بسم الله الرحمٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | Lecture 12

Glycolysis Pt.3

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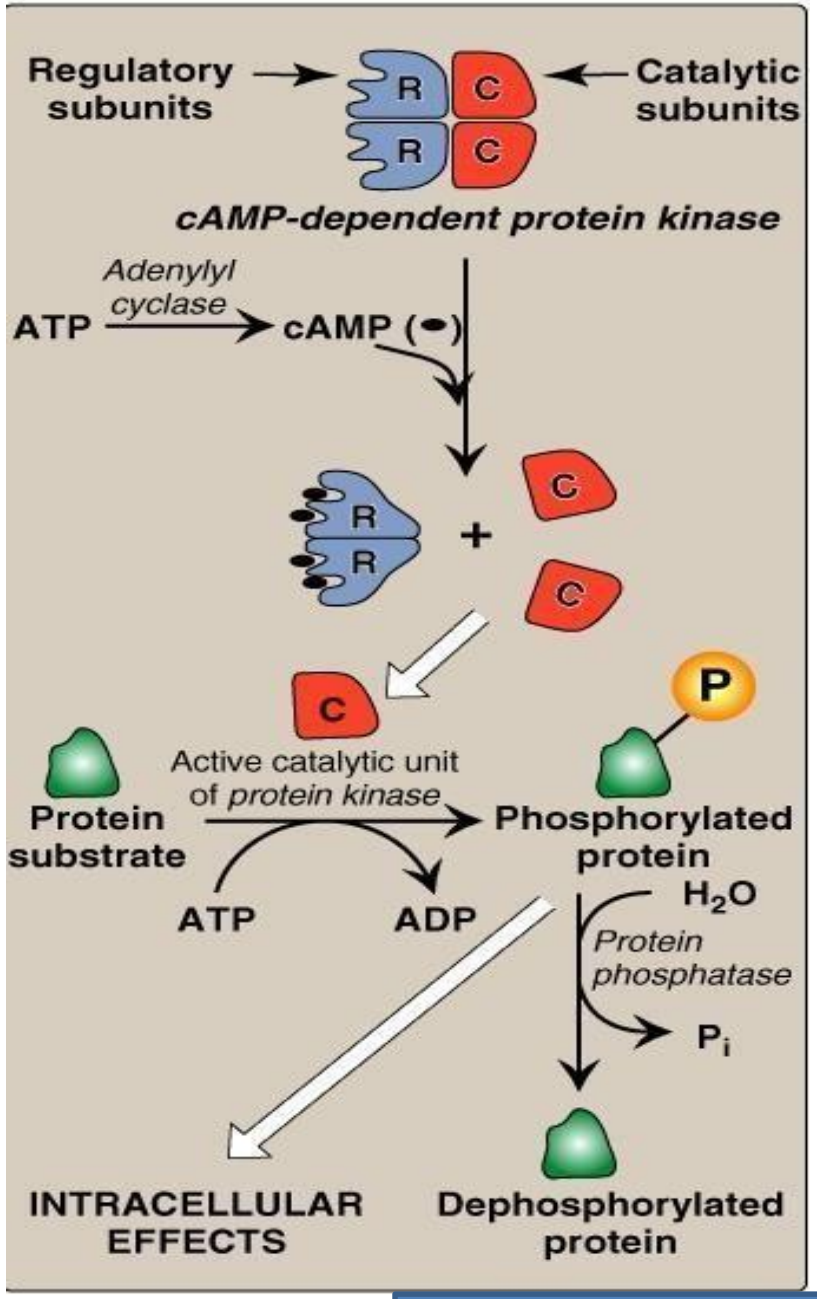
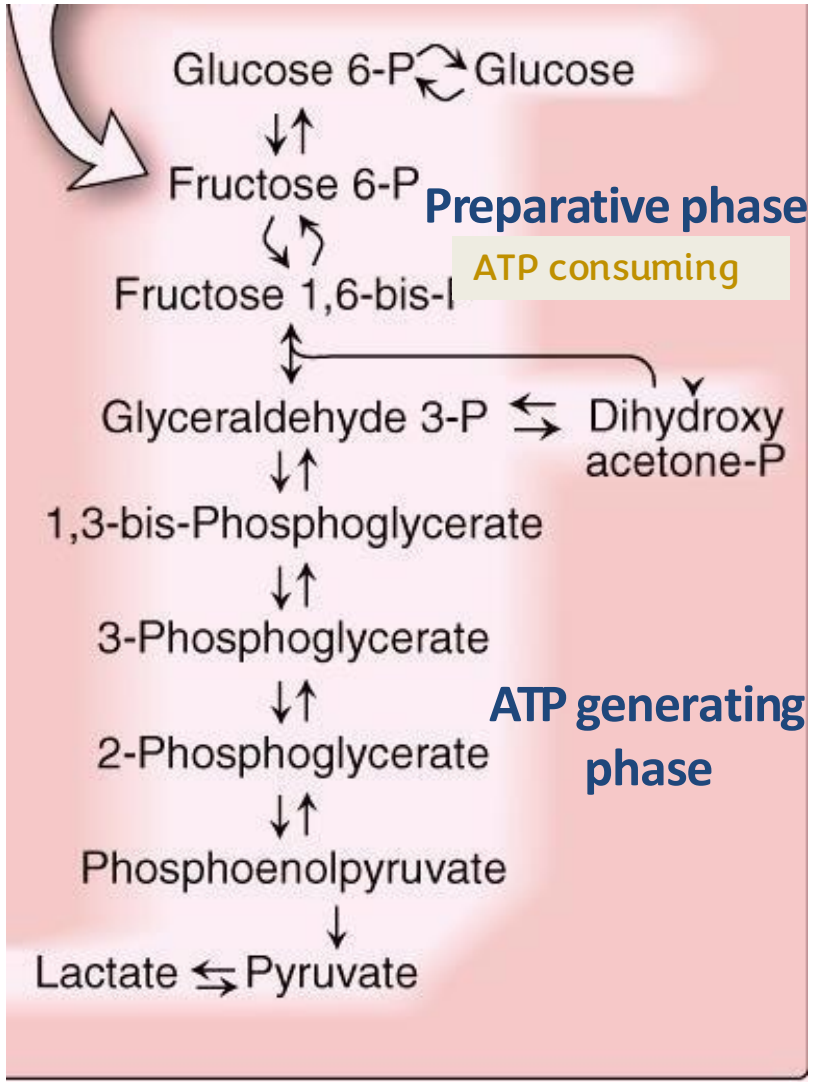


INTRACELLULAR EFEECTS

- ✓ Activated enzymes
- ✓ Inhibited Enzymes
- ✓ Cell's ion channels
- ✓ Bind to promoter



GLYCOLYSIS

The breakdown of glucose through a linear (the product of one step is a reactant of the next step) 10-step pathway.

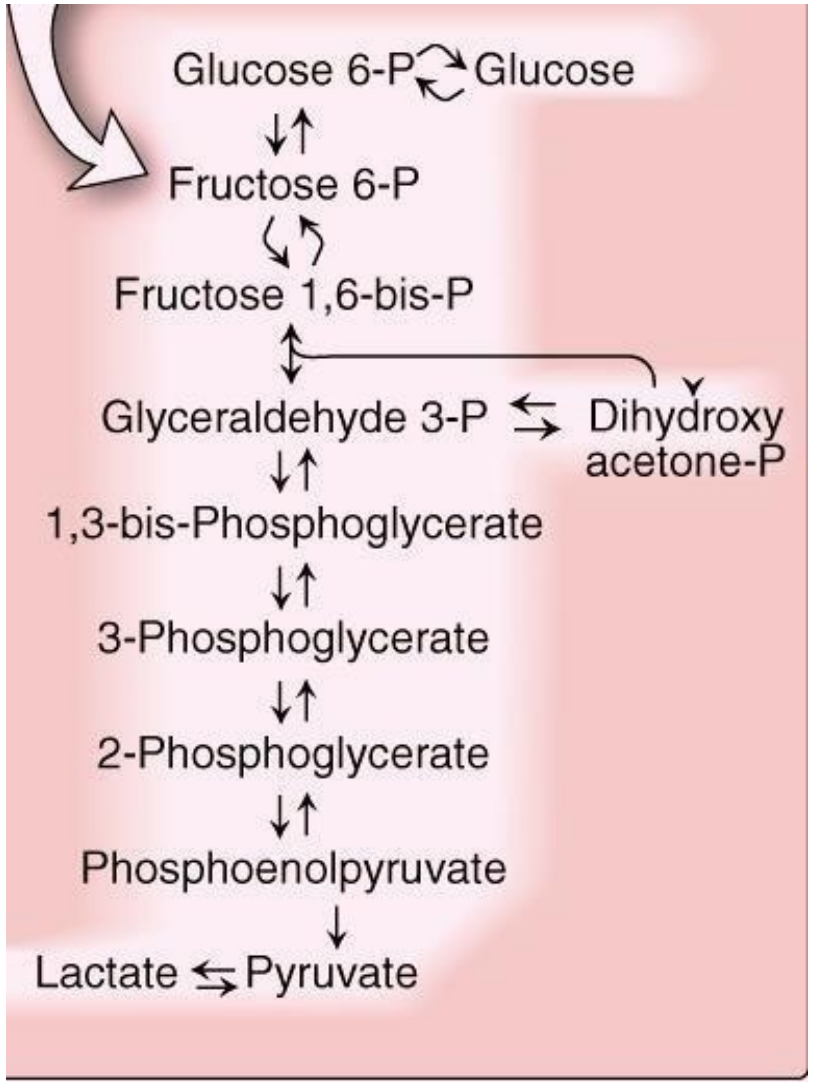
✓ Breakdown of glucose to pyruvate Pathway characteristics

- Universal Pathway: In all cell types (in the cytosol)
- Generation of ATP
- With or without O_2
- The product of one reaction is the substrate of the next reaction

Reversibility:

Out of the 10 steps, 7 are reversible and 3 are irreversible. Understanding which steps are irreversible is crucial for regulation, as these are the key control points.

Catabolism of glucose and anabolism of intermediates that can feed into other metabolic pathways



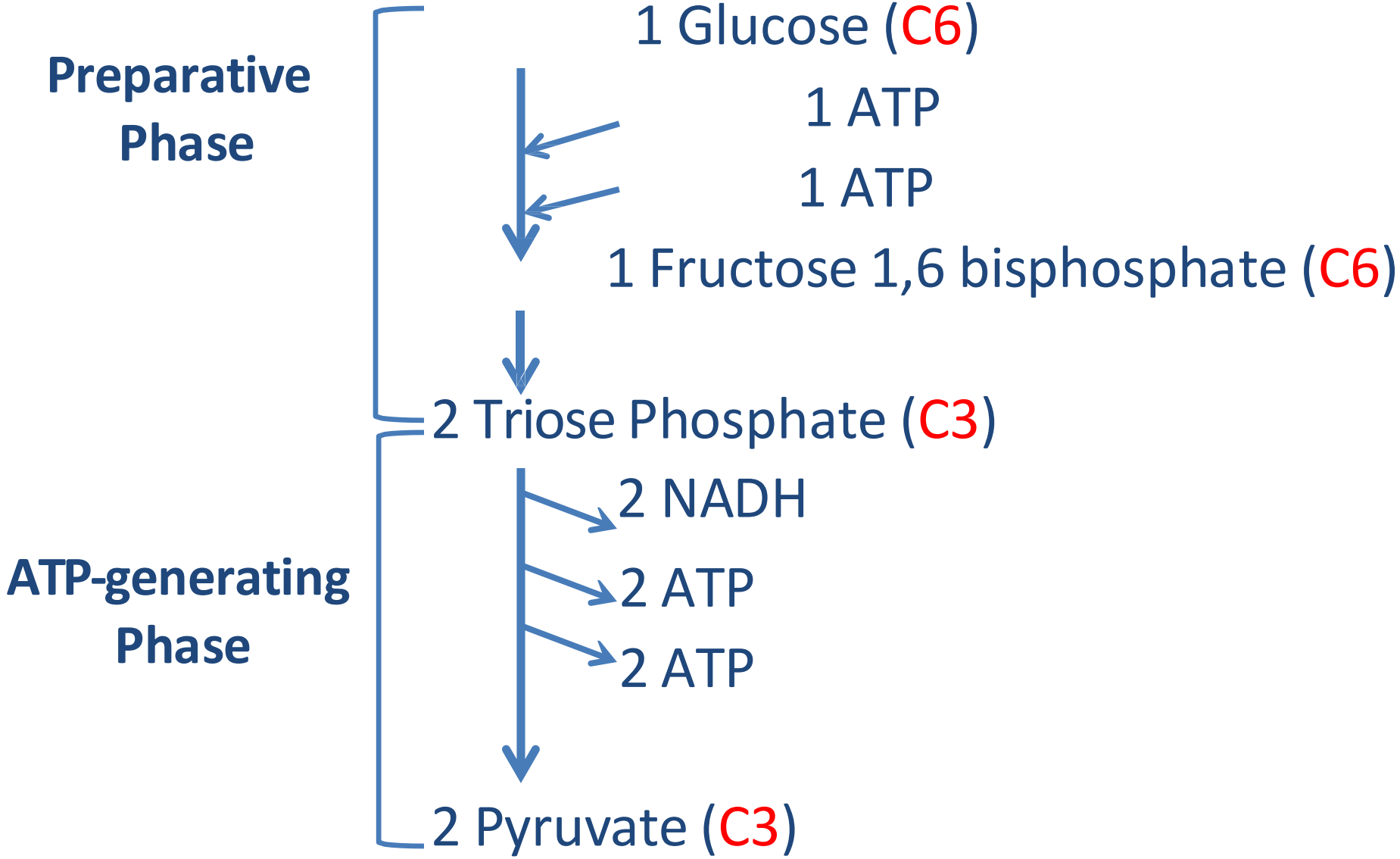
- Divided into two phases:

Preparative Phase – consumes energy (ATP).

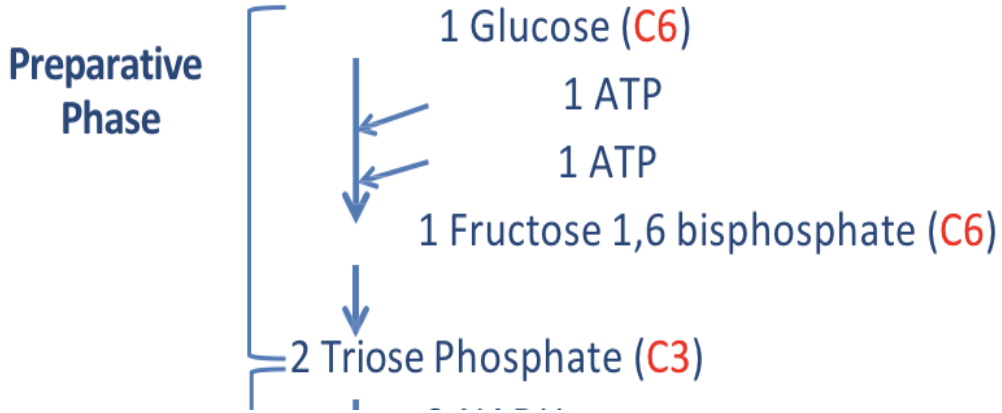
ATP-Generating Phase – produces energy (ATP and NADH).

- At the end of glycolysis, glucose is converted into two molecules of pyruvate, each containing three carbons.
- Throughout the pathway, some steps consume ATP while others produce it. However, the net result of glycolysis is a gain of ATP. Ultimately, the final product of glycolysis is pyruvate

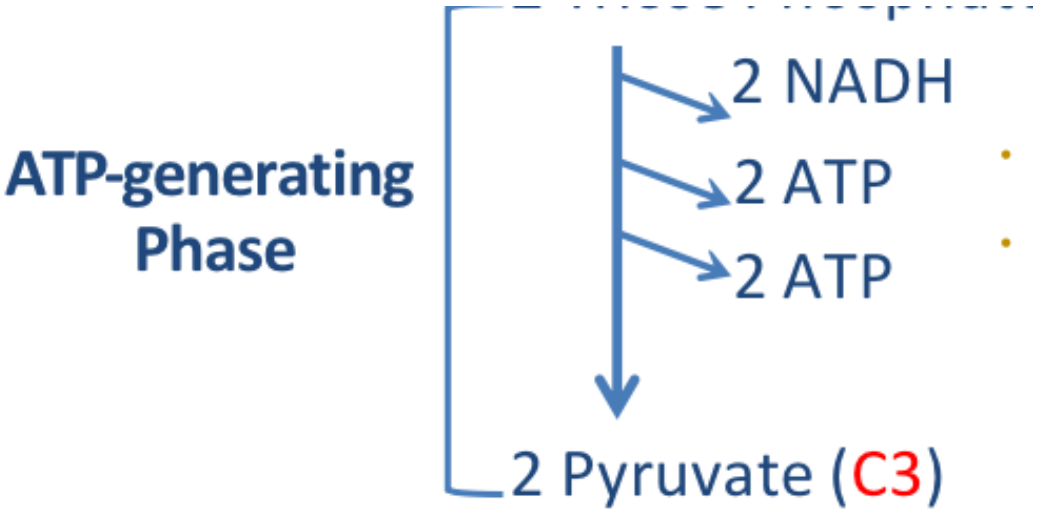
The Two Phases of the glycolytic Pathway



- Begins with glucose and converts it into fructose-1,6-bisphosphate.
- This phase involves the formation of intermediates and leads to the cleavage of the six-carbon molecule into two three-carbon molecules called triose phosphates



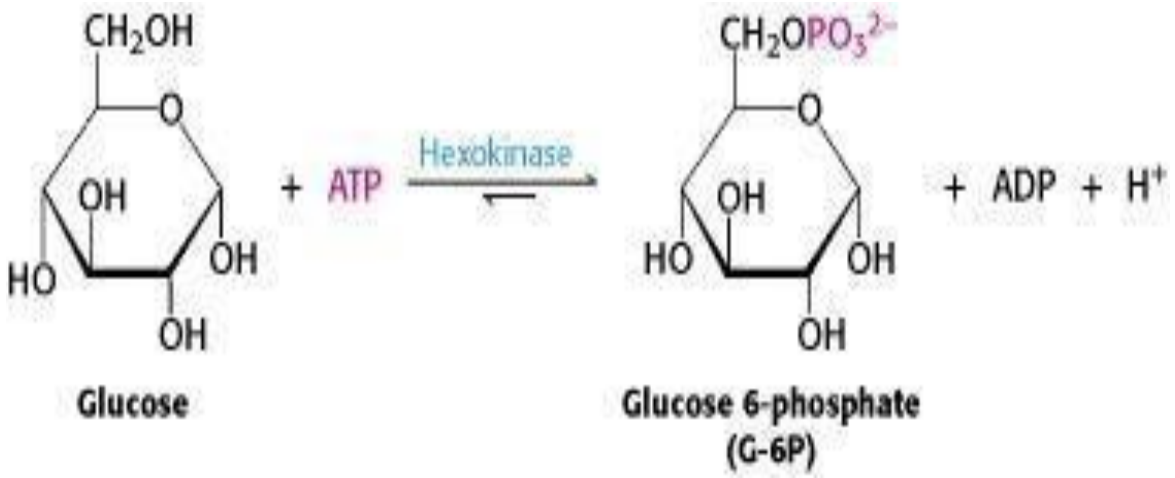
- Each triose phosphate undergoes a series of reactions that ultimately produce pyruvate.
- Although the preparative phase reactions occur once per glucose molecule, the ATP-generating phase reactions happen twice, because each glucose molecule is split into two triose phosphates.



Steps of Glycolysis

- **Primary Energy Source:** The body relies on glucose as the main energy source(it's consumed as long as it's available). When glucose is scarce (not enough amount), the body shifts to alternative energy sources like lipids.
- **Well-Fed State:** After eating, glucose is absorbed into the bloodstream, raising blood sugar levels. This increase in blood glucose stimulates the secretion of insulin.
- **Role of Insulin:** Insulin helps increase the number of GLUT4 transporters on cell surfaces, allowing more glucose to enter cells. This ensures that glucose is trapped inside the cells by phosphorylation, preventing it from leaving and maintaining energy storage.

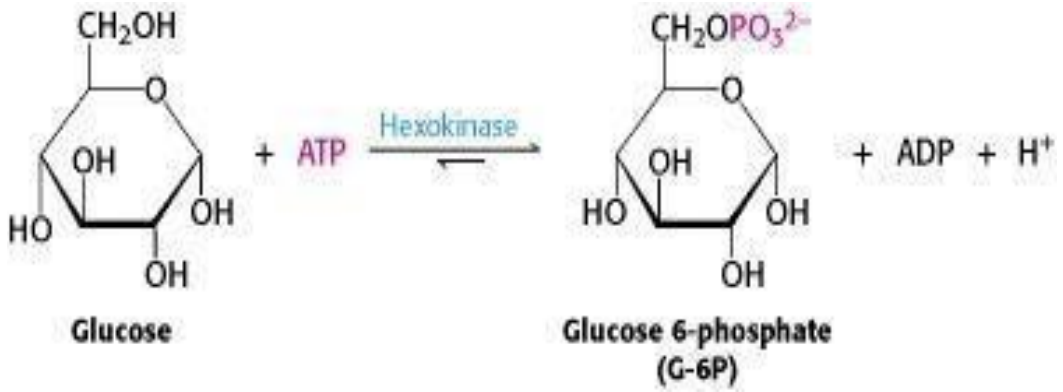
Step 1: Irreversible but Not Committed



Phosphorylation

	Hexokinase	Glucokinase
Occurrence	In all tissues	In liver
Km	< 0.02 mM	10-20 mM
Specificity	Glc., Fruc, Man, Gal	Glc.
induction	Not induced	↑ insulin, Glc
Function	At any glucose level	Only > 100 mg/dl

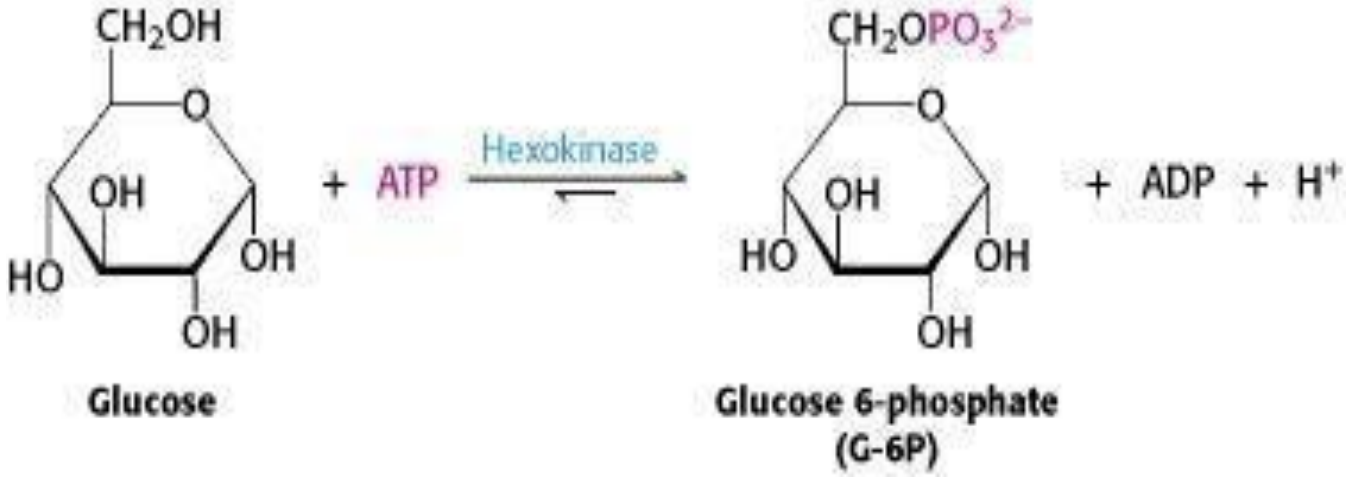
The phosphorylation of glucose to glucose-6-phosphate is irreversible, but it's not the committed step since glucose-6-phosphate can enter other pathways



Transport and Enzymes: Glucose-6-phosphate cannot exit the cell via GLUT transporters. Additionally, key enzymes like **hexokinase** and **glucokinase** play crucial roles in this step.

Phosphate groups can come from ATP or from other organic phosphate sources depending on the energy requirement.
If the reaction is energetically favorable (negative ΔG), phosphate can be transferred without ATP.
But if it's not favorable (positive ΔG), ATP is used to drive the reaction, By coupling the unfavorable reaction with ATP hydrolysis, the overall process becomes energetically favorable ,allowing the phosphate group to be transferred.

Step 1: Irreversible but Not Committed



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Hexokinase vs. Glucokinase

1. Hexokinase:

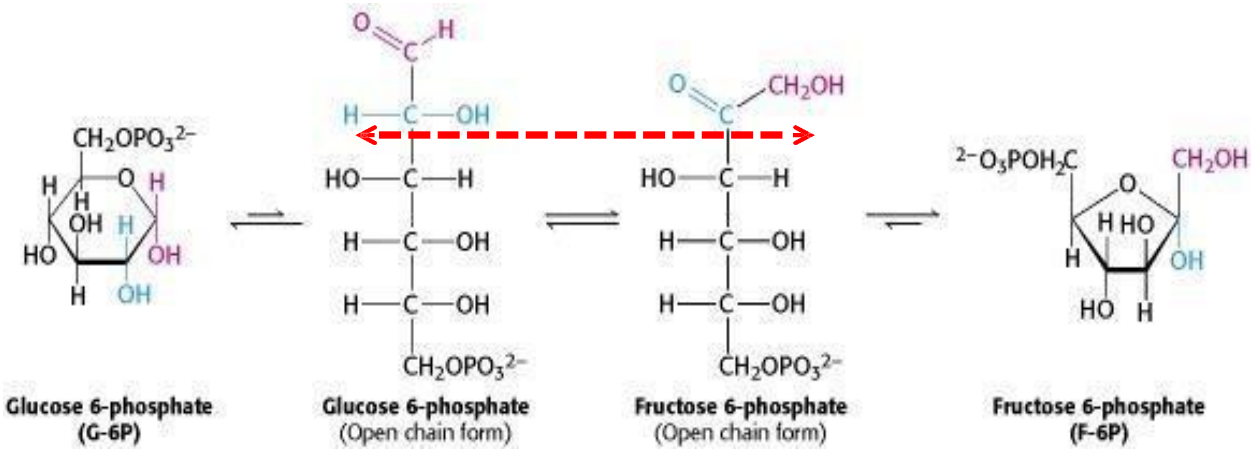
- **Broad Substrate Range:** Acts on all hexoses (six-carbon sugars), not just glucose.
- **Tissue Distribution:** Found in nearly all tissues, making it widely distributed.
- **Affinity:** Has a low Km, meaning it reaches half-maximal activity at low glucose concentrations, indicating high affinity.
- **Induction:** Not inducible; its activity remains relatively constant.
- **Function:** Efficient at trapping glucose in the cell even at low glucose levels, ensuring it's phosphorylated quickly.

2. Glucokinase:

- **Specificity:** Primarily found in the liver and pancreatic beta cells; specific to glucose.
- **High Km:** Requires higher glucose concentrations to reach half-maximal activity, reflecting a lower affinity for glucose.
- **Induction:** Induced by insulin, increasing its activity in response to high blood glucose levels.
- **Function:** Helps in glucose storage when glucose levels are high, ensuring that the liver can store glucose as glycogen.

Step 2: reversible

Isomerization



Phosphoglucose Isomerase

Phosphorylation Precedes Isomerization: To prevent easy exit of glucose from the cell, glucose is phosphorylated first, forming glucose-6-phosphate.

Ring and Open Chain Forms: Glucose-6-phosphate can exist in a ring form, but for isomerization to occur, the molecule must briefly switch to an open-chain form

Mechanism: The open-chain form allows the transfer of the carbonyl group from the first carbon to the second carbon, converting glucose-6-phosphate into fructose-6-phosphate.

The reaction is catalyzed by phosphoglucose isomerase, which facilitates the interconversion between the phosphorylated forms of glucose and fructose

Step 3: Irreversible and Rate-Limiting Phosphorylation

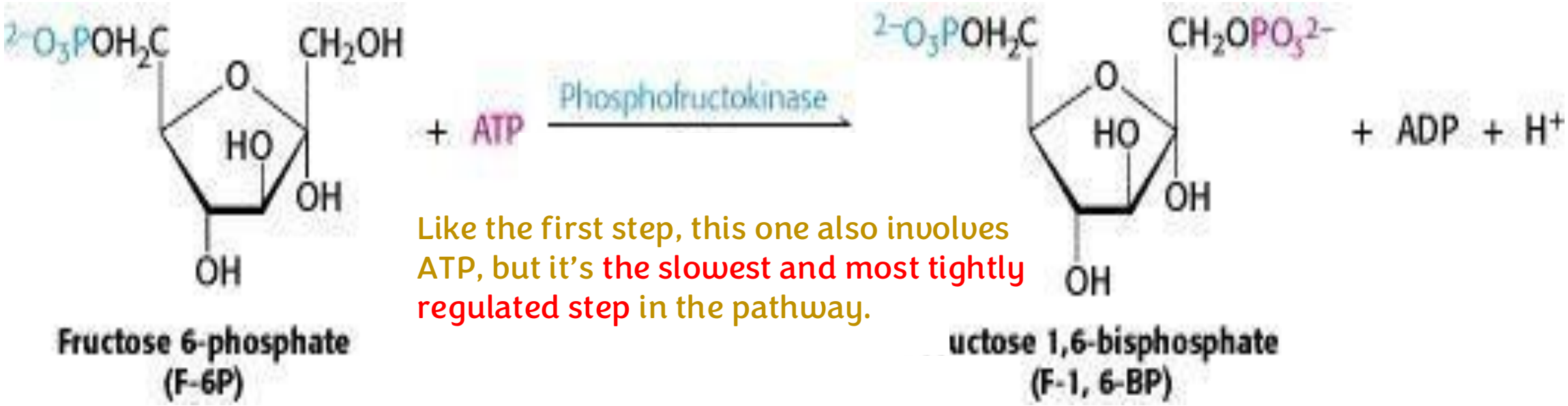
Fructose 6-phosphate (F-6P)

uctose 1,6-bisphosphate (F-1, 6-BP)

This phosphorylation step uses ATP, similar to the first phosphorylation step

The enzyme that catalyzes this reaction is phosphofructokinase-1 (PFK-1), a key regulatory enzyme in glycolysis.

While PFK-1 is central to catalysis, phosphofructokinase-2 (PFK-2) is more involved in the regulation of glycolysis, and we'll delve into that in more detail later



Fructose 6-phosphate
(F-6P)

uctose 1,6-bisphosphate
(F-1, 6-BP)

Like the first step, this one also involves ATP, but it's the slowest and most tightly regulated step in the pathway.

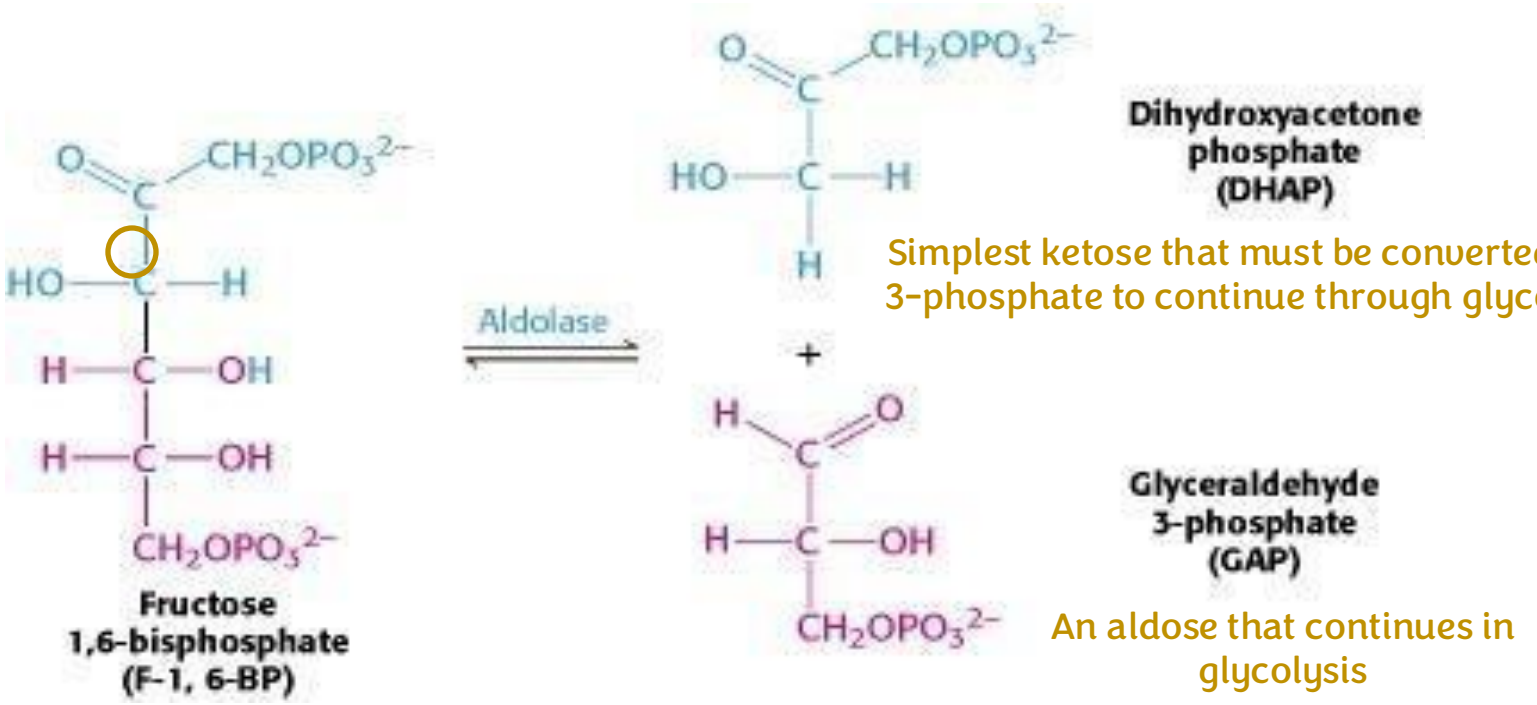
This step is irreversible and is considered the rate-limiting step of glycolysis (the slowest step) , meaning it regulates the overall pace of the pathway

Why it's committed step ?
Because, Once fructose-6-phosphate is phosphorylated to fructose-1,6-bisphosphate, it's committed to continuing through glycolysis and cannot easily divert to other pathways.

Step 4: reversible

Cleavage

The ring structure of fructose-1,6-bisphosphate is temporarily opened to enable cleavage.



Simplest ketose that must be converted into glyceraldehyde-3-phosphate to continue through glycolysis because it can't .

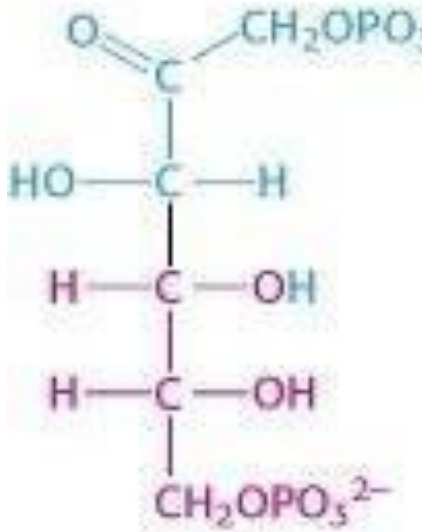
An aldose that continues in glycolysis

Aldolase is the enzyme responsible for this cleavage, playing a crucial role in glycolysis

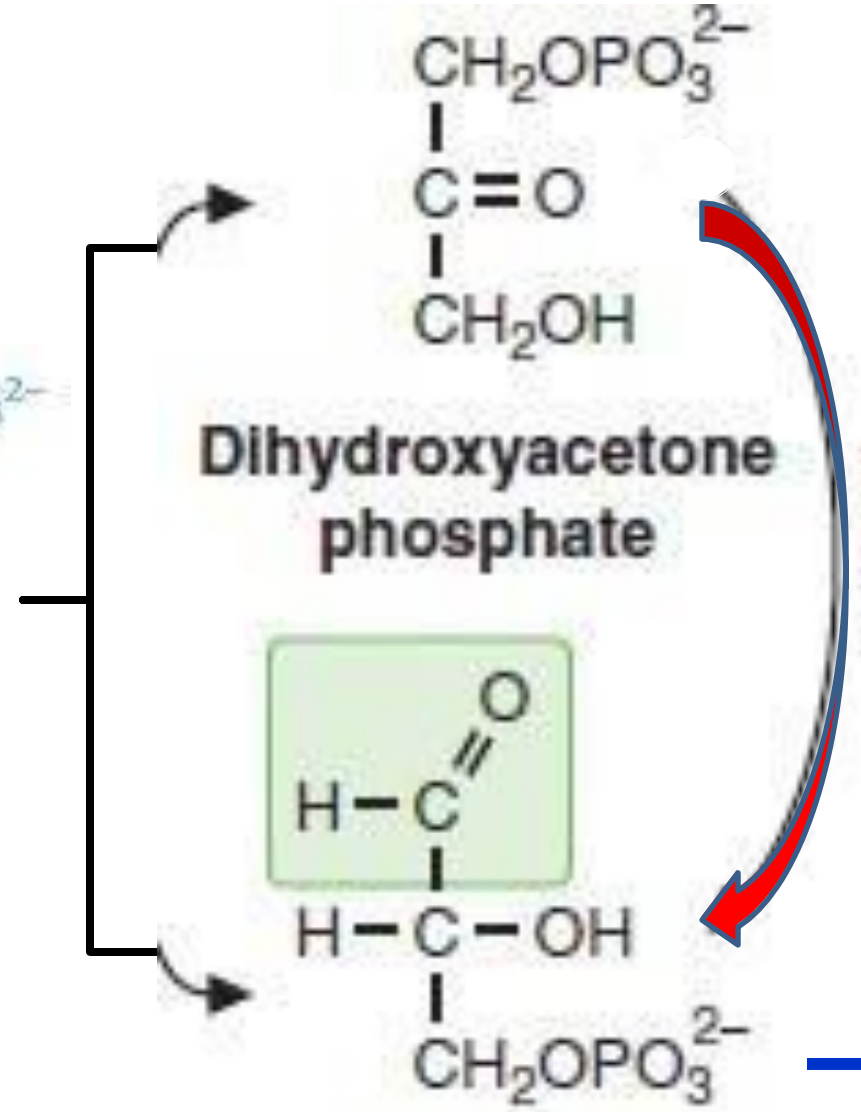
- **Cleavage Mechanism:** The enzyme aldolase catalyzes the cleavage of fructose-1,6-bisphosphate into two three-carbon molecules: dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP).

Step 5:reversible

- **Enzyme:**
  The reaction is catalyzed by **triose phosphate isomerase**.
- **Reversibility:**
  This step is **reversible**, meaning DHAP can convert to GAP and vice versa.



Fructose 1,6-bisphosphate (F-1, 6-BP)



DHAP is converted into GAP by an isomerization reaction, ensuring that both products can continue in the pathway

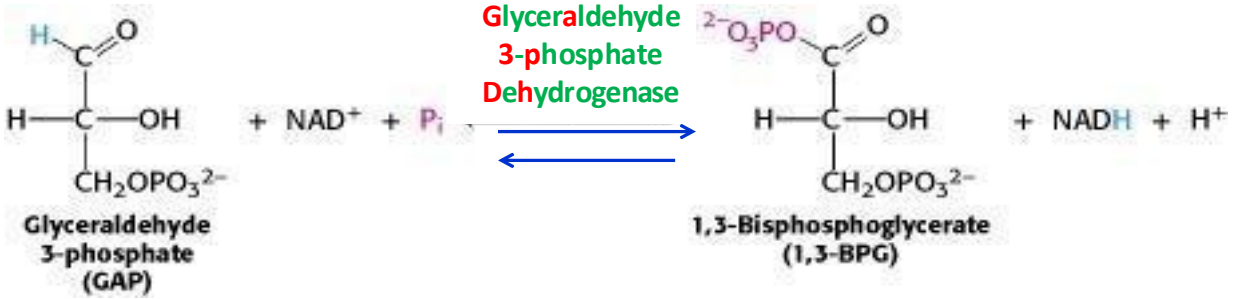
Isomerization

- **Equilibrium Shift:**
  Under normal conditions, the equilibrium favors **DHAP**.
  However, since **GAP is continuously consumed** in the next glycolytic steps, the reaction keeps shifting **toward GAP formation**, ensuring the pathway continues forward.

- **Importance:**
  This reaction ensures that **both three-carbon molecules** (from the cleavage step) are funneled into the same pathway – glycolysis – maximizing energy yield.

Step 6: reversible

Oxidation-reduction



Glyceraldehyde-3-phosphate (GAP) is oxidized by **glyceraldehyde-3-phosphate dehydrogenase**.

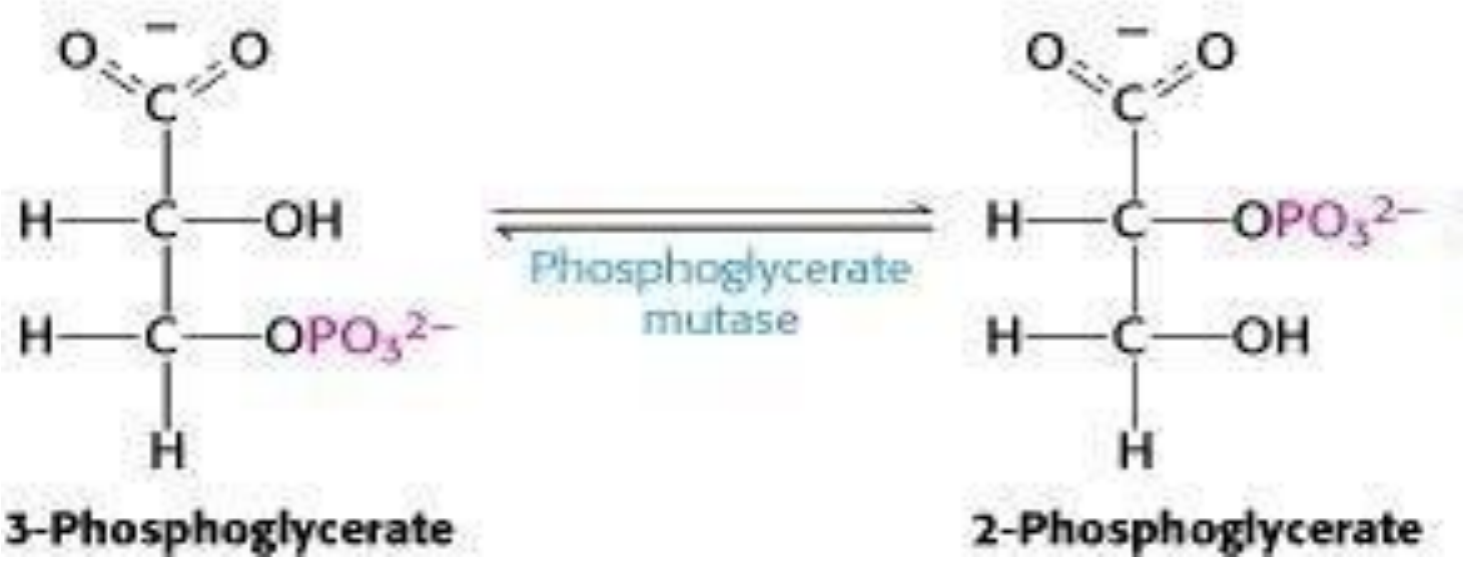
- **Oxidation-Reduction Process:**
  During this step, the aldehyde group of GAP is oxidized, losing a hydrogen atom (H), which is transferred to **NAD^+**, reducing it to **NADH**.
- **Phosphate Addition:**
  An inorganic phosphate (Pi) is added to the oxidized GAP, forming **1,3-bisphosphoglycerate (1,3-BPG)**.
- **Energy Consideration:**
  This process doesn't require ATP hydrolysis. Instead, energy is captured in the form of the high-energy bond of 1,3-BPG.
- **Subsequent ATP Production:**
  The energy stored in 1,3-BPG will later be used to generate ATP in a subsequent step

Step 7:

Phosphorylation

- **1,3-Bisphosphoglycerate (1,3-BPG)** donates a phosphate group to ADP, catalyzed by **glycerate kinase**.
- **ATP Generation:**
  This reaction converts ADP to ATP, producing **one ATP molecule** for each 1,3-BPG.
- **Key Point:**
  This is the **first ATP generated** in glycolysis, and it occurs twice per glucose molecule since glycolysis produces two molecules of 1,3-BPG.
- **Net ATP Consideration:**
  Although we generate two ATP molecules in this step, we previously consumed two ATP molecules in the preparatory phase. Therefore, the **net ATP gain so far is zero**.

Steps 8-10

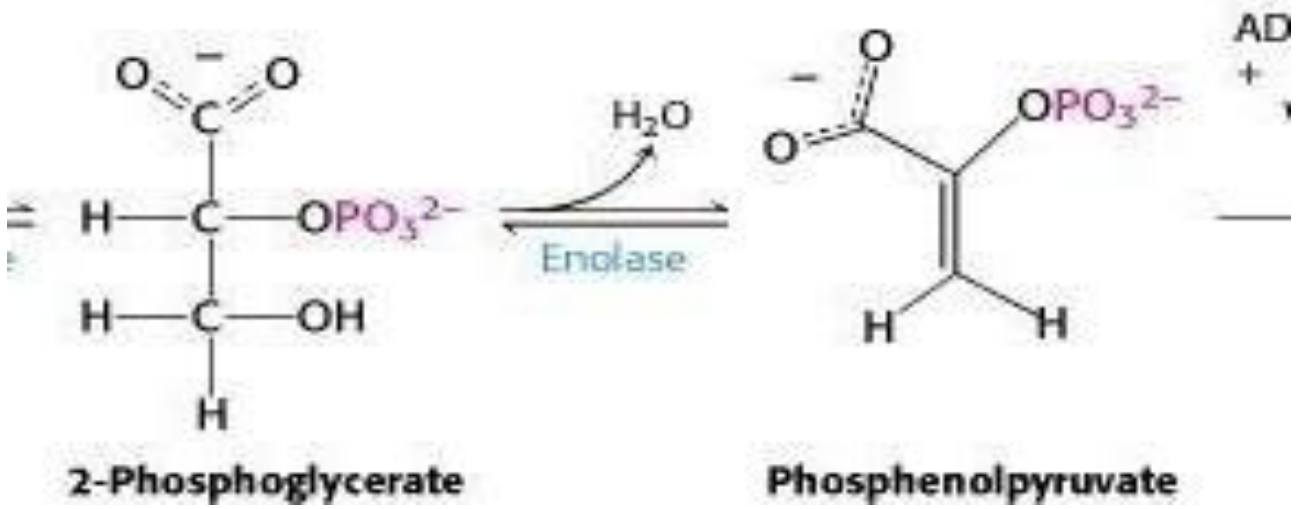


Phosphoryl-shift isomerization

- Step 8: reversible

Isomerization of 3-Phosphoglycerate

- 3-Phosphoglycerate is converted to 2-Phosphoglycerate by the enzyme phosphoglycerate mutase.
- Purpose:
  This isomerization shifts the phosphate group from the third carbon to the second carbon, preparing the molecule for the next dehydration step.



Dehydration

Step 9: reversible

Dehydration of 2-Phosphoglycerate

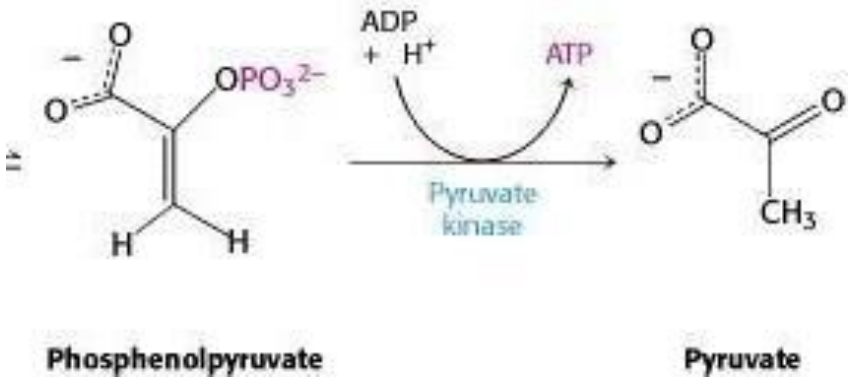
- Reaction Overview:
  2-Phosphoglycerate is converted to phosphoenolpyruvate (PEP) by the enzyme enolase.
- Process:
  During this step, a water molecule is removed, creating a high-energy enol form of pyruvate. This dehydration is crucial for the subsequent ATP generation.

Step 10: irreversible
ATP Generation and Pyruvate Formation

- **Phosphoenolpyruvate (PEP)** donates a phosphate group to ADP, catalyzed by **pyruvate kinase**, forming **pyruvate** and generating a second ATP molecule.
- **Key Point:**
  This is the final ATP-producing step of glycolysis, and since we have two molecules of PEP per glucose, we get two ATP molecules in total.
- **Net ATP Yield:**
  Although we used two ATP in the preparatory phase, we now generate four ATP in the later steps, leading to a net gain of two ATP per glucose molecule.

  - **ATP:** 4 produced – 2 consumed = **2 ATP (net)**
  - **NADH:** 2 molecules produced
  - **End product:** 2 Pyruvate

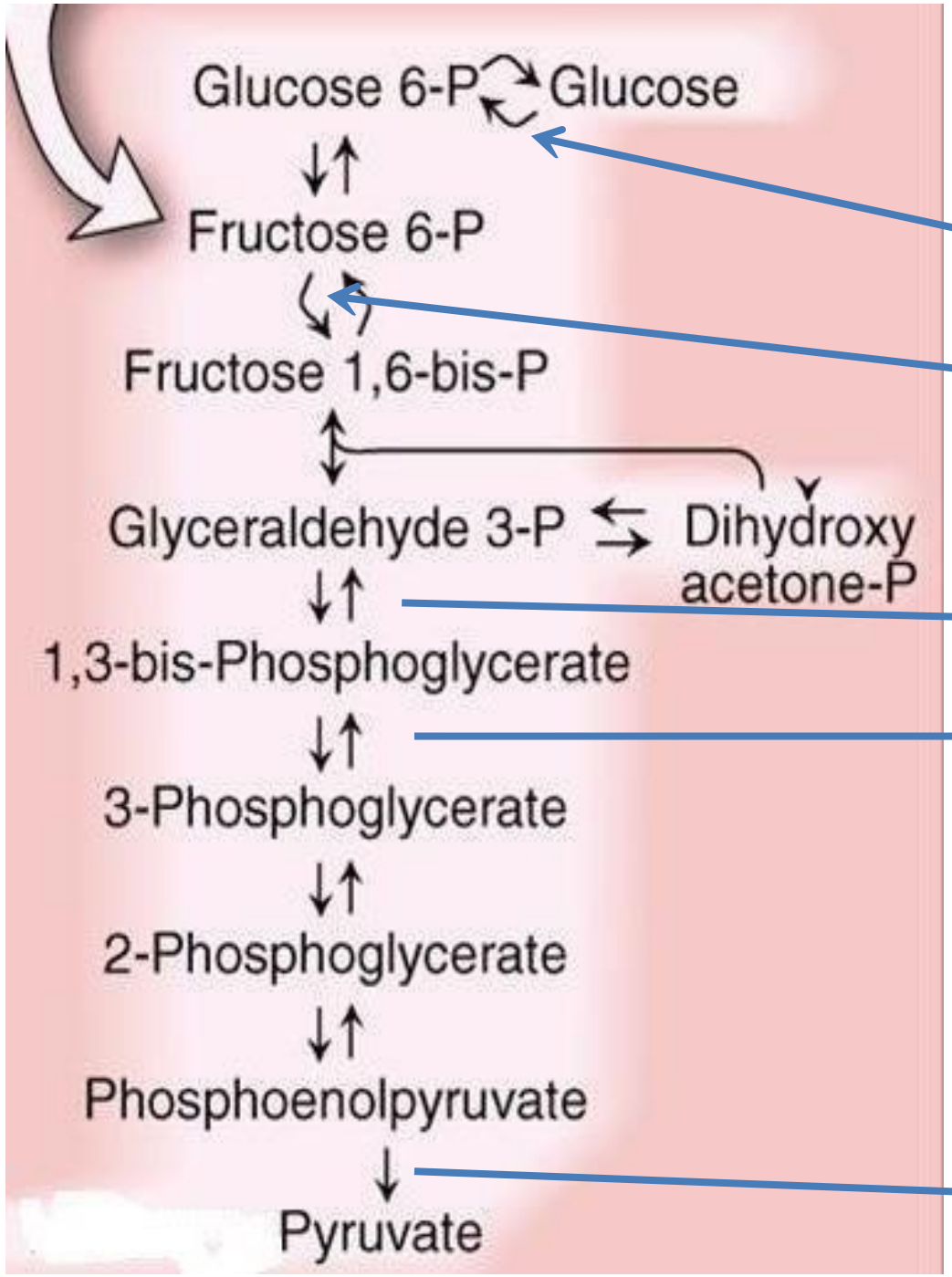
Phosphorylation



Steps 1,3,10 all are irreversible steps and catalyzed by kinases (hexokinase, glucokinase, phosphofructokinaze.1 and pyruvate kinase)

Summary

Step	Reaction / Process	Enzyme	Substrate → Product	ATP / NADH Change	Reversible?	Key Notes
1	Phosphorylation of Glucose	**Hexokinase / Glucokinase**	Glucose → Glucose-6-Phosphate	**-1 ATP used**	❌ Irreversible	Traps glucose inside the cell; regulatory step.
2	Isomerization	**Phosphoglucose Isomerase**	Glucose-6-Phosphate → Fructose-6-Phosphate	–	✅ Reversible	Converts aldose to ketose form.
3	Second Phosphorylation (Rate-limiting Step)	**Phosphofructokinase-1 (PFK-1)**	Fructose-6-Phosphate → Fructose-1,6-Bisphosphate	**-1 ATP used**	❌ Irreversible	Major regulatory and slowest step; activated by AMP, inhibited by ATP/citrate.
4	Cleavage	**Aldolase**	Fructose-1,6-Bisphosphate → G3P + DHAP	–	✅ Reversible	Splits 6C sugar into two 3C molecules.
5	Isomerization	**Triose Phosphate Isomerase**	DHAP → G3P	–	✅ Reversible	Ensures both 3-carbon molecules proceed as G3P.
6	Oxidation & Phosphorylation	**Glyceraldehyde-3-Phosphate Dehydrogenase**	G3P → 1,3-Bisphosphoglycerate	**+2 NADH produced**	✅ Reversible	First energy-yielding step; involves oxidation-reduction.
7	ATP Generation (Substrate-Level Phosphorylation)	**Phosphoglycerate Kinase**	1,3-BPG → 3-Phosphoglycerate	**+2 ATP formed**	✅ Reversible	First ATP-producing step.
8	Isomerization	**Phosphoglycerate Mutase**	3-Phosphoglycerate → 2-Phosphoglycerate	–	✅ Reversible	Prepares for high-energy intermediate formation.
9	Dehydration	**Enolase**	2-Phosphoglycerate → Phosphoenolpyruvate (PEP)	–	✅ Reversible	Water removed; forms high-energy PEP.
10	ATP Formation (Final Step)	**Pyruvate Kinase**	PEP → Pyruvate	**+2 ATP formed**	❌ Irreversible	Final ATP-producing step; regulated by energy status.



Energy Need and Production

-ATP

-ATP

2 NADH

2 ATP

Is Oxygen needed?

2ATP

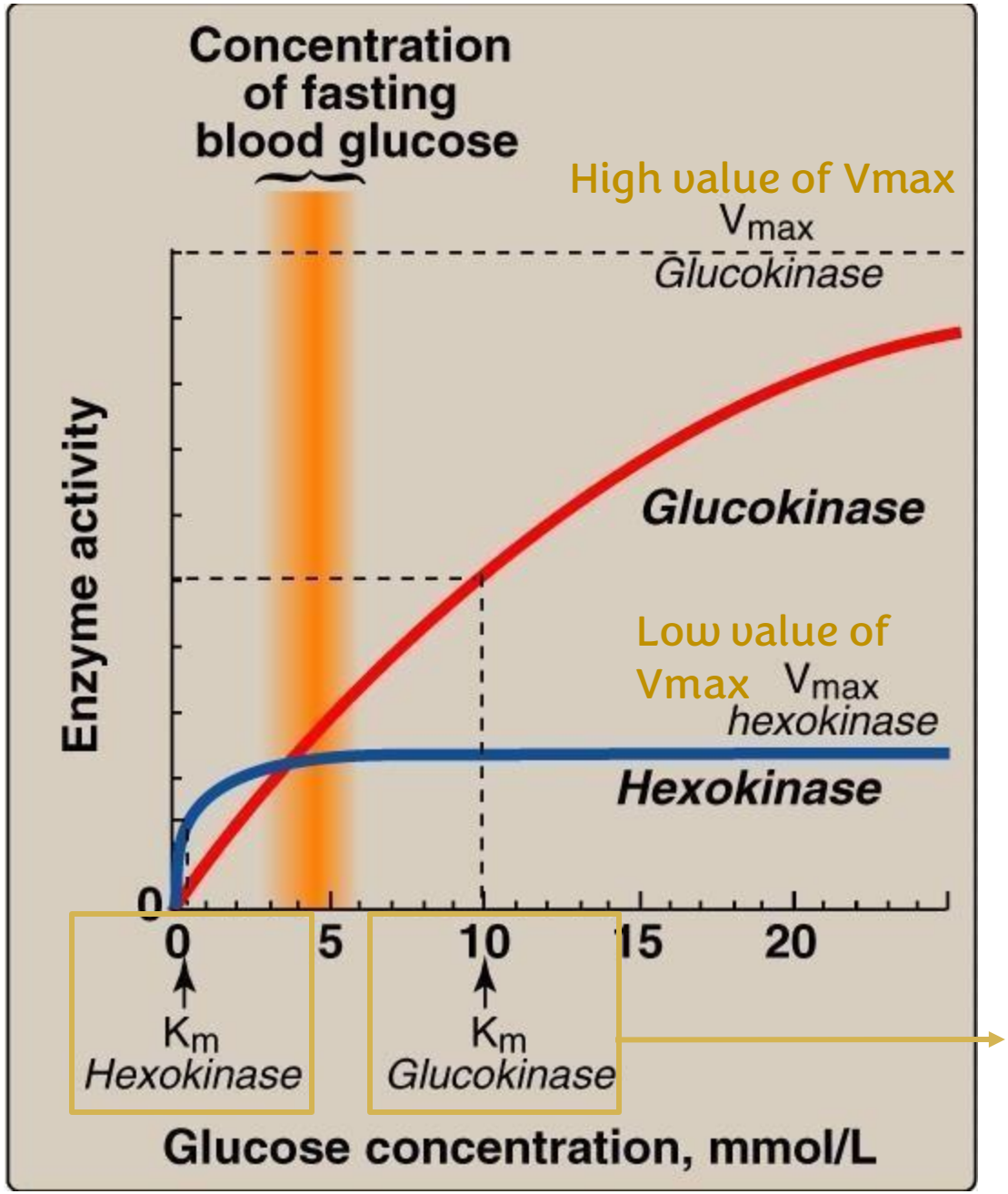
NADH is indeed more than just an immediate energy carrier. It plays a crucial role in various metabolic pathways. In some reactions, NADH is oxidized back to NAD+, which helps maintain the redox balance in the cell. So, it's really a dynamic cycle of reduction and oxidation, rather than just a one-way energy source

Regulation of Glycolysis

Regulation occurs at the irreversible steps of the pathway.

These key steps are:

- Hexokinase/Glucokinase (Step 1)
- Phosphofructokinase-1 (PFK-1) (Step 3)
- Pyruvate kinase (Step 10)



Glucokinase and Hexokinase Activity

Km reflects affinity, higher km means lower affinity

Orange area ?

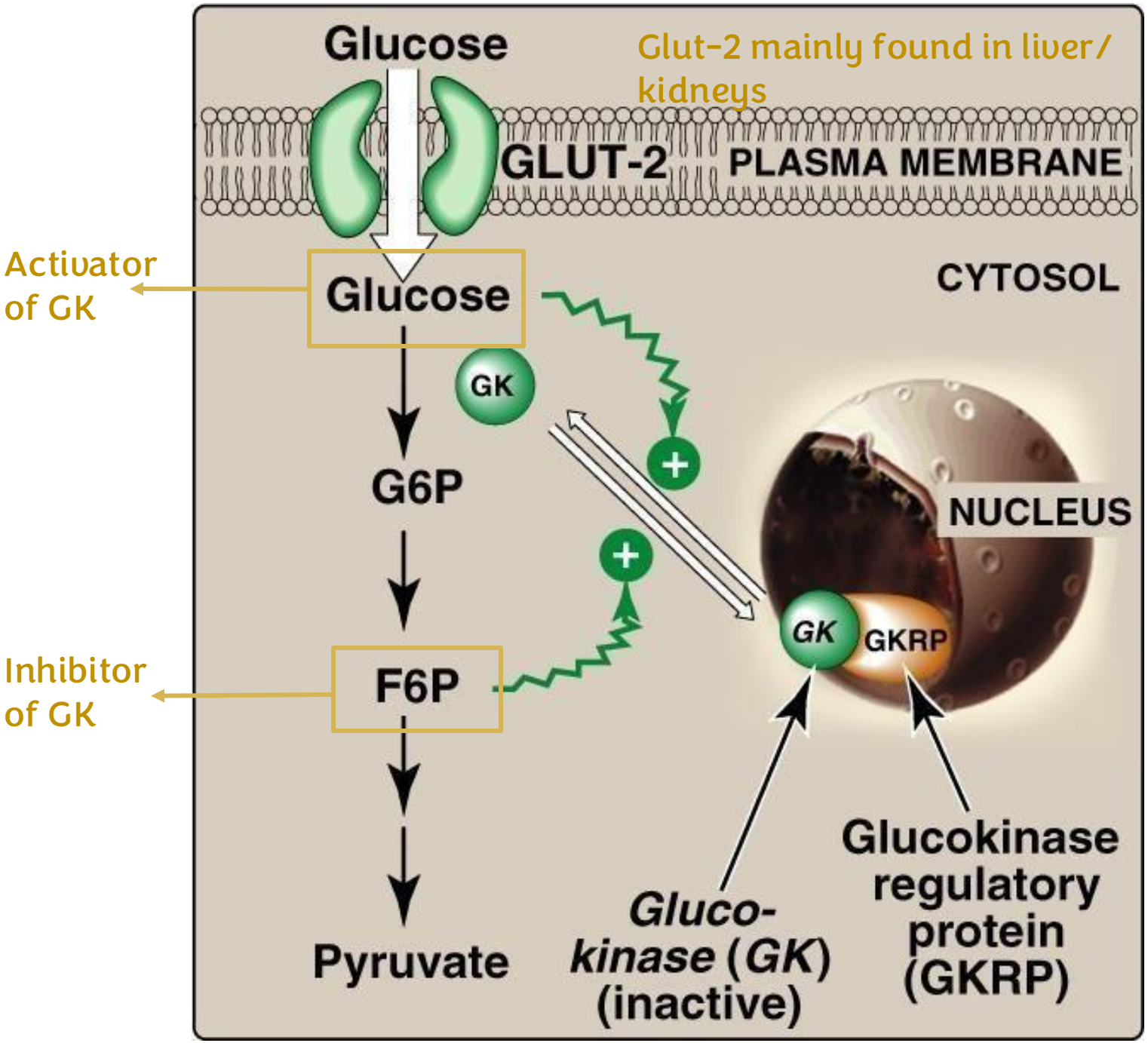
- It represents the concentration of Glucose while fasting, but this does not necessarily mean the person is fasting. Even if you eat, blood glucose eventually returns to this “fasting” range after insulin acts to lower blood sugar.
- After eating, blood glucose rises.
- Insulin is released to reduce glucose levels.
- Glucose does not drop to zero, it returns to the normal baseline range, thus your blood glucose level stays the same whether its 2 hours after eating or 4 hours or even while fasting

Why doesn't blood sugar drop to zero?

- Because sugar is a solute in the blood.
  If glucose concentration drops too much, it will affect the osmotic pressure and the kinetics of water molecules, which will eventually affect blood pressure (BP).
  → We don't want to decrease its concentration too much.
- Another reason:
  Some tissues are exclusively dependent on glucose as a source of energy, especially the brain.
  Therefore, we must keep a reservoir of glucose in the blood so these tissues can take from it when needed.

At fasting glucose levels:

- Hexokinase is already at Vmax
  → even without eating, hexokinase stays fully active.
- Glucokinase is only ~25% active
  → becomes significantly active only when glucose levels increase after eating.



(Regulation of step 1 of glycolysis)

Glucokinase Regulation

Where is glucokinase (GK) before glucose enters the cell?

- GK is stored in the nucleus, bound to Glucokinase Regulatory Protein (GKRP).
- GKRP traps GK inside the nucleus and keeps it inactive.

When glucose is high

- Glucose enters the cell through GLUT-4 mainly (GLUT-2 mentioned only as an example)
- High glucose causes glucokinase (GK) to separate from GKRP in the nucleus
- GK leaves the nucleus and moves to the cytosol
- GK phosphorylates glucose to G6P → glucose becomes trapped in the cell
- Glycolysis then starts
- Overall result: glycolysis ON

When F6P is high

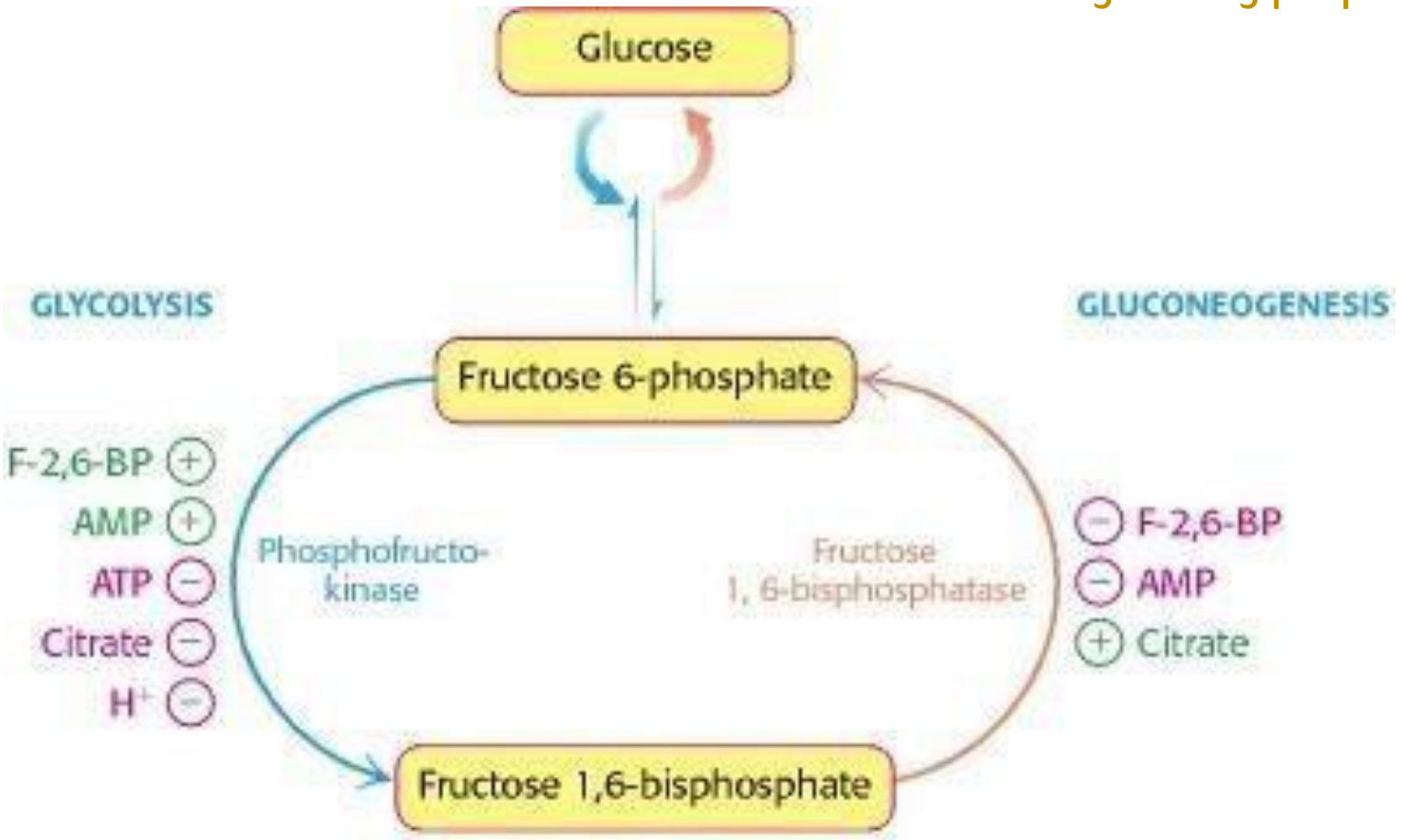
- High F6P indicates glycolysis intermediates are accumulating
- F6P activates GK sequestration back to the nucleus
- GK binds again to GKRP and becomes inactive
- GK stays trapped in the nucleus
- Overall result: glycolysis decreases (OFF switch)

What is sequestration? Trapping or isolating glucokinase (GK) inside the nucleus so it cannot act in the cytoplasm

(Regulation of step 3 of glycolysis)

Allosteric Regulators of PFK1

There is another type of PFK, PFK2 which is used for regulatory purposes



Opposite Regulation of Glycolysis and Gluconeogenesis

- Gluconeogenesis is the synthesis of glucose.
- Glycolysis breaks down glucose for energy.
- These two pathways have opposite regulation(the same molecule that activates glycolysis, inhibits gluconeogenesis for example), especially at the irreversible steps.

Why do they have opposite regulation?

- To prevent both pathways from running at the same time.
- Running both would waste energy and create a futile (pointless) cycle.
- The body regulates them so only one pathway is active depending on metabolic needs.

Why can't both pathways operate simultaneously (especially irreversible steps)?

- Irreversible steps do not depend on equilibrium like reversible reactions.
- If both irreversible directions were active at once, it would waste ATP and energy reserves.
- The cell chooses one pathway based on conditions like ATP, AMP, citrate, and F-2,6-BP levels.

Simple idea

- If energy is low → glycolysis ON, gluconeogenesis OFF
- If energy is high → gluconeogenesis ON, glycolysis OFF

F-2,6-BP (+)

This is a regulatory molecule and NOT an intermediate in glycolysis, it activates PFK1 and we will discuss it later in the slides

AMP (+)

- AMP indicates that the cell has low energy.
- When AMP levels are high, it means ATP is low.
- Therefore, the cell activates glycolysis to produce more ATP and restore energy levels.

ATP (−)

ATP has the opposite effect of AMP. When ATP levels are high, it signals that the cell already has plenty of energy. In this case, there is no need to break down more glucose, so ATP inhibits PFK-1 and slows glycolysis. The cell can then use its energy for other purposes instead of producing more.

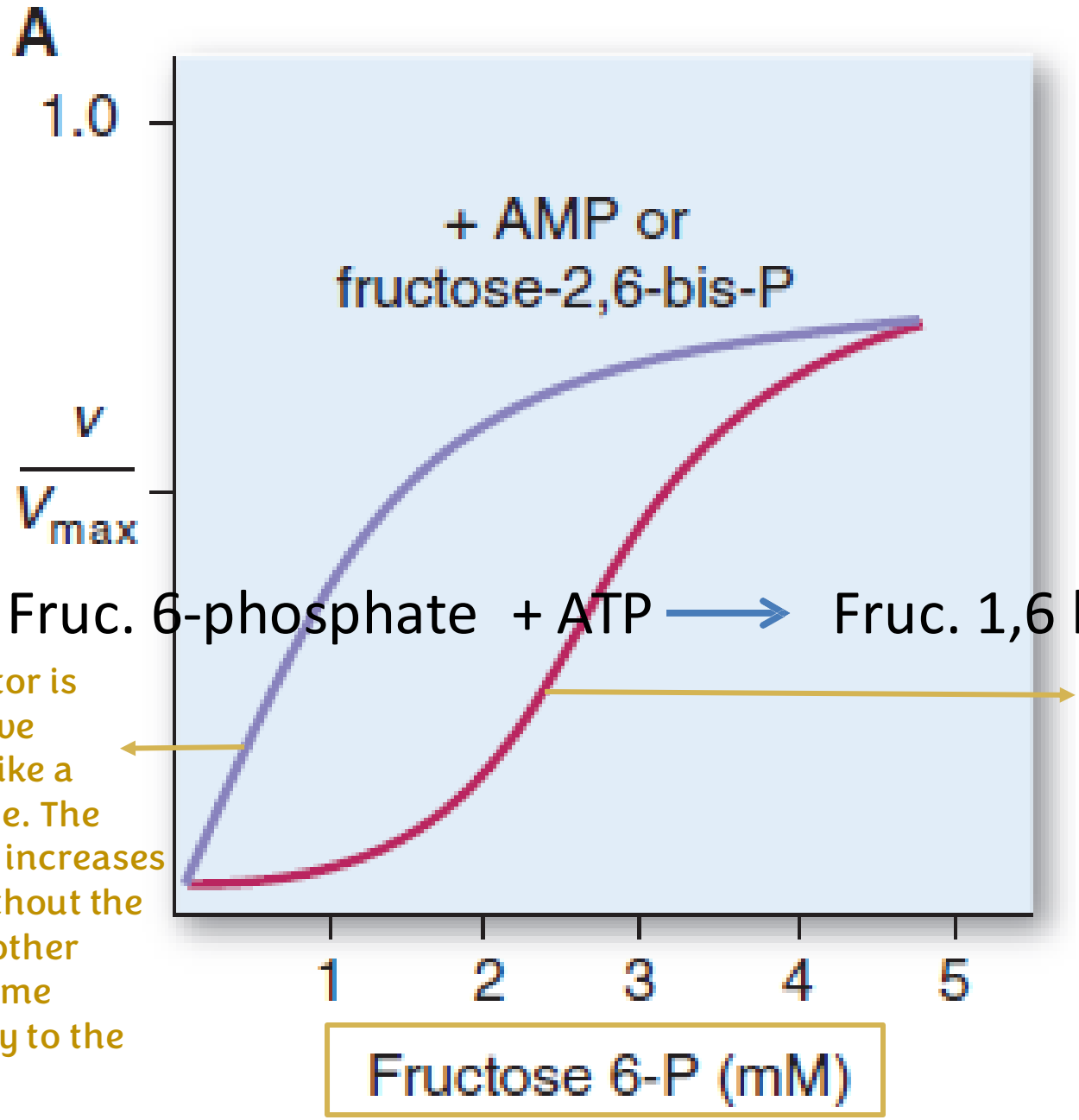
Citrate (−)

Citrate acts as an inhibitor because high citrate levels mean the Krebs cycle is very active and producing plenty of energy. This indicates that the cell is already in a high-energy state, so glycolysis is turned off to avoid unnecessary glucose breakdown

H^+ (−)

High hydrogen ion (H^+) levels indicate that the TCA cycle and electron transport chain are already working actively and producing plenty of energy

Regulation of PFK1 by Fructose 2,6-bisphosphate



Fruc. 6-phosphate + ATP → Fruc. 1,6 bisphosphate + ADP

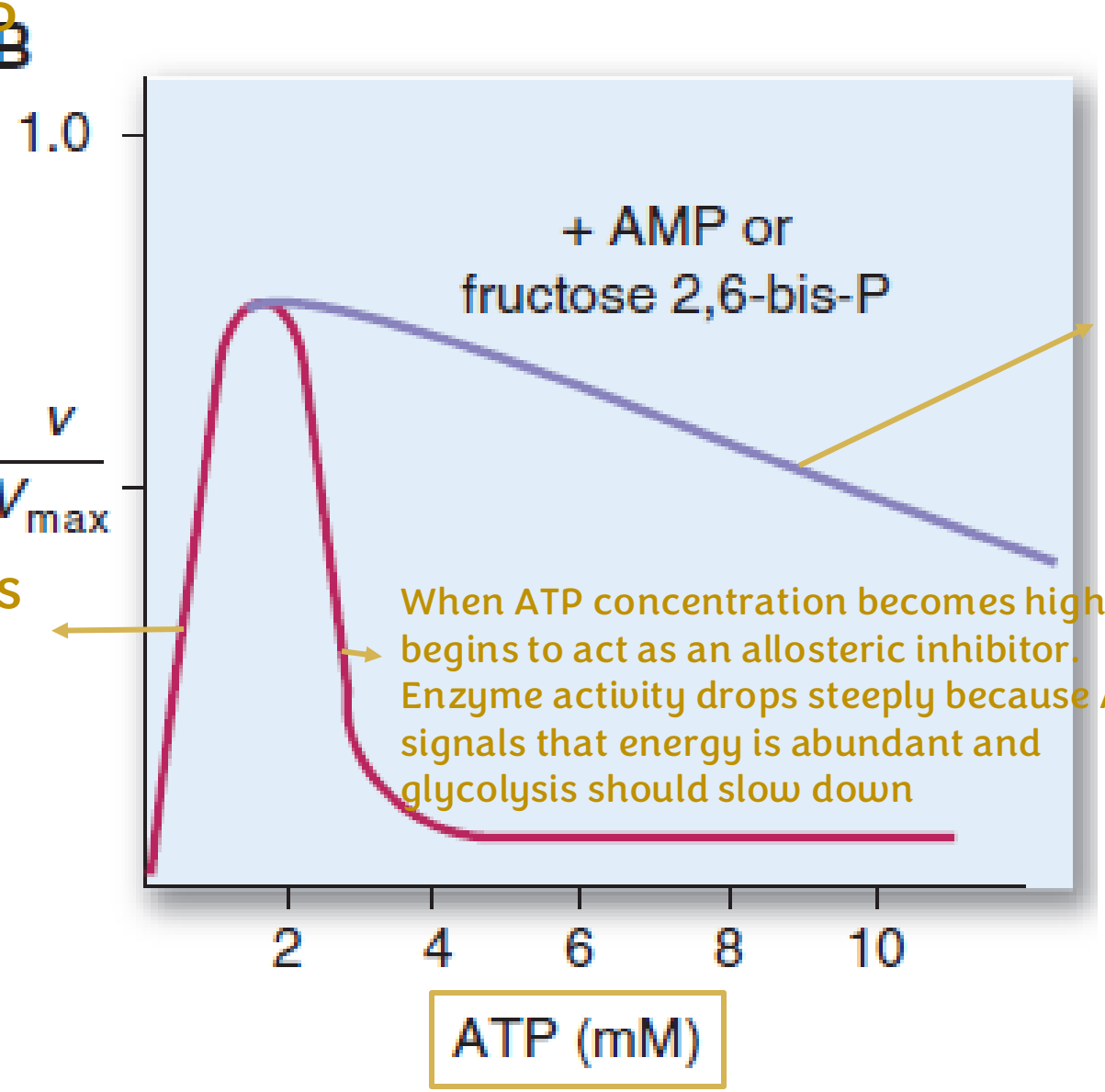
When an activator is present, the curve becomes more like a hyperbolic shape. The enzyme activity increases immediately without the initial delay. In other words, the enzyme responds quickly to the substrate.

This is the original enzyme activity curve. It has a sigmoidal shape, meaning there is a slight delay at the beginning before the enzyme activity increases. At low substrate concentration, the velocity (V/Vmax) rises slowly, and then it increases more rapidly as more substrate becomes available. This reflects cooperative enzyme behavior in the absence of activators

(ATP concentration on the x-axis!)

How about the other substrate?

This curve shows normal enzyme behavior. At low ATP concentration, ATP simply acts as a substrate, so it does not inhibit the enzyme yet. As Dr. Nafez mentioned, a molecule must be in excess to function as a regulator. Therefore, at low ATP levels, it cannot act as an inhibitor.



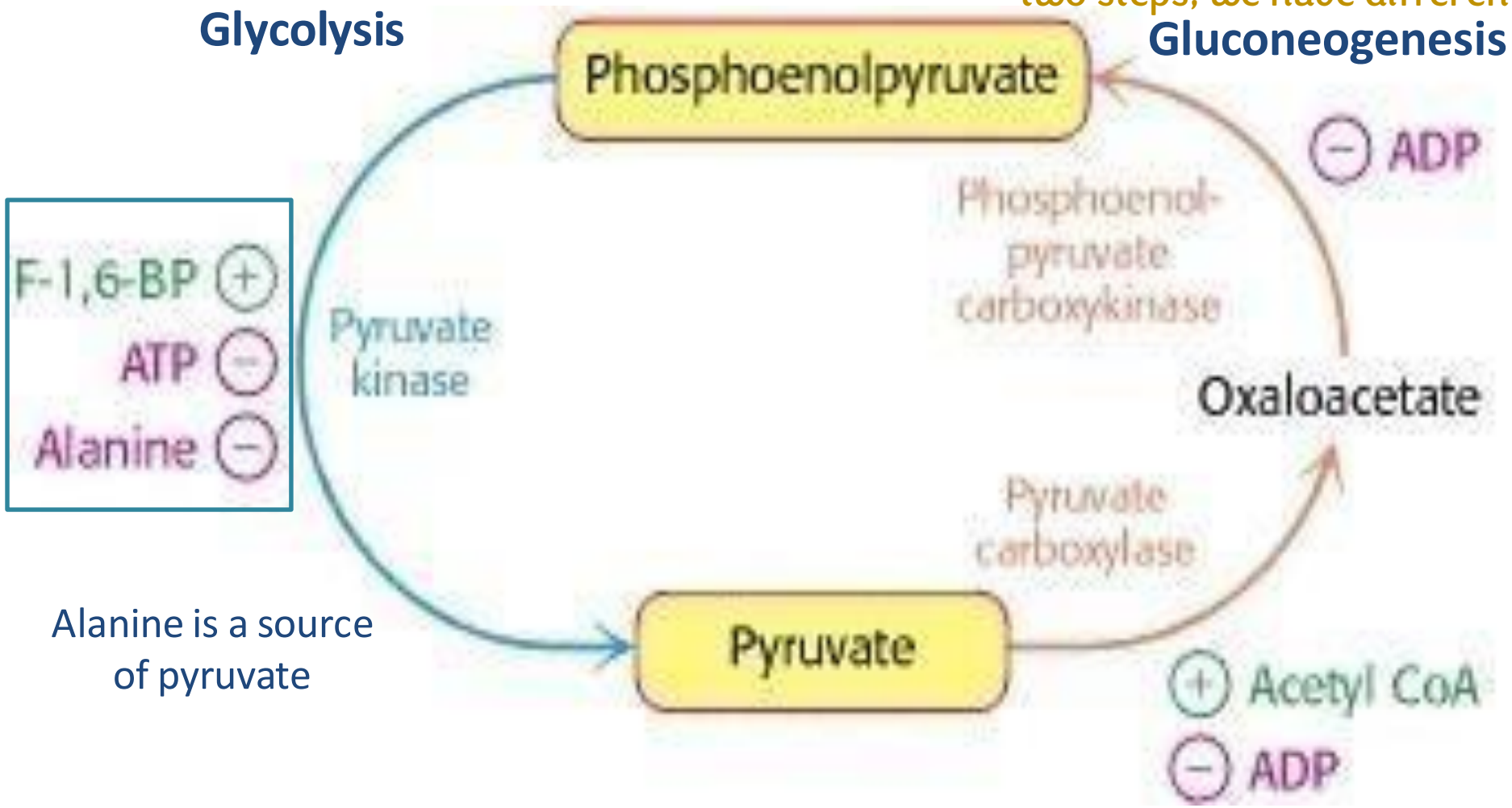
when AMP or fructose-2,6-bisphosphate is added, the curve changes. These molecules indicate low-energy status, so they counter the inhibitory effect of ATP. As a result, the drop-in enzyme activity becomes slower. Even though ATP still eventually inhibits the enzyme, the presence of AMP or F-2,6-BP delays inhibition

When ATP concentration becomes high, ATP begins to act as an allosteric inhibitor. Enzyme activity drops steeply because ATP signals that energy is abundant and glycolysis should slow down

(Regulation of step 10 of glycolysis)

Regulation of Pyruvate Kinase

In gluconeogenesis step 10 is reversed in two steps, we have different regulators



Alanine is a source of pyruvate

Why ATP inhibits pyruvate kinase

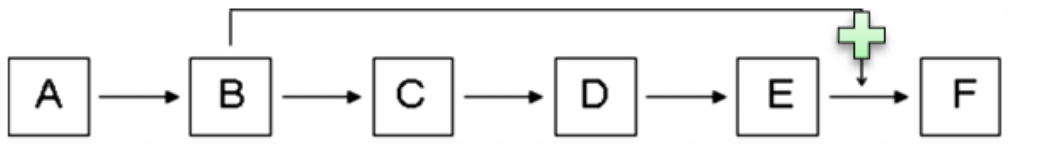
ATP inhibits the last step of glycolysis because high ATP levels signal that the cell already has enough energy. When the energy state of the cell is high, there is no need to continue glycolysis, so ATP turns this step off.

Why alanine inhibits pyruvate kinase

Alanine also inhibits pyruvate kinase. When the amino group is removed from alanine, it becomes pyruvate. Therefore, alanine is a source of pyruvate. High alanine means high pyruvate, which signals that glycolysis does not need to produce more, so glycolysis is inhibited.

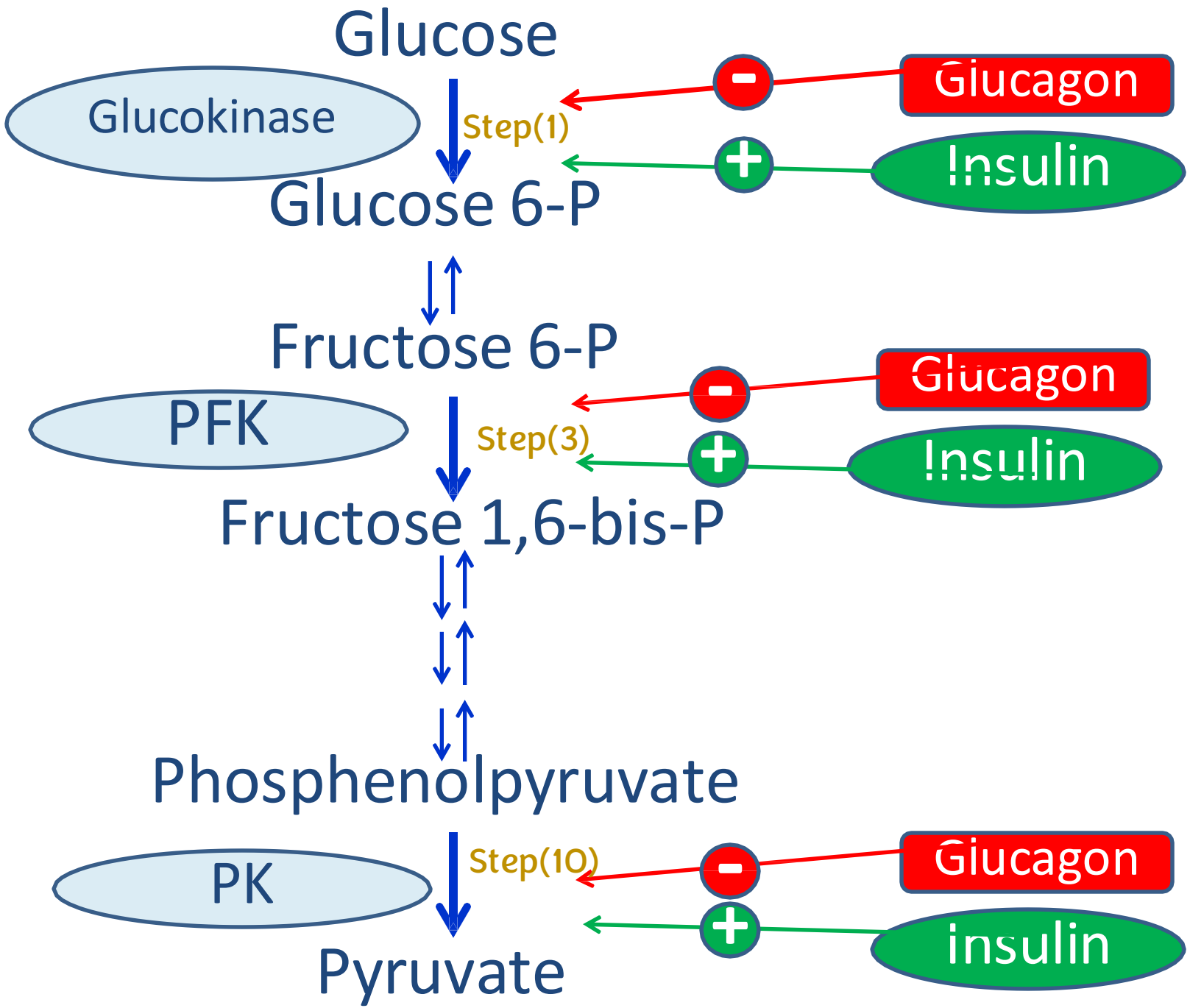
Why F-1,6-BP activates pyruvate kinase

Fructose-1,6-bisphosphate is produced in step 3 of glycolysis, and it serves as a positive regulator of the last step (pyruvate kinase). Once the pathway commits to glycolysis at step 3, F-1,6-BP signals the cell to continue forward and complete the pathway. This is what we call feed-forward regulation



Hormonal Regulation

The examples we mentioned before are classified as allosteric regulation, now we will discuss hormonal regulation. Insulin and glucagon are the main hormonal regulators



Why insulin activates glycolysis

Insulin is released when blood glucose levels are high, such as after eating. The body's goal in this situation is to use the available glucose for energy and store the excess. Therefore, insulin activates glycolysis so that glucose can be broken down and used by the cells

Why glucagon inhibits glycolysis

Glucagon is released during fasting or low-glucose states. In this condition, the body needs to save glucose for organs that depend on it (especially the brain) and switch to other fuel sources like fats. For this reason, glucagon inhibits glycolysis to prevent glucose breakdown and promotes glucose production instead

Do insulin and glucagon enter the cell?

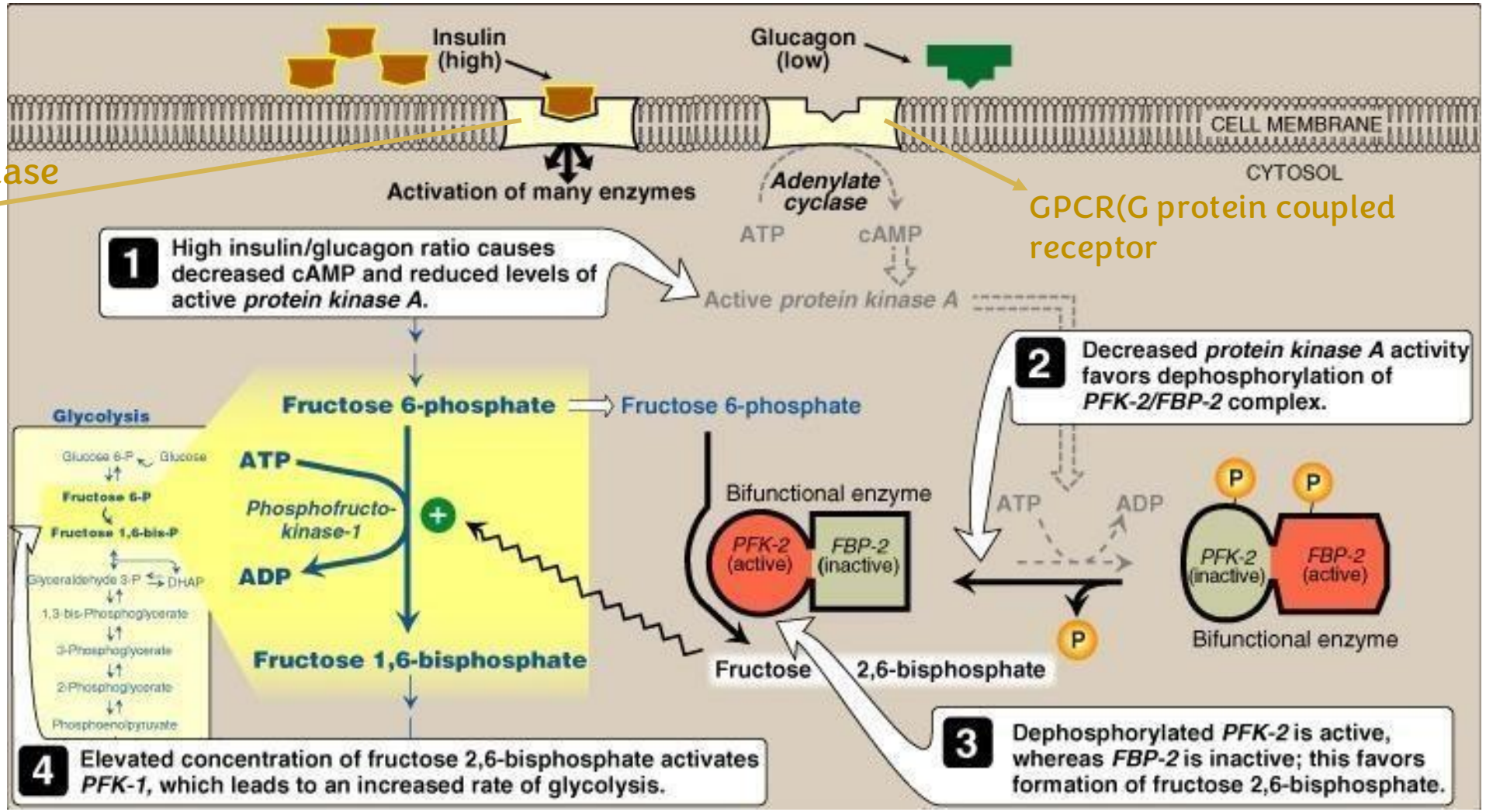
No. Insulin and glucagon do not enter the cell. They bind to receptors on the cell surface, which activates internal signaling pathways. These pathways then lead to activation or inactivation of glycolytic enzymes inside the cell.

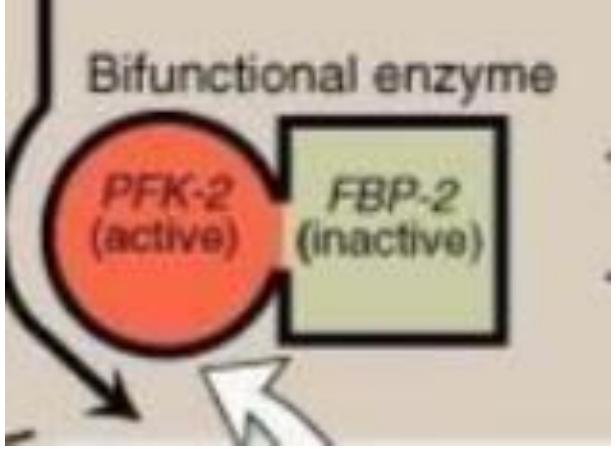
Hormonal Regulation of Phosphofructokinase

This represents a well-fed/ high glucose state since the Adenylyl cyclase pathway is faded

Tyrosine kinase receptor

GPCR(G protein coupled receptor





- PFK-2 = Phosphofructokinase-2

Function: makes Fructose-2,6-BisPhosphate (glycolysis activator) from fructose-6-phosphate → F-2,6-BP strongly activates PFK-1(step 3 enzyme in glycolysis) → glycolysis increases.

- FBP-2 = Fructose bisphosphatase-2

Function: breaks Fructose-2,6-BisPhosphate back to fructose-6-phosphate → F-2,6-BP falls → glycolysis decreases.

This bifunctional enzyme has two opposite functions. One side is active while the other side is inactive – they cannot both be active at the same time, and they cannot both be inactive. Only one function operates at a time, switching depending on the metabolic state.

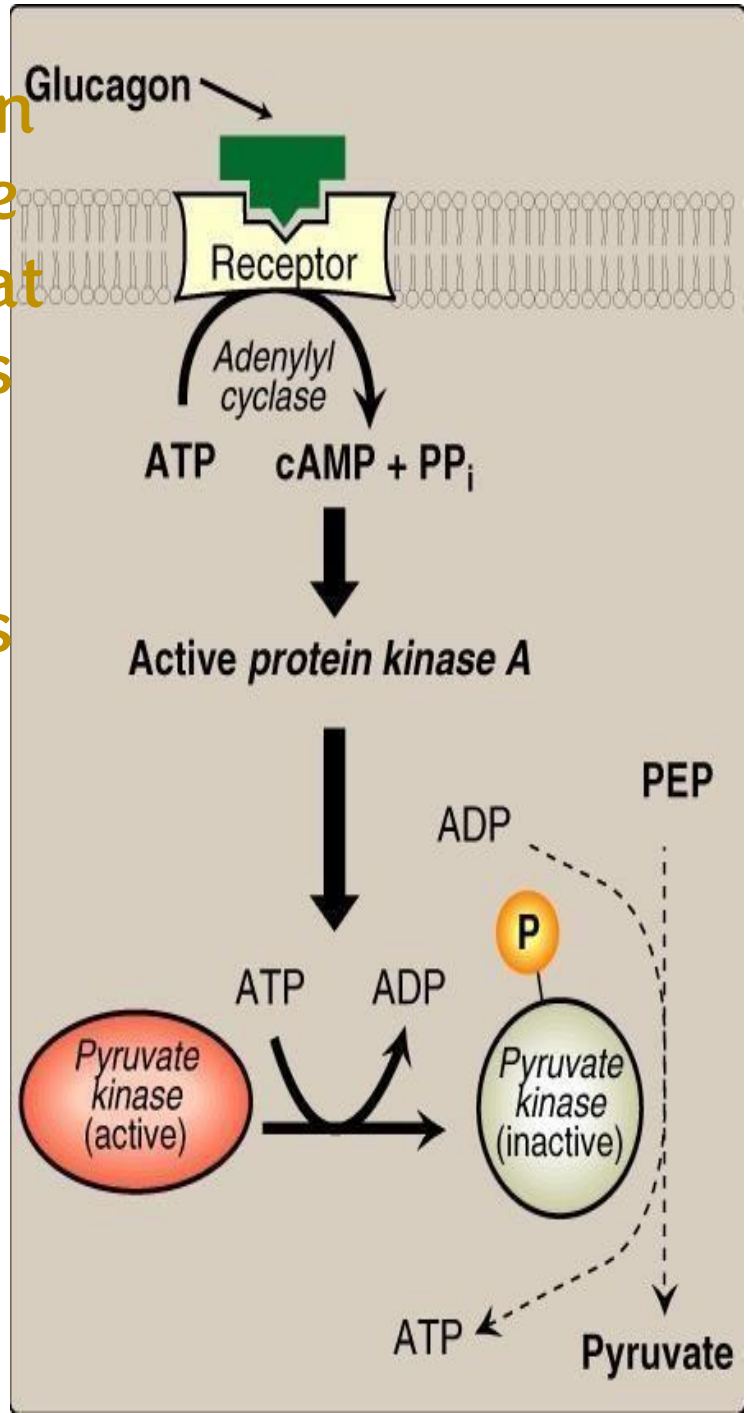
Insulin (Well-fed / high glucose state)

- High glucose → insulin increases.
- Insulin receptor activates tyrosine kinase signaling → activation of many enzymes and molecules
- High insulin / low glucagon, the pathway related to glucagon is inactivated.
- Decreased PKA activity causes dephosphorylation of the PFK-2/FBP-2 enzyme complex.
- Dephosphorylation activates PFK-2 and inactivates FBP-2.
- PFK-2 produces fructose-2,6-bisphosphate (F-2,6-BP).
- F-2,6-BP activates PFK-1 → glycolysis increases.

Glucagon (Fasting / low glucose state)

- Low glucose → glucagon increases.
- Glucagon binds GPCR → ↑ cAMP → activates Protein Kinase A (PKA).
- PKA phosphorylates many protein substrates, one of them is the PFK-2/FBP-2 enzyme complex.
- Phosphorylation inactivates PFK-2 and activates FBP-2.
- FBP-2 breaks down F-2,6-BP.
- Low F-2,6-BP → PFK-1 inhibited → glycolysis decreases.

Another example on a substrate protein that PKA works on (when the GPCR pathway is activated)

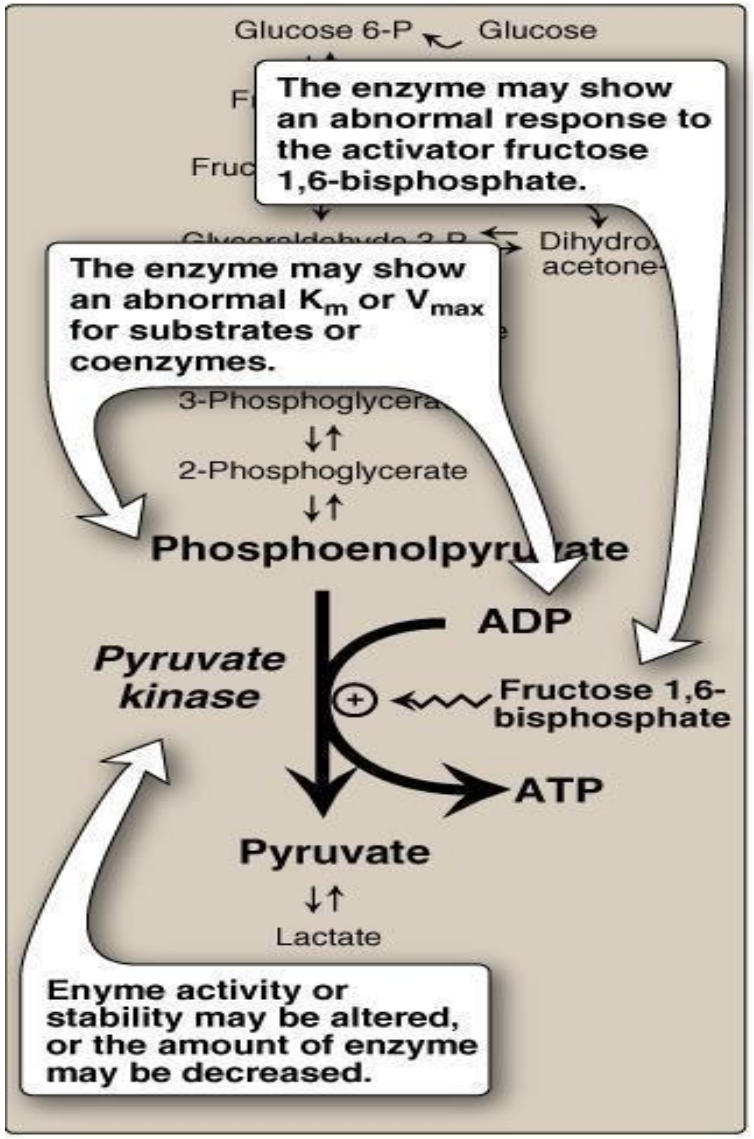


Hormonal Regulation of Pyruvate Kinase

This regulation occurs mainly during fasting. Glucagon binds to its receptor on the cell surface and activates adenylate cyclase, increasing cAMP. The rise in cAMP activates protein kinase A (PKA). PKA then phosphorylates pyruvate kinase (the last enzyme in glycolysis), which inactivates it. By inactivating pyruvate kinase, glycolysis slows down so that glucose can be conserved for important tissues like the brain during fasting.

Clinical Hint: Pyruvate Kinase Deficiency

- The most common among glycolytic enzyme deficiencies
- RBCs are affected
- Mild to severe chronic hemolytic anemia
- ATP is needed for Na+/K+ pump➔ maintain the flexible shape of the cell
- Low ATP ➔ premature death of RBC
- Abnormal enzyme; mostly altered kinetic properties (Km/Vmax)



Alterations observed with various mutant forms of pyruvate kinase

Pyruvate kinase deficiency

Pyruvate kinase is one of the most susceptible glycolytic enzymes to mutations. It catalyzes the final step of glycolysis, which is the step that produces ATP. If this enzyme is missing or defective, the last step cannot occur, and ATP will not be produced. This step is especially important because it generates the net gain of 2 ATP in glycolysis.

Why red blood cells are especially affected

RBCs rely completely on glycolysis for ATP because they have no mitochondria. Even though their energy need is low, glycolysis is their only source of ATP. With pyruvate kinase deficiency, ATP production drops significantly, so RBCs are the first cells to suffer.

Effects of no ATP in RBCs

When RBCs cannot produce ATP, they cannot carry out essential functions. Two major consequences occur:

- Oxidative stress and ROS accumulation
  Without energy, RBCs cannot remove reactive oxygen species (ROS). These toxic by-products damage cell components and shorten RBC lifespan. ROS are by-products of many metabolic reactions, and because they are highly reactive, they can interact with cellular components and damage their structure and function. This oxidative damage contributes to cell injury and is associated with various conditions, including cancer and inflammatory diseases
- Failure of the Na^+/K^+ pump
  RBCs lose the ability to run the Na^+/K^+ pump, which normally maintains ion balance and preserves the cell's shape.

Why RBC shape matters

Normal RBCs are biconcave discs. This shape provides:

- Large surface area → efficient gas exchange
- Flexibility → allows RBCs to squeeze through narrow capillaries

When ATP is low and Na+/k+ pump is damaged, RBCs lose their biconcave shape and flexibility. They become rigid and cannot deliver oxygen efficiently.

Outcome

Damaged RBCs die early (before their normal lifespan of ~120 days). This premature destruction, combined with oxidative stress, leads to chronic hemolysis. The bone marrow attempts to replace the lost cells, but when RBC destruction exceeds regeneration, hemolytic anemia develops.

External (non-physiological) Inhibitors of Glycolysis

These are compounds from the external environment (not made inside the body) that can interfere with and inhibit the glycolysis pathway. In other words, they are substances you may be exposed to outside the body that can block or disrupt glycolysis and therefore energy production in cells.

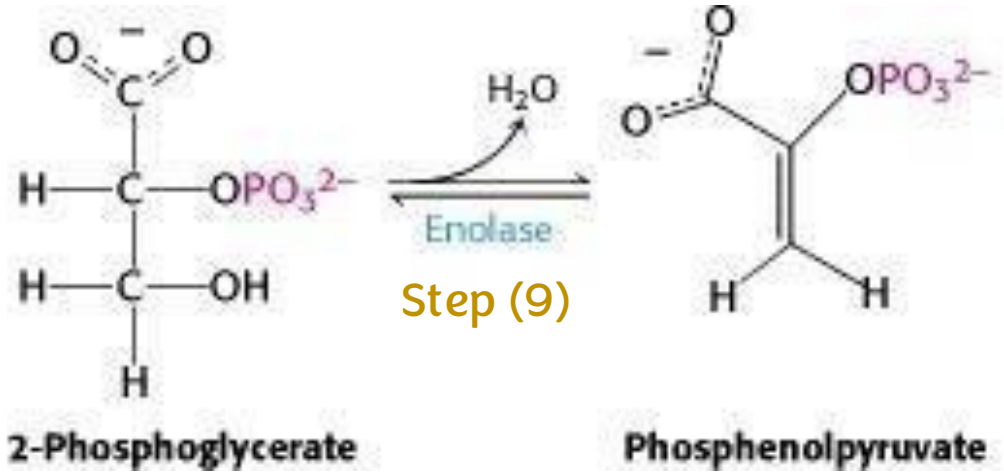
Inorganic Inhibitors of Glycolysis, non-physiologic

Fluoride

Fluoride is found in toothpaste and even in drinking water in small safe amounts. It mainly protects teeth from tooth decay and bacterial damage.
Fluoride does not build the tooth structure – that's the job of calcium.
Instead, fluoride strengthens and improves tooth quality and helps prevent bacterial growth.

- Fluoride inhibits Enolase

  In bacterial glycolysis



Fluoride inhibits enolase, so glycolysis stops in bacteria.
With no glycolysis, bacteria cannot produce energy, and because they lack energy, they cannot divide or grow.
This results in reduced bacterial activity, making fluoride bacteriostatic (it stops bacteria from multiplying rather than killing them directly)

Fluoridated water → ↓ bacterial enolase →

Prevention of Dental Carries

Also, prevention of dental cavity formation

Inorganic Inhibitors of Glycolysis, non-physiologic

(Arsenic: A toxic metalloid / transition-like element)

➢ Arsenic Poisoning

☆–Pentavalent Arsenic (Arsenate) competes with phosphate as as a substrate for GA3PDH (Glyceraldehyde 3-phosphate dehydrogenase)

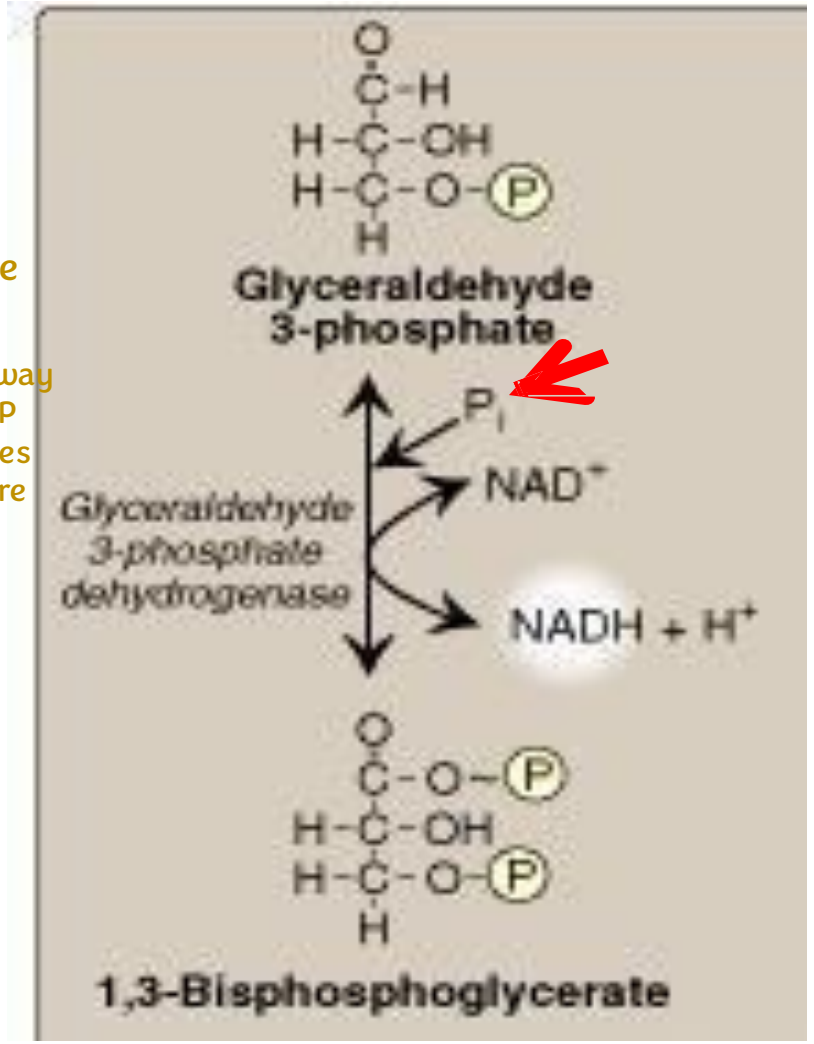
↓ATP synthesis

When arsenate is used instead of phosphate, the pathway cannot form ATP at a later step, leading to reduced ATP synthesis. As a result, glycolysis continues but produces no usable energy, contributing to cellular energy failure

☆–Trivalent Arsenic (Arsenite) Forms stable complex with-SH of lipoic acid Pyruvate Dehydrogenase

↓α ketoglutarate Dehydrogenase

↓➔Neurological disturbances…….**DEATH**



Trivalent Arsenic (Arsenite) more dangerous!

Inhibition of Pyruvate Dehydrogenase Complex

This toxin binds to the -SH (sulfhydryl) groups of lipoic acid preventing its oxidation, reduction, and regeneration, lipoic acid is an essential co-enzyme inside the pyruvate dehydrogenase complex.

When lipoic acid is blocked, the entire enzyme complex becomes inactive, and pyruvate cannot be processed properly → severe drop in energy production.

Additionally, there other enzyme complexes in the body with very similar structures and use the same co-enzymes, including lipoic acid – for example, α-ketoglutarate dehydrogenase complex (Krebs cycle).

Although these complexes work on different substrates, they both rely on lipoic acid.

So when this co-enzyme is inhibited, both complexes are affected.

As a result, multiple important reactions stop, → significant energy failure, → serious neurological effects, and can eventually lead to death.

Additional Resources:

رسالة من الفريق العلمي:

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			