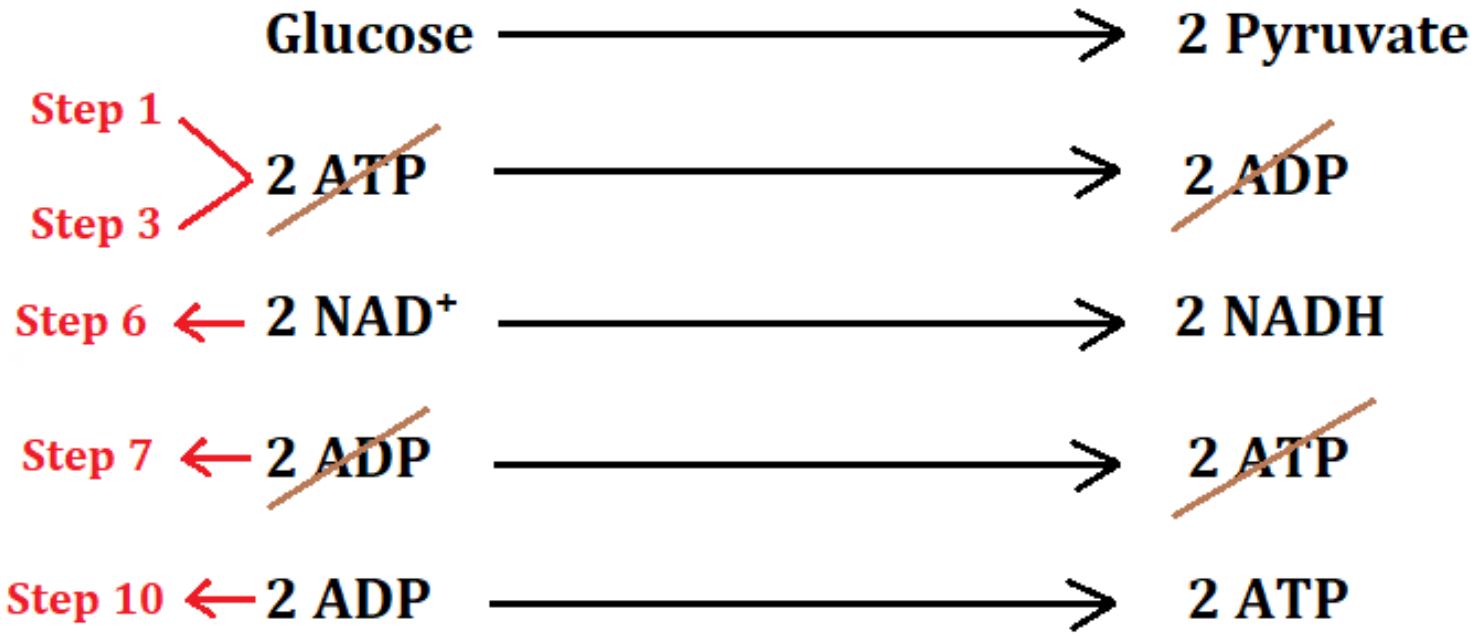
Clinical Note:

- Liver and RBCs have the same PK isozyme
- Mutation of liver and RBCs PK gene will make the enzyme less active "deficiency" (enzyme deficiency can be in the enzyme activity not in it's concentration)
- However, PK deficiency affect only RBCs leading to low ATP production and Hemolysis → Anemia why?
- liver cells can synthesize more PK to compensate (لتعويض) the low activity of the enzyme also liver cells can generate ATP from fat by oxidative phosphorylation.
- While RBCs cannot synthesize more PK (No nucleus/no genes) also RBCs has no mitochondria to generate ATP from oxidative phosphorylation → less ATP produced → Hemolysis → Anemia
- severity of anemia depends on the degree of enzyme deficiency
- BUT, PK deficiency in RBC → accumulation of 2,3-BPG → RBCs release more Oxygen

  خلايا الدم الحمراء تتكسر بسرعة لكنها كريمة اكثر في اعطاء الاكسجين
- Individuals heterozygous for PK deficiency have ***resistance to the most severe forms of malaria***.

Glucokinase (Step1), PFK I (Step3) and Pyruvate Kinase (Step10) all genetically controlled by Insulin → activate their genes
Glucagon → Inhibit their genes

Overall reaction of Glycolysis



Glycolysis is an ***amphibolic pathway***; means that it has a role in both catabolism and anabolism

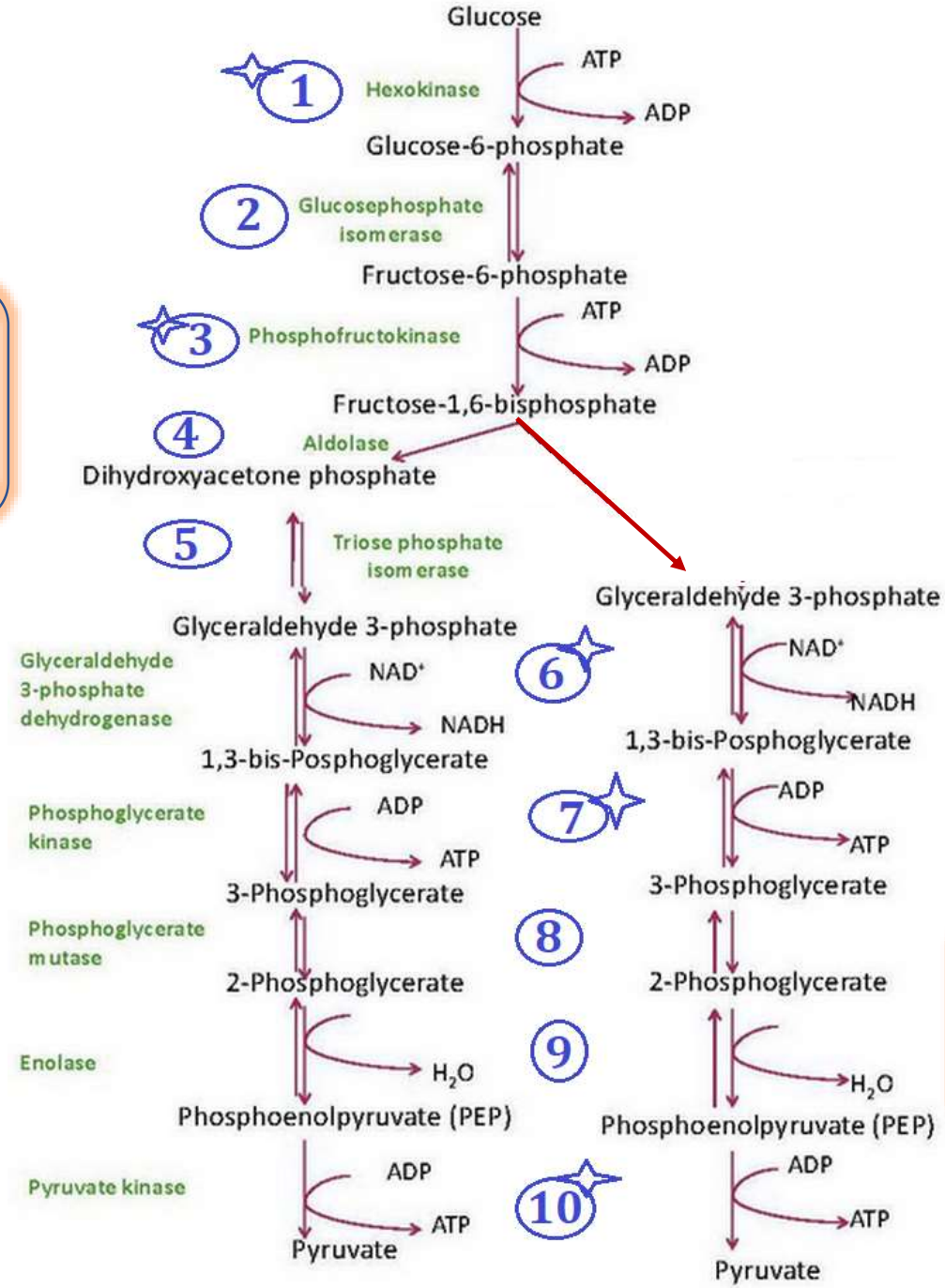
- Its important for carbohydrate catabolism
- The intermediates formed during glycolysis can be used as precursors to synthesize fat and amino acids for example:
  - 3-phosphoglycerate is a precursor for serine synthesis
  - Pyruvate is a precursor for alanine
  - DHAP can be converted to Glycerol-3-P for fat synthesis

$$\text{Glucose} + 2NAD^+ + 2ADP + 2Pi \longrightarrow 2\text{Pyruvate} + 2NADH + 2ATP$$

$$+ 2H_2O$$

Q: What are the products of one molecule of Glyceraldehyde-3-P when converted to pyruvate??

1 NADH
2 ATP



Q: Starting from Glucose-6-P to 2 pyruvate; what is the net ATP produced??

3 ATP

Q: Starting from Fructose 1,6-Bisphosphate to 2 Pyruvate; what is the net ATP produced??

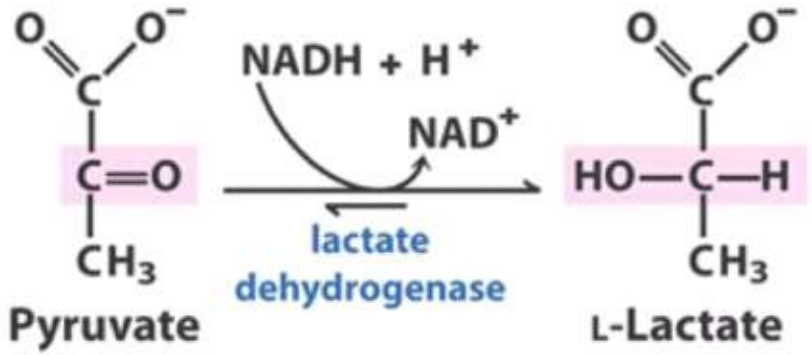
4 ATP

Anaerobic Glycolysis

This occurs in:

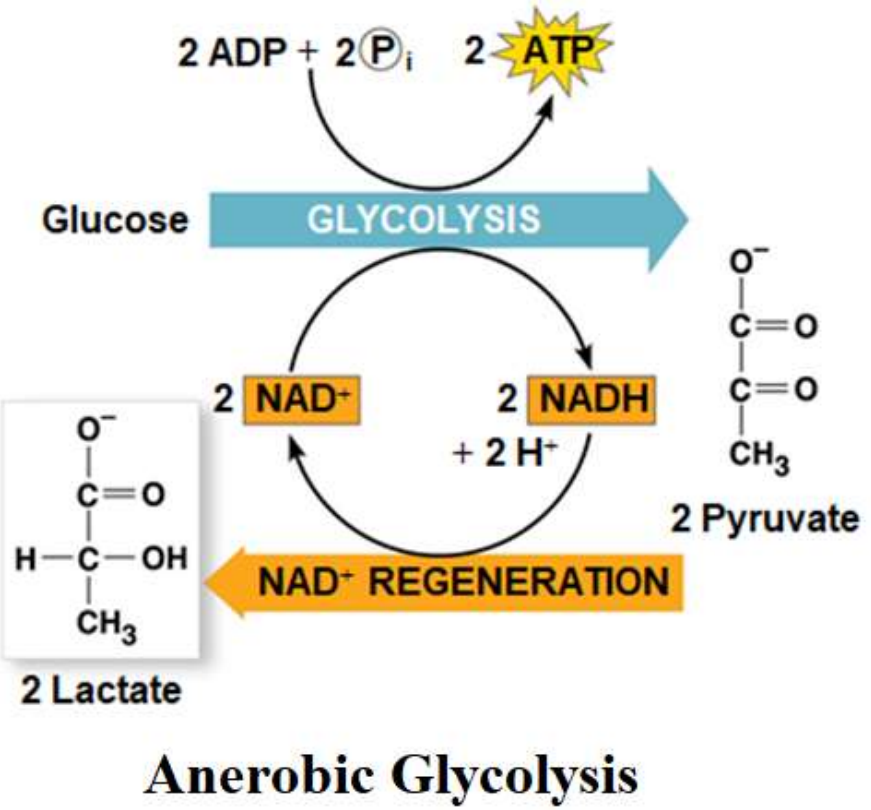
a. Cells that lack (تفتقر) mitochondria such as RBCs
b. Cells of avascular tissues such as eye lens/cornea and kidney medulla where low oxygen level (Hypoxia)
c. Exercising muscle where more ATP is needed

خطوة اضافية



What is the aim of this step?

We know that during glycolysis in step 6 we need NAD^+ to be reduced to NADH, this NADH must be reoxidized back to NAD^+ in order to have a continuous supply of NAD^+
(we must regenerate NAD^+)
In **Aerobic** glycolysis NADH is reoxidized when it gives it's electron to Oxygen through ETC
But in RBCs where no ETC or when Oxygen is not available how can we regenerate NAD^+??
This is done by reducing Pyruvate to Lactate, this step regenerate NAD^+



Q: the final electron acceptor in Anerobic Glycolysis is ……Pyruvate……

This step is ***reversible*** and the direction depends on the relative concentration of reactants and products (Pyruvate/lactate ratio and more important NADH/NAD^+ ratio) high **NADH/NAD^+** ratio stimulate reduction of pyruvate to lactate

Now what is the fate of Lactate??

Lactate is diffused out of the cells to blood to be transported to the liver cells, where lactate is converted back to pyruvate which is used for glucose synthesis "Gluconeogenesis"

Lactic acid (Lactate) is an **Acid** which lowers the pH, during heavy exercise lactic acid is accumulate in muscles and blood this leads to:

- Muscle cramp (تشنج) and pain
- Lactic acidosis (low blood pH due to high Lactic acid)

- Any thing ↑ production of Lactic acid or ↓ Lactic acid utilization (استهلاك) will cause Lactic acidosis

Causes:

1. Impaired O_2 transport as in **Myocardial Infarction (MI)** (السكتة القلبية)

→ Collapsed (فشل / انهيار) Circulatory system → Hypoxia → increase Lactic acid production

2. Respiratory Failure as in **Pulmonary Embolism** جلطة رئوية

↓O_2 → Hypoxia → increase Lactic acid production

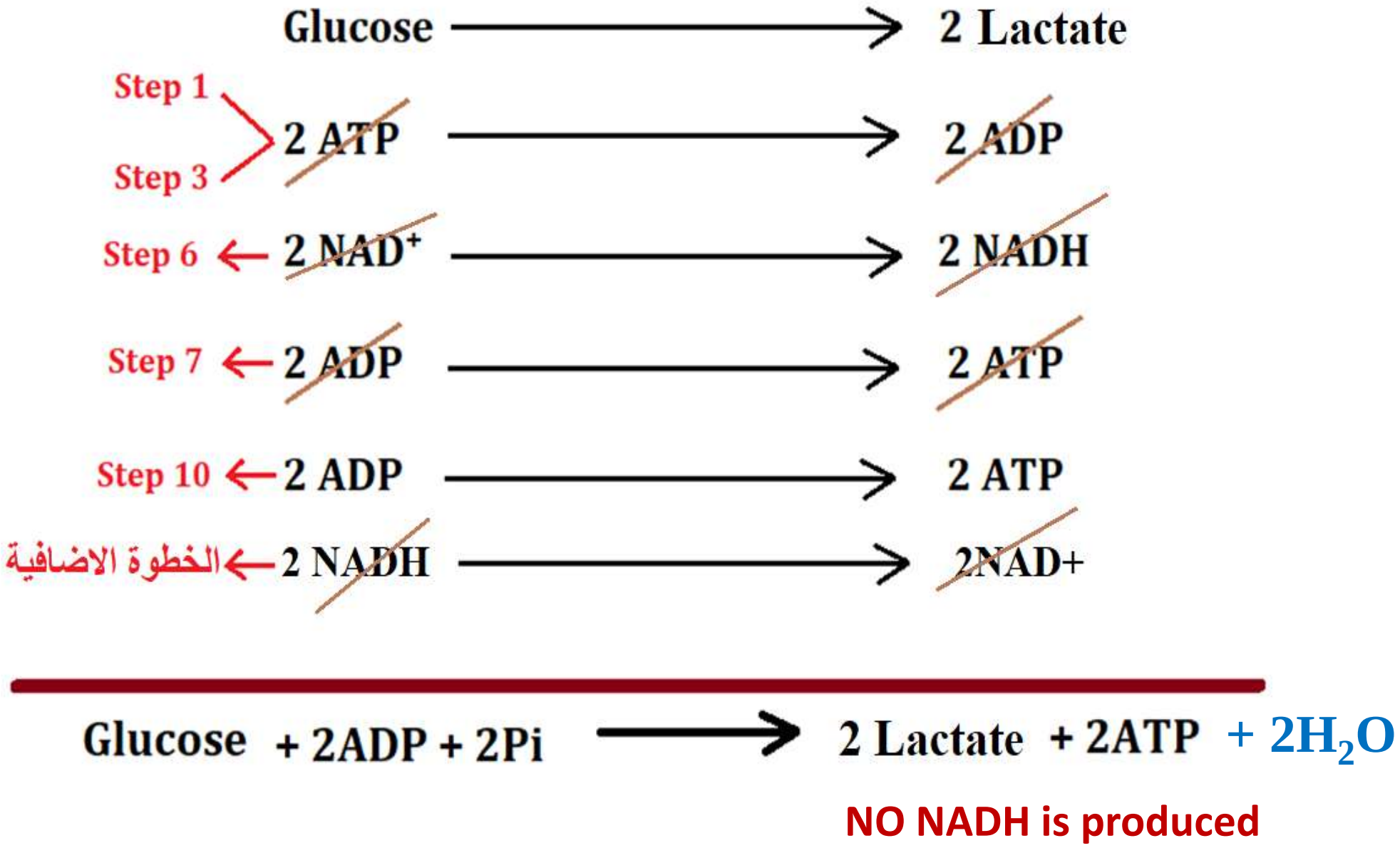
3. **Uncontrolled Hemorrhage** نزيف حاد

↓Blood Pressure → ↓ Circulation → ↓O_2 → Hypoxia → increase Lactic acid production

4. **Alcohol Intoxication** leads to increase NADH/ NAD^+ ratio

او اي سبب منطقي يؤدي لتراكم ال **Pyruvate** او ال **NADH**

Overall Reaction of Anaerobic Glycolysis



NO NADH is produced

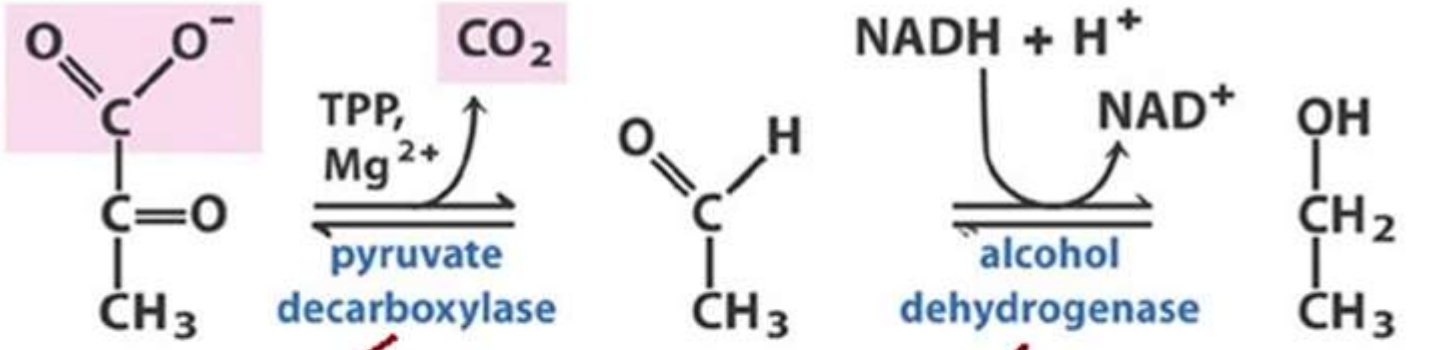
Alcoholic Fermentation in Yeast and some Bacteria

Pyruvate (3C) ⟶ **Ethanol + CO_2** (2C)

This occurs in 2 Steps:

Aim: Regenerate NAD^+ to be used in Step 6

Q: The final electron acceptor in alcoholic fermentation is ……Acetaldehyde……



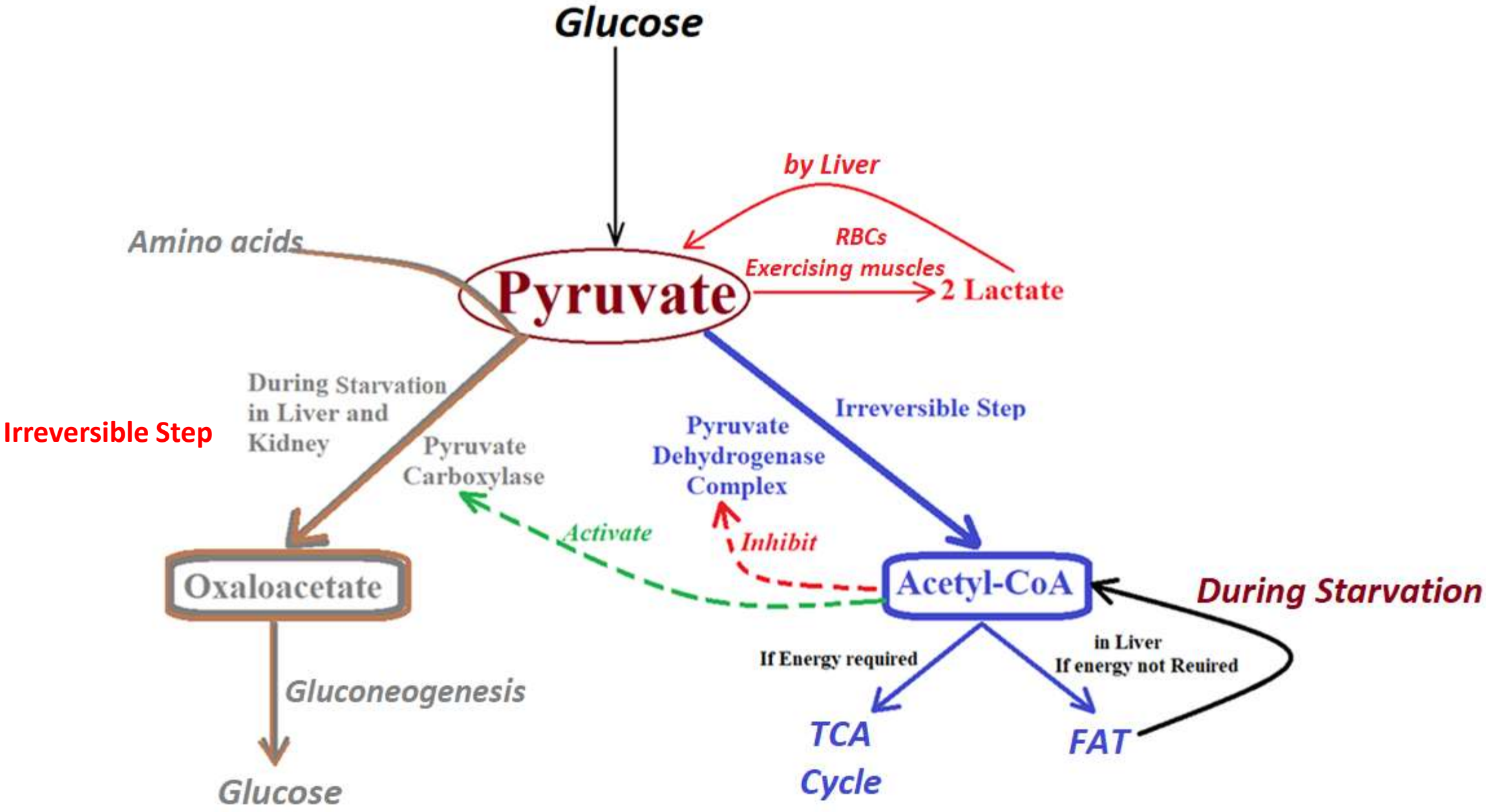
Require Thiamine Pyrophosphate (B1)
This enzyme NOT found in human
as separated enzyme

موجود في الانسان

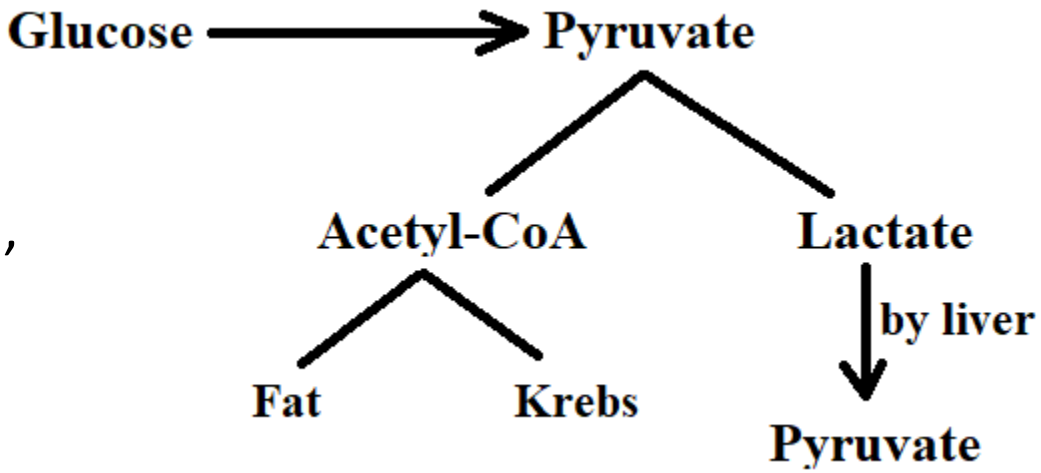
Overall Reaction of Alcoholic Fermentation

Glucose + 2ADP + 2Pi ⟶ 2 Ethanol + 2 CO_2 + 2ATP

your body during Fed state and during Starvation



In words



When glucose is available (Fed State)

Glucose is converted to pyruvate by glycolysis

Pyruvate is converted to lactate in RBCs and Hypoxic tissues , then lactate is converted back to pyruvate in the liver

In other tissues pyruvate is Oxidatively decarboxylated to Acetyl-CoA by Pyruvate dehydrogenase complex

Acetyl-CoA:

a. Oxidized by TCA cycle if energy is required
b. Converted to Fatty acids (FAT) if energy is not required

When Glucose is NOT available (Starvation)

➢Fat is degraded to Acetyl-CoA ↑↑↑

- High level of acetyl-CoA indicate that you are starved and Fat is catabolized in large amounts

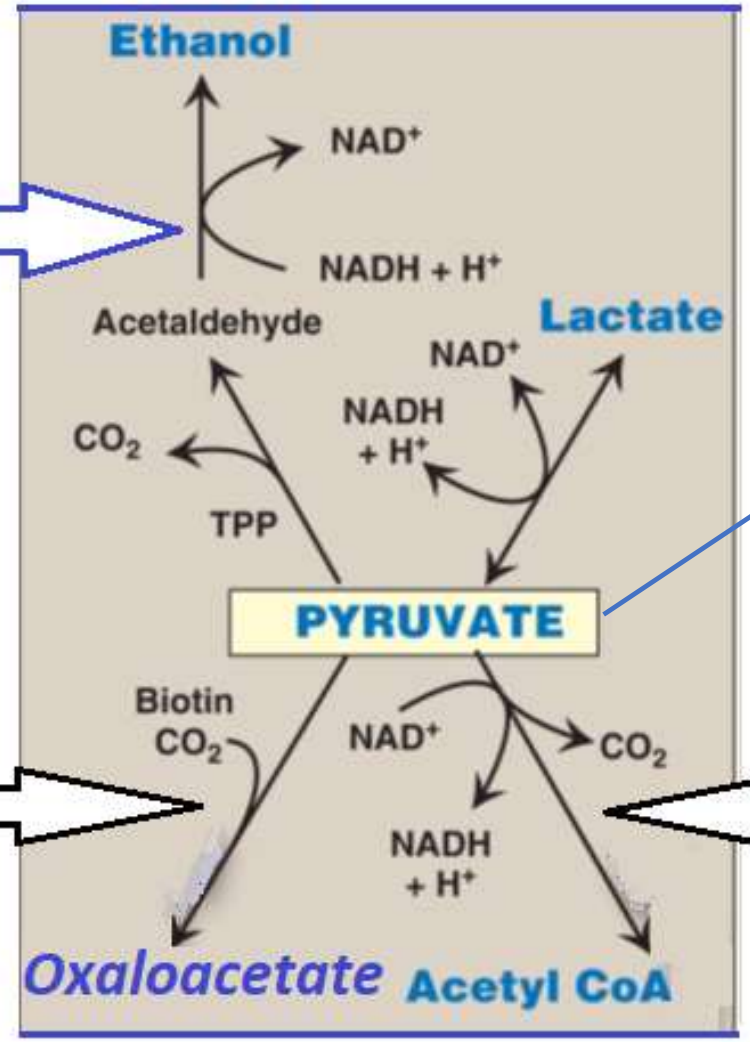
➢In the liver Pyruvate must **not** be converted to acetyl-CoA but to oxaloacetate by ***Pyruvate Carboxylase*** which require Biotin (B7) as cofactor, then oxaloacetate is used to synthesize glucose “Gluconeogenesis” ; so High acetyl-CoA inhibit pyruvate Dehydrogenase and activate Pyruvate Carboxylase

-During Starvation Pyruvate come mainly from amino acids

Fates of pyruvate

ETHANOL SYNTHESIS

- Occurs in yeast and some bacteria (including intestinal flora)
- Thiamine pyrophosphate–dependent pathway



Alanine

PYRUVATE CARBOXYLASE

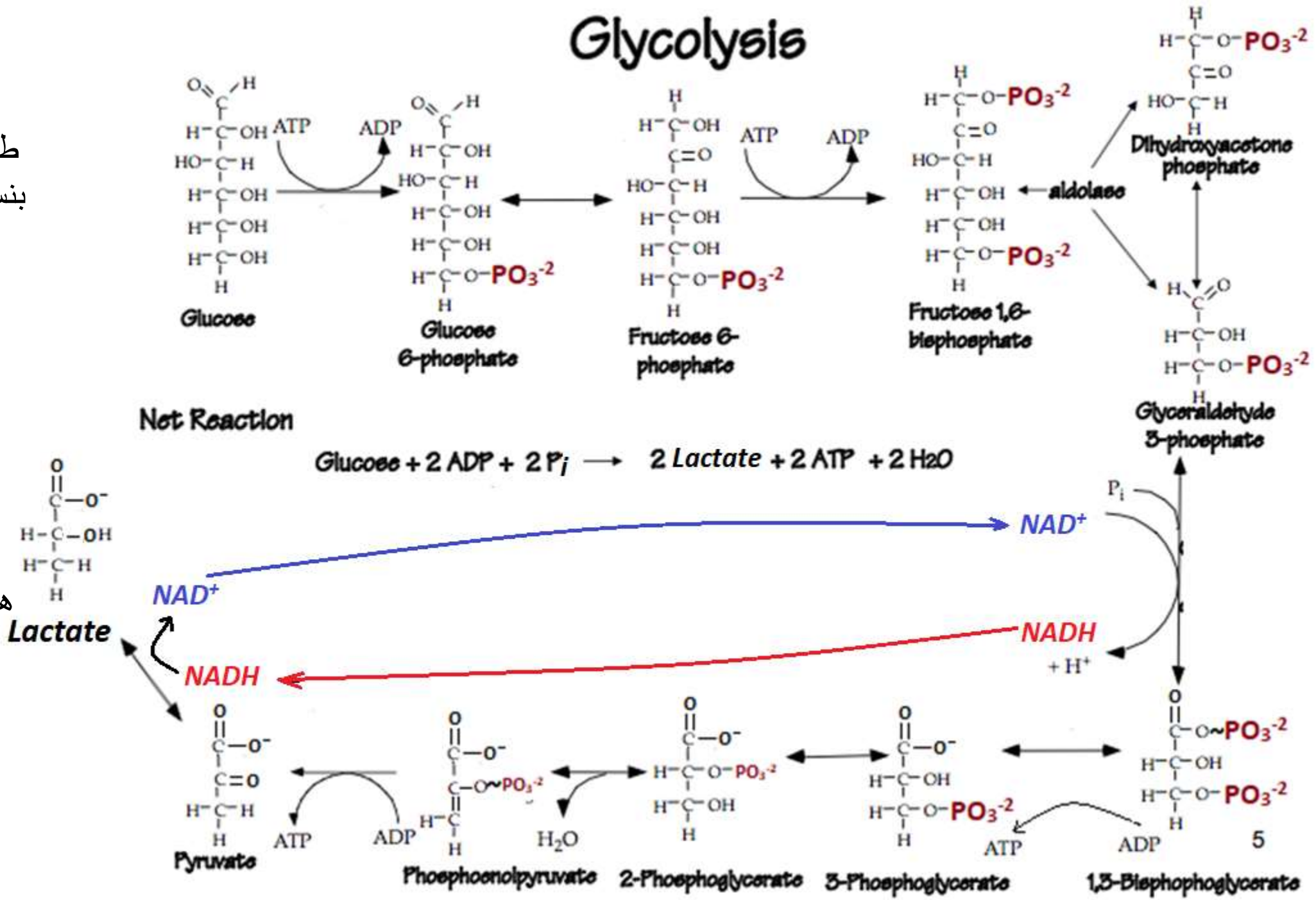
- Activated by acetyl CoA
- Replenishes intermediates of the TCA cycle
- Provides substrates for gluconeogenesis
- An irreversible reaction

PYRUVATE DEHYDROGENASE COMPLEX

- Inhibited by acetyl CoA
- Source of acetyl CoA for TCA cycle and fatty acid synthesis
- An irreversible reaction

تمييز الخطوات عن
طريق ال Structures
بنسأل عنه الدكتور لنتأكد
+

ما في ارقام خطوات
بالامتحان
يعني ما في
Step1
Step2
Step3
هاد كان لتسهيل الشرح



Glucose Oxidation pathways:

1. Anaerobic
2. Aerobic

- Major oxidation pathways
- For Energy production

3. Hexose Monophosphate shunt "HMS"
4. Uronic acid pathway

- Minor oxidation pathways
- NOT for Energy production

Importance of Glycolysis for RBCs:

1. Provide energy 2ATP (RBCs require ATP for Na-K pump to maintain its normal shape and fixability)
2. Reduction of methemoglobin (Fe^{+3}) to normal Hemoglobin (Fe^{+2}), this is done by Cytochrome b5-Methemoglobin Reductase which use NADH formed by glycolysis as a reducing agent
3. Formation of 2,3 BPG