SHEET NO.
8 part 1

METABOLISM

DOCTOR 2019 | MEDICINE | JU

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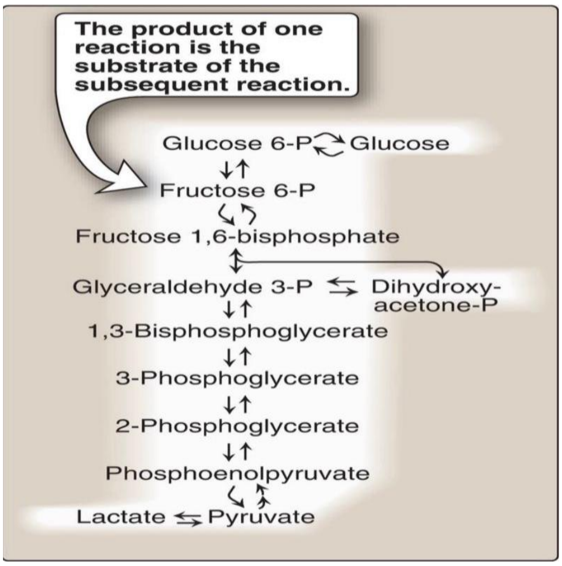
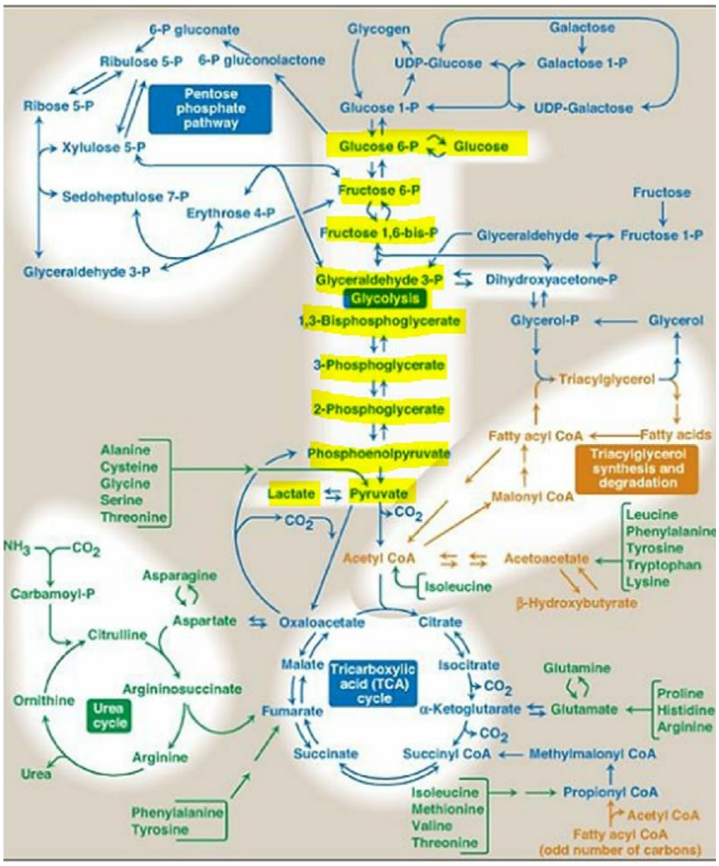
Metabolism Overview

Metabolic pathways do not occur in **isolation**. Instead, they are organized into multistep sequences called **pathways**, such as that of **glycolysis**. In a pathway, the product of one reaction serves as the substrate of the subsequent reaction.

Different pathways can intersect forming an integrated and purposeful network of chemical reactions. Metabolism is the sum of all the chemical changes occurring in a cell, tissue, or the body.



The Metabolic Map ↓



A metabolic map containing the important central pathways of energy metabolism is presented here. ←

The pathways of metabolism must be **coordinated** so that the production of energy or the synthesis of end products meets the **needs** of the cell.

Glycolysis (Gly: sugar, lysis: degradation)

Among the thousands of enzymes within the **cytosol** are those responsible for glycolysis, a chemical process involving **10-separate sequential reactions** that break down the simple **six** carbon sugar molecule, glucose, into two pyruvic acid molecules, each of which contains **three** carbons.

The intermediates in this pathway are either 6 carbons (glucose 6-P, fructose 6-P,…) or 3 carbons (glyceraldehyde 3-P,…)

The glycolytic pathway is used in all tissues -and in all cell types- for the oxidation of glucose to provide **energy** (as ATP) and **intermediates** for other metabolic pathways

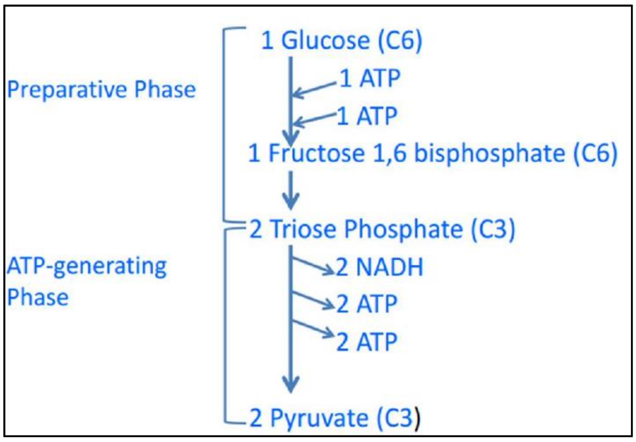
– in other words; glycolytic intermediates are used as precursors for many anabolic pathways- .

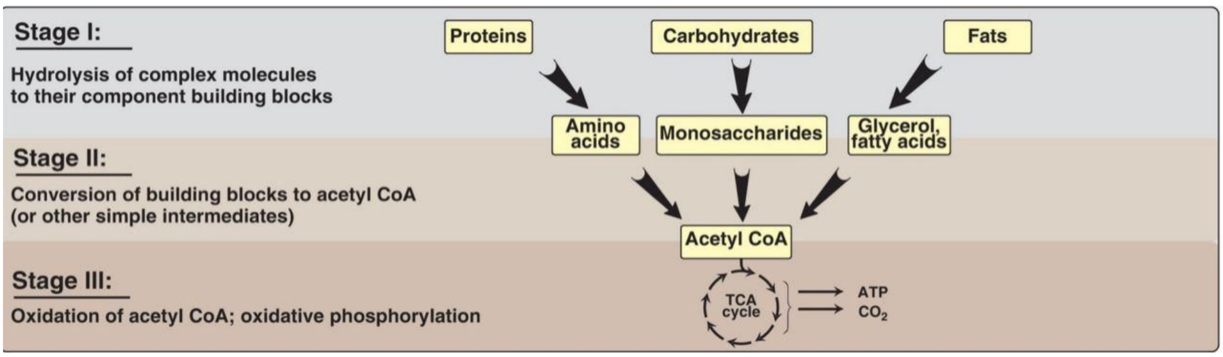
Glycolysis can occur **aerobically** or **anaerobically** depending on whether oxygen is available;

-**Aerobic glycolysis** takes place in cells with mitochondria and adequate supply of O2. Its end product is pyruvate (the ionized form of pyruvic acid).

-**Anaerobic glycolysis** takes place in cells that lack mitochondria (like RBCs), or in cells deprived of sufficient O2 (hypoxia). Its end product in mammalian cells is lactate.

The oxidation of glucose into pyruvate occurs through 2 phases: **Energy- investment phase (**AKA **preparative phase**), in which phosphorylated intermediates are synthesized at the expense of 2 ATP. **Energy-generation phase** in which 4 ATP molecules are produced by **substrate-level phosphorylation.**





∮Types of glycolytic reactions:

1. Phosphoryl-transfer reactions: Transfer of the phosphoryl group (PO3) from one **molecule** to **another**.

2. Isomerization reactions: One molecule is transformed into another molecule which has **exactly** the same **atoms, but the atoms have a different arrangement**. (ketose↔aldose)

3. Cleavage reactions: Covalent bond breakage in a molecule leading to the formation of smaller molecules. (6 carbons-molecule ↔ two (3 carbons) molecules)

4. Oxidation-reduction (redox) reactions: Transfer of **electrons** from one molecule to another.

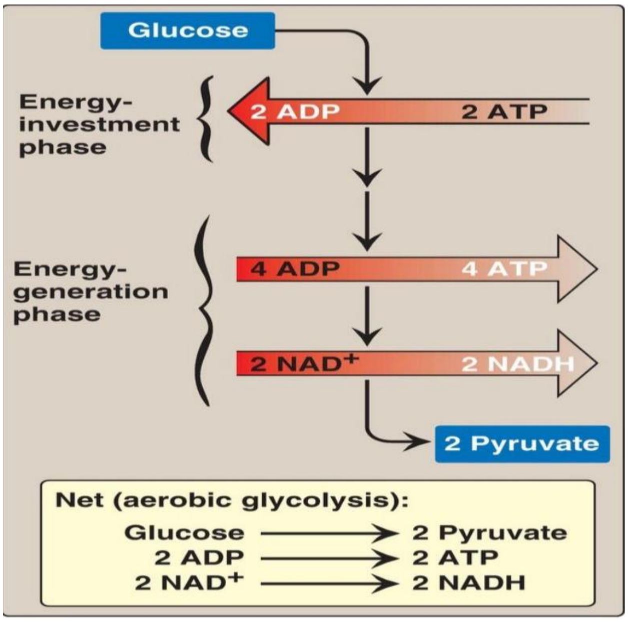
5. Phosphoryl-shift reactions: The **intra-molecular** shift of a phosphoryl group from one **atom** to another within the **same molecule**.

6. Dehydration reactions: Loss of water from the reacting molecule

Tissues with an absolute or high requirement for Glucose

- Brain
- RBCs
- Cornea lens and retina
- Kidney Medulla
- Testis
- Leukocytes
- White muscle fiber

Know that energy investment phase needs ATP to occur, in otherwise energy generation phase release 4 ATP, so the net result will be 2 ATP, 2 pyruvate and 2 NADH.
And pyruvate has 2 ways, either go to TCA cycle OR lactic cycle.



In the **energy-investment phase**, glucose is phosphorylated by **hexokinases**; which are found in most tissues or **glucokinases**; found in liver cells, each one of them has different function.

- Hexokinase (has several isoenzymes), has a high affinity (low Km) for glucose. This permits the efficient phosphorylation and subsequent metabolism of glucose even when tissue concentrations of glucose are low↓.

-Glucokinase has a lower affinity (higher Km) for glucose, requiring a **higher** glucose concentration for half-saturation. Thus, glucokinase functions **only** when the intracellular concentration of glucose in hepatocytes is elevated↑, in order to store it as glycogen for a later time when your body needs it.

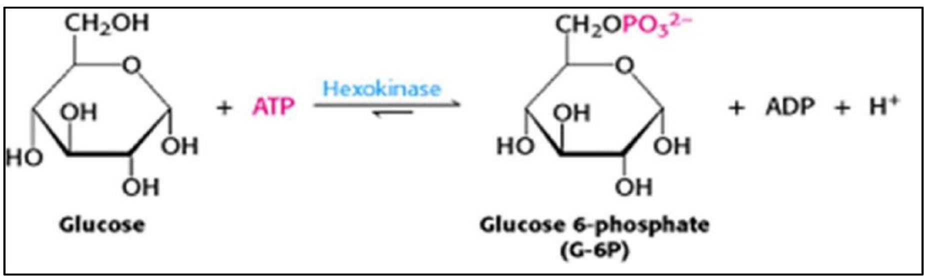
-Unlike hexokinase, glucokinase is induced by insulin (↑insulin → ↑G6P).

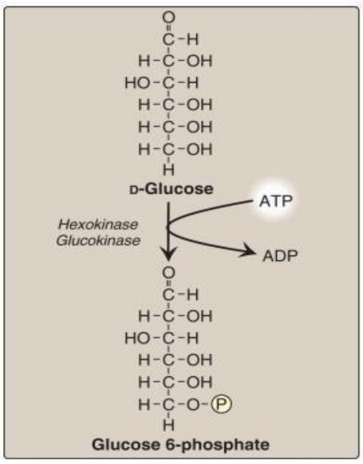
	Hexokinase	Glucokinase
Occurance	In all tissues	In liver
Km	< 0.02 mM	10-20 mM
Specificity	Glc., Fruc, Man, Gal	Glc.
induction	Not induced	↑ insulin, Glc
Function	At any glucose level	Only > 100 mg/dl

Glycolysis Reaction

- The Glycolysis reaction consists of 10 reactions, the first five reactions correspond to an **energy-investment phase (preparative phase),** and the subsequent reactions constitute an **energy-generation phase.**
- Glycolysis (aerobic) produces **2 ATP**, **2 NADH,** and **2 Pyruvate molecules, and that what we talked about.**

1. Glucose phosphorylation (first rate-limiting step)

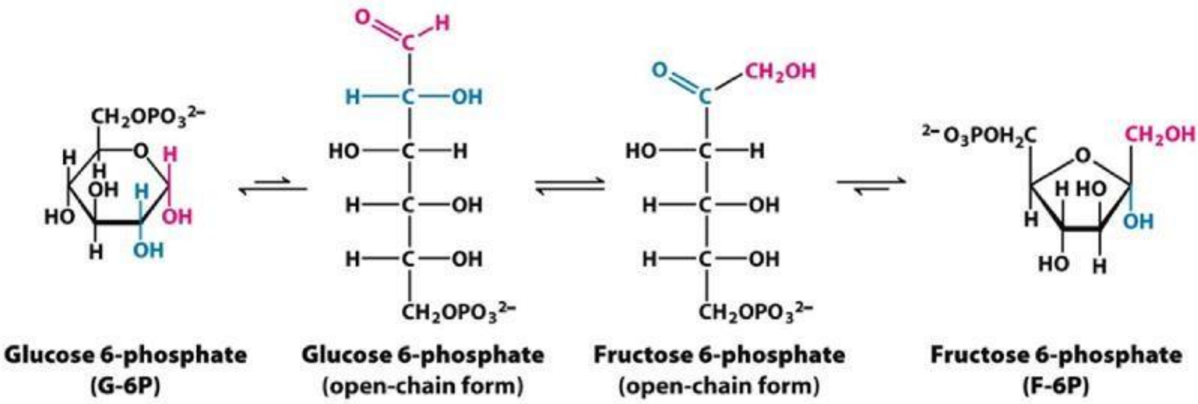




- The enzymes that catalyze this reaction are **Hexokinase, Glucokinase, (kinase: transfer phosphate group from substrate to another)**
- Glucose accept phosphate group, and added to C6, ATP → ADP.
- This reaction is **the first irreversible reaction** since a high energy bond in ATP is broken, and a low energy bond (ester bond) is formed in Glucose 6-P. This is considered as an exergonic reaction.
- Since the reaction is exergonic and irreversible, it should be **regulated**.
- Phosphorylated sugar molecules do not penetrate cell membrane (no longer able to leave the cell ''trapped'') since:
  - ✓ there are no specific transmembrane carriers for these compounds
  - ✓ they are too polar (Phosphate group is highly negative charged).

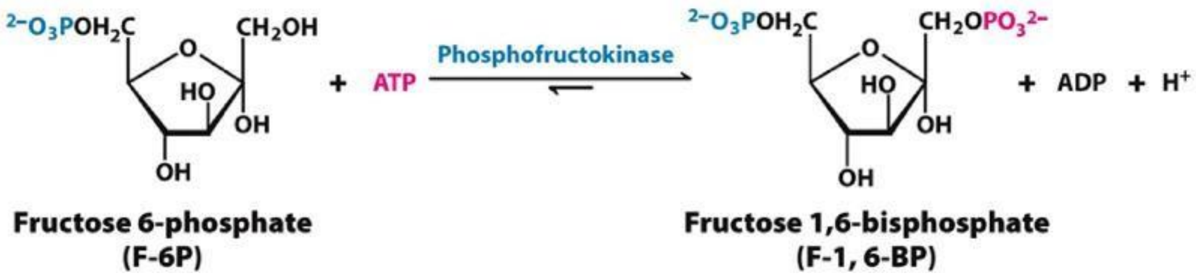
- **as a result,** Phosphate group will be trapped also, because adding Phosphate group will decrease the level of free Phosphates which is required for ATP synthesis (ATP required ADP+Pi).
- The consequence of having phosphate group on glucose that the glucose can get out of the cell, but once it is phosphorylated, glucose 6-phosohate can't leave the cell because it's highly polar molecule (negative charge) and there's no transporter for it.
- Hexokinase is found in all over cells, because glycolysis is too.
- In general, phosphorylation reactions are **endergonic** ($\Delta G>1$) because they **require** energy to form **additional** bonds with the **new** phosphate groups. However, during glucose phosphorylation, glucose is converted into glucose 6-phosphate using an ATP molecule as a **source** of <u>**energy**</u> (ATP hydrolysis) and a <u>**phosphate group.**</u> **ATP is** a **substrate** in the reaction that has a **high** chemical energy within its bonds, this makes the G(substrates) > G(products) and therefore $\Delta G<1$.

2. Glucose 6-P isomerization



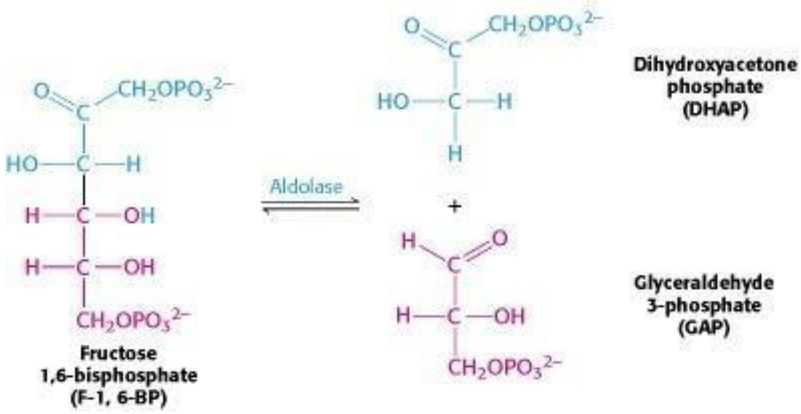
- The enzyme that catalyzes this reaction is **Phosphoglucose isomerase**.
- The reaction is freely reversible and doesn't require regulation.
- The isomerization from a 6 membered ring (G-6P) to 5 membered ring (F-6P) is done through the open-chain form.

3. Fructose 6-phosphate phosphorylation (the committed step)



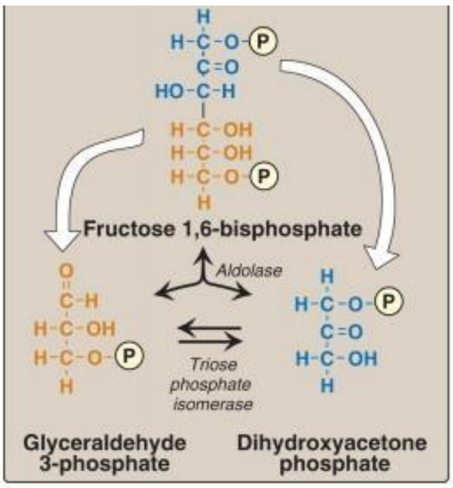
- The enzyme that catalyzes this reaction is **Phosphofructokinase-1 (PFK-1)**.
- In this reaction another Phosphate group is added to C1 of F-6P.
- This reaction is **the second irreversible reaction.** Therefore, it is highly regulated, [note: The conversion from F-6P to F-1,6-BP cannot be done spontaneously through the enzyme since it's an endergonic reaction. However, coupling the reaction with ATP produces and overall **exergonic reaction**, thus making it possible for the enzyme. (**large - ΔG**)].
- This reaction is a **rate-limiting** and **committed step** because F-1,6-BP is only used in Glycolysis unlike G-6P which can be used in more than one reaction.
- Bis → adding 2 groups of phosphate are attached to the same molecules but not next to each other. Di → 2 groups of phosphate are joined together.

4. Fructose 1,6-bisphosphate cleavage



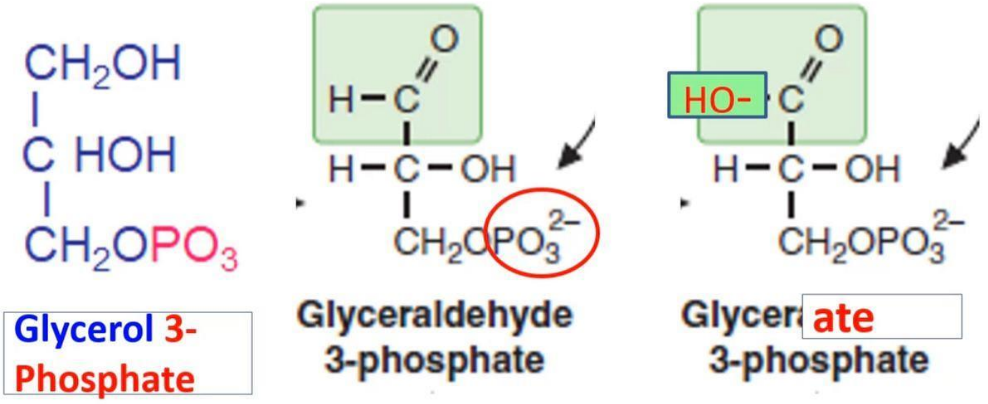
- The cleavage of **F-1,6-BP** into **Glyceraldehyde 3-Phosphate (GAP)** and **Dihydroxyacetone Phosphate (DHAP)** is catalyzed by an enzyme called **Aldolase** (aldole cleavage: ketone group next to hydroxyl group)
- The cleavage occurs between C3 and C4.
- The cleavage does not require water since it's a lyase reaction not hydrolyses.
- This reaction is reversible and not regulated.

5. Dihydroxyacetone phosphate isomerization



- The enzyme that catalyzes this reaction is **Triose phosphate isomerase,** which interconverts **DHAP** and **GAP.**
- **DHAP** must be isomerized to **GAP** for further metabolism by glycolytic pathway.
- This isomerization results in the net production of **2 molecules of GAP.**
- **F-1,6-BP** will convert to 2 GAP by 2 reactions: cleavage and isomerization.

Look at the following structures to understand the oxidation of glyceraldehyde 3-Phosphate



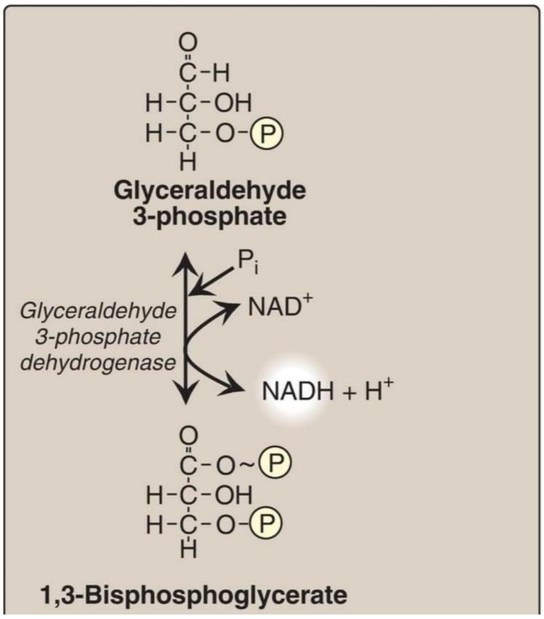
What is the difference between Glycerol 3-phosohate and Glyceraldehyde 3-phosphate?

Functional group at C1, Glycerol 3-phosphate → Hydroxyl group, Glyceraldehyde → aldehyde group, Glycerate 3-phosphate → carboxylic acid.

- if you oxidized alcohol → aldehyde → oxidized aldehyde → carboxylic acid

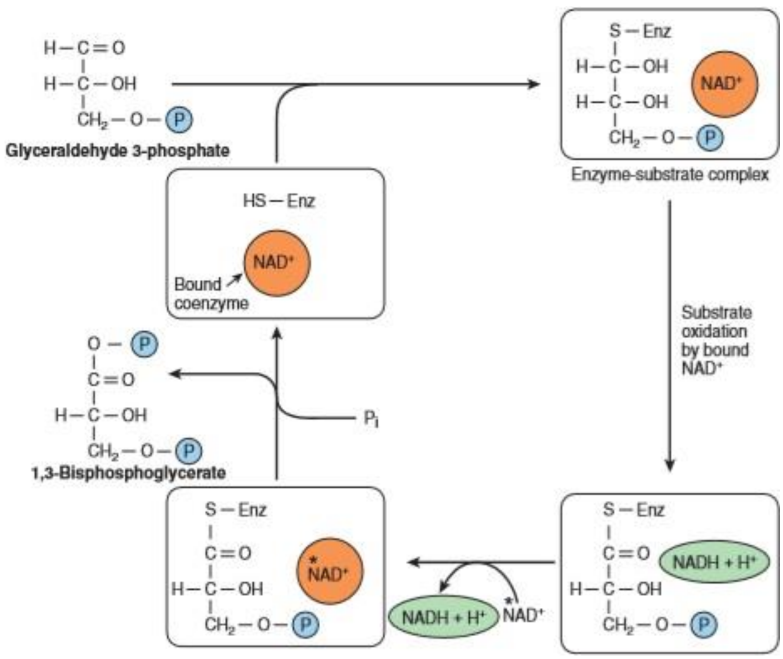
6. Glyceraldehyde 3-phosphate oxidation (the first oxidation-reduction reaction)

Compound $+H_2O$	Product + phosphate	$\Delta G°$
Phosphoenol pyruvate	Pyruvate	-14.8
1,3 bisphosphoglycerate	3 phosphoglycerate	-11.8
Creatine phosphate	Creatine	- 10.3
ATP	ADP	- 7.3



- The enzyme that catalyzes this reaction is **Glyceraldehyde 3-phosphate dehydrogenase (GAP dehydrogenase).**
- GAP dehydrogenase converts **GAP** to **1,3-bisphophoglycerate (1,3-BPG).**
- **A phosphate group** is added to **GAP** to produce **1,3-BPG**. This phosphate group linked to **C1** of the **1,3-BPG** by a high energy bond, conserves much of the free energy produced by the oxidation of GAP.
- In this step, **1 (NADH)** is produced **per 1 (GAP)**, So, for each glucose (2 GAP), **the net production is 2 NADH.**
- Transfer phosphate group from 1,3-bisphosphoglycerate to ADP to make it ATP needs -4.5.

➢ How does this reaction occur?



Mechanisms of action:
E and S form covalent linkage

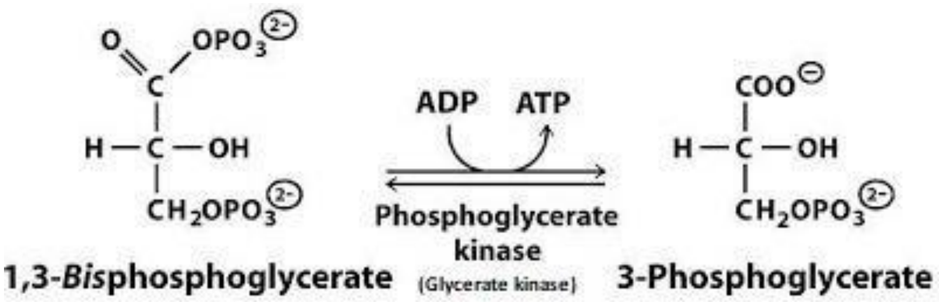
S is oxidized and NADH is formed NADH is released

Pi attacks the thioester bond releasing the product

- ✓ The enzyme has **cysteine** at its side chain which make a covalent bond with the substrate producing **thiohemiacetal** **(organic functional group with the general formula RCH(OH)SR).**
- ✓ **Thiohemiacetal** is oxidized and loses two hydrogen atoms to form an **acyl thioester intermediate**, which is highly energy bound (due to the thioester bond), and **NAD^+** is reduced to **NADH** (the compound is still bound to the enzyme).
- ✓ **Now** a **phosphate group** attacks the enzyme-substrate complex (thioester bond), which leads to release of **the product** (**1,3-BPG**) and **the free enzyme** (the released energy from thioester bond is used to bind the **Phosphate group**).

Note: If **H2O** attacks the thioester bond (abnormal), **3-Phosphoglycerate (3-PG)** is produced and the energy is released in the form of **heat.** This leads to the decrease in the amount of ATP produced by this pathway, because in this reaction, the step that produces ATP is **bypassed** (will be discussed later).

7. 3-Phosphoglycerate synthesis and ATP production



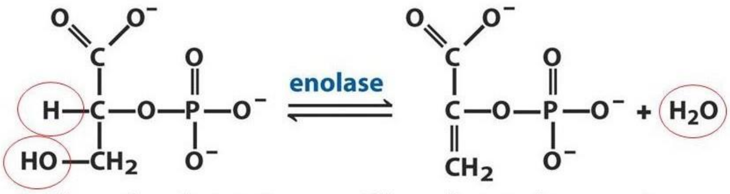
- The enzyme that catalyzes this reaction is **Phosphoglycerate kinase (Glycerate kinase).**
- It's a reversible reaction and has a large negative ΔG.
- In this reaction **1,3-BPG** is converted to **3-Phosphoglycerate (3-PG)**, **the high energy Phosphate group** of 1,3-BPG (at C1) is used to synthesize **ATP from ADP**.
- This Kinase, unlike most kinases, **is physiologically reversible**; which means that **it phosphorylates ADP to ATP**.
- This reaction is an example of **substrate level phosphorylation**, in which the energy needed to produce high energy Phosphate comes from a substrate rather than from **ETC** (Electron Transport Chain).
- In this step **1 (ATP)** is produced **per 1 (1,3-BPG)**, so for each glucose molecule (2 of 1,3-BPG), **the net production is 2 ATP.**

8. Phosphate group Shift

3-Phosphoglycerate 2-Phosphoglycerate

- The enzyme that catalyzes this reaction is **Phosphoglycerate mutase,** which **shifts** the Phosphate group from C3 to C2.
- So, 3-Phosphoglycerate (3-PG) is **isomerized** into 2-phosphoglycerate (2-PG).
- It's a freely reversible reaction.

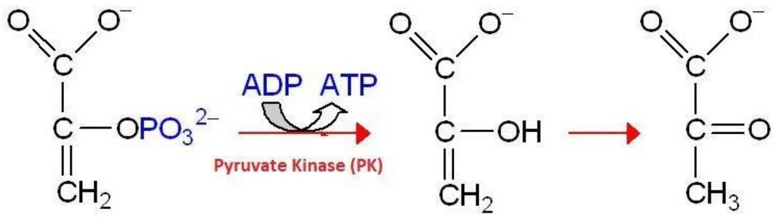
9. 2-Phoshoglycerate dehydration



2-Phosphoglycerate Phosphoenolpyruvate

- The enzyme that catalyzes this dehydration reaction is **Enolase (phosphopyruvate hydratase).** It dehydrates 2-Phosphoglycerate (2-PG) by removing water (OH from C3 and H from C2) (enolase redistributes the energy).
- Removing water produces a compound with a double bond called **Phosphoenolpyruvate** (PEP).
- The product (PEP) contains a high energy enol Phosphate.
- This reaction is reversible **despite** the high energy nature of the product.

10. Pyruvate synthesis and ATP production



PEP enolpyruvate pyruvate

- The enzyme that catalyzes this reaction is **Pyruvate kinase**.
- This reaction is **the third irreversible reaction.**
- **PEP** has a high energy, thus, the Phosphate group has a high ability to **transfer to ADP to form ATP (substrate level phosphorylation) and it's -7.5.**
- When **PEP** loses its Phosphate group, it will be converted to **enol pyruvate** (OH

and double bond at the same carbon ''C2'') which is a highly unstable molecule. Therefore, rearrangement occurs to produce **Pyruvate.**

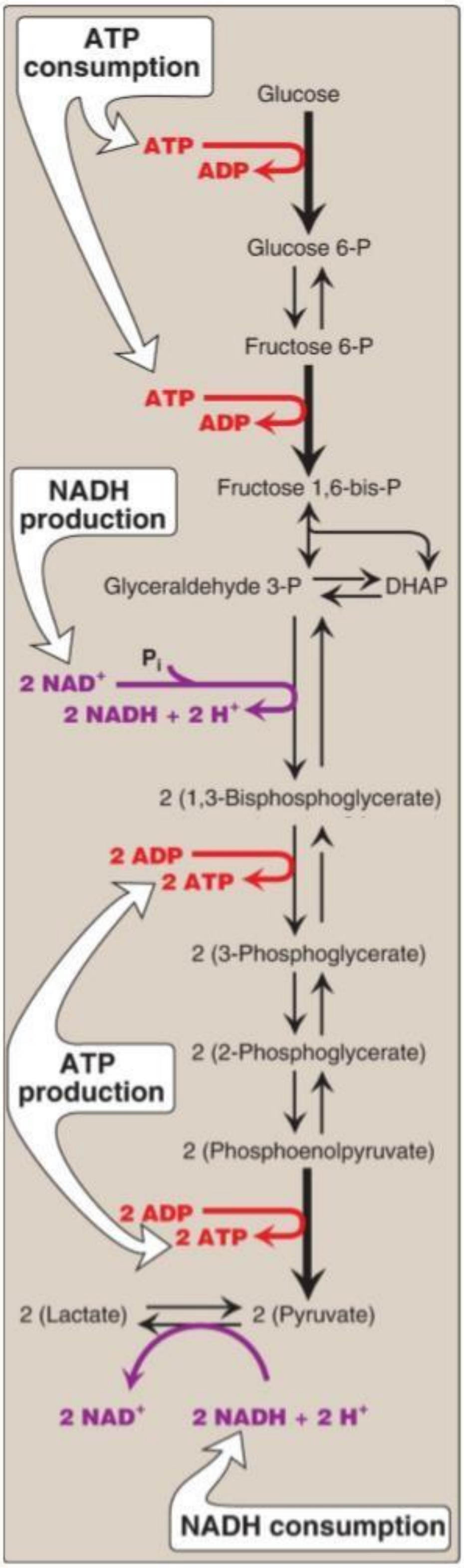
- In this step **1 (ATP)** is produced **per 1 (PEP)**, So for each Glucose molecule (2PEP) **the net production is 2 ATP.**

❖ Summary for Glycolysis →

- **Step 1:** consumes 1 ATP molecule
- **Step 3:** consumes 1 ATP molecule
- **Step 6:** Production of 2 NADH
- **Step 7:** Production of 2 ATP molecules
- **Step 10:** Production of 2 ATP molecules

➢ **The net production of Glycolysis is**

✓ **2 ATP & 2 NADH (after cleavage, we have 2 molecules).**



❖ **Is Oxygen Needed?**

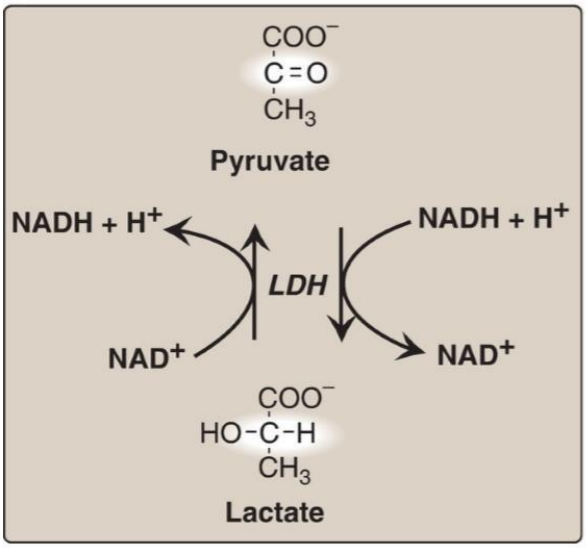
- **It depends**

✓ **Yes,** if the pathway was **Aerobic Glycolysis,** because **Glucose** concentration is much **higher** than **NAD^+** concentration **(limited amount).** Glucose reduces **NAD^+** to **NADH**, after a while all **NAD^+** will be consumed to form **NADH** which means that this pathway won't go any further. So, to keep the process going **NADH** should be **reoxidized** to **NAD^+** and that is done by **ETC** in the presence of **Oxygen**.

✓ **NO,** if the pathway was **Anaerobic Glycolysis**, by reducing the **Pyruvate** (the end product) to **Lactate** (reduction of **ketone** group produces **secondary alcohol**) which leads to the oxidation of **NADH** (reducing agent) to **NAD^+**.

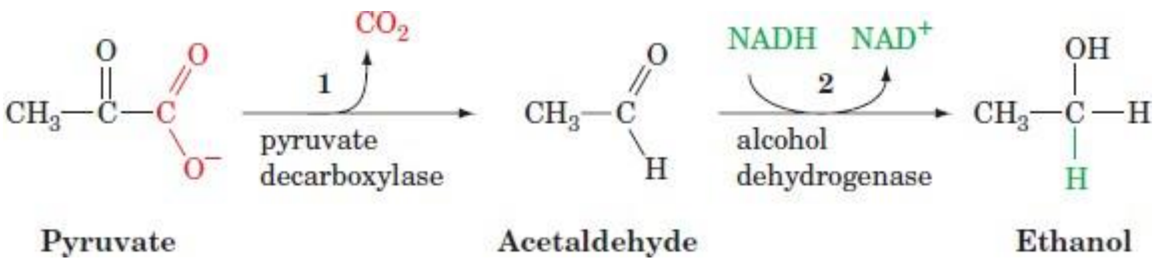
- This process mainly happens in **RBCs** because they don't have mitochondria (NO ETC).
- The net products of Anaerobic Glycolysis are **2 ATP**, **ZERO NADH**, and **2 Lactate molecules.**

Pyruvate reduction to lactate

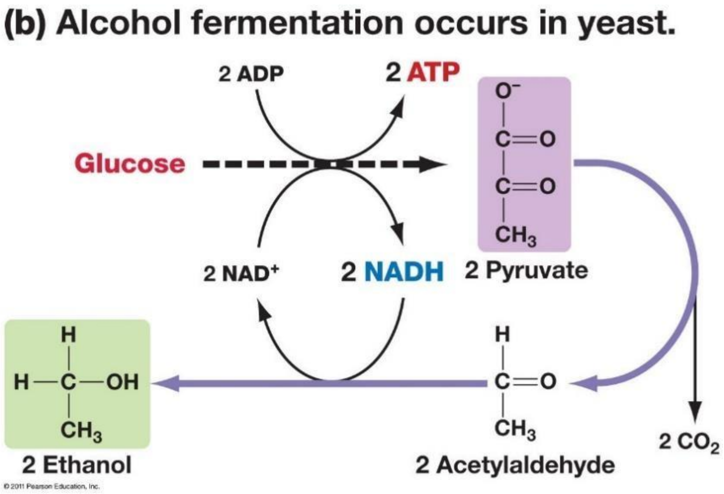


- The enzyme that catalyzes this reaction is **Lactate dehydrogenase (LDH).**
- Pyruvate is reduced to lactate, and NADH converted to NAD+ (available for glycolysis to continue)
- **LDH** is used for the diagnosis of **Myocardial infarction.**
- **Lactate** (حامض اللبن) is the final product of anaerobic glycolysis in **eukaryotic cells.**
- Lactate is the major fate of pyruvate in **poorly vascularized tissues** and **RBCs.**
- 2 NADH produced by aerobic condition, but in anerobic conditions is zero.

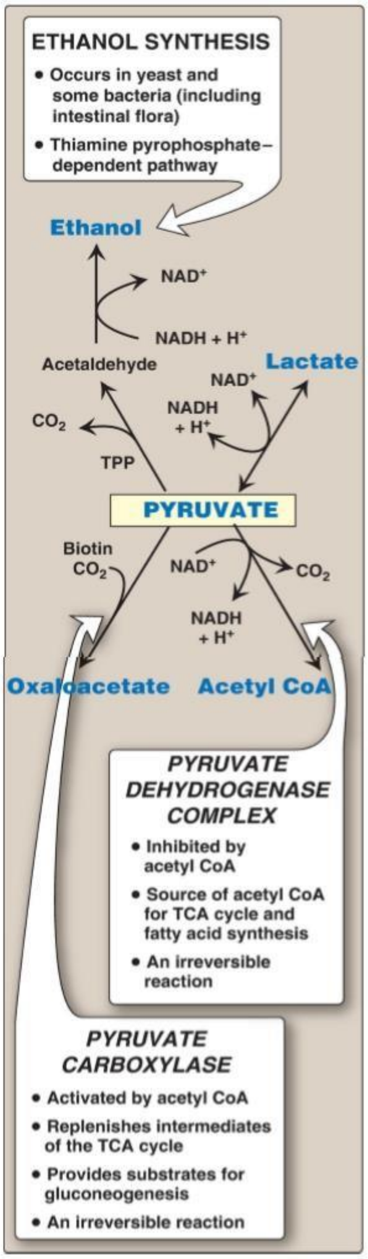
Fermentation



- It's another way to reoxidise **NADH** to **NAD$^+$**.
- This reaction is catalyzed by **2 enzymes**
  - ✓ **Pyruvate decarboxylase** (converts Pyruvate to Acetaldehyde)
  - ✓ **Alcohol dehydrogenase** (converts Acetaldehyde to Ethanol)
- This reaction occurs in two steps
  - ➢ **First** reaction is catalyzed by Pyruvate decarboxylase in which Pyruvate **is decarboxylated** into Acetaldehyde with the **release of CO2**.
  - ➢ **Second** reaction is catalyzed by Alcohol dehydrogenase in which **NADH** reduces Acetaldehyde to Ethanol (primary alcohol).



- This reaction doesn't take place in humans, but it happens in yeast and some bacteria, and this is why we use yeast in bread making to make dough rise (because of the presence of CO2 ''Gas''). Also, it happens in the making of wine and vinegar.



Fate of pyruvate

- **Pyruvate** has several fates, we are already discussed more than one of them
  - ✓ **Pyruvate to Acetyl CoA**
  - ✓ **Pyruvate** to **Lactate (in muscle, cornea and etc..)**
  - ✓ **Pyruvate** to **Ethanol**
  - ✓ **Pyruvate** to **Oxaloacetate** (Note: **Oxaloacetate** can be converted to **Aspartate**)
  - ✓ **Pyruvate to Alanine** (amino acid)
- So, Glycolysis has other routes other than providing energy. It also provides building blocks for making variety of substances.

Lactate production

- ➢ **Cells with low energy demand**, like **RBCs (there's no synthesis or contraction)**, only for Na+/Ka+ atpase.
- ➢ **To cope increased energy demand** in rigorously exercising muscle, like in a case of running, escaping. Which lead to the increase of the lactate 5 to 10 folds.

- **Hypoxia** in the case of collapse circulatory system, stop of the heart beat (to survive brief episodes of hypoxia), and that will lead to Lactic Acidosis.

Lactic Acidosis

- **Decrease PH in the plasma**, so Lactic Acidosis can be treated by bicarbonate (that will correct the PH in the plasma).
- **The most common cause of metabolic acidosis is**
  - Increase the production of lactic acid.
  - Decrease the utilization of lactic acid.

  $$\text{Pyruvate} + \text{NADH} \rightleftharpoons \text{Lactate} + \text{NAD}^+$$

  - High lactate, oxygen debt.
  - Lactate will be absolutely back to pyruvate.
- **Most common causes**: impairment of oxidative metabolism due to collapse of circulatory system.
- Impaired O2 transport
- Respiratory failure
- Uncontrolled hemorrhage

- **Direct inhibition of oxidative phosphorylation.**
- **Hypoxia in any tissue.**
- **Alcohol intoxication (high NADH/NAD$^+$),** which will lead to **inhibition** and **the stop of**
  - Glycolysis
  - Gluconeogenesis
  - Pyruvate Dehydrogenase
  - TCA cycle activity
  - Pyruvate carboxylase

[Note: the first step in using alcohol as a sores of energy in the body is oxidize alcohol by **Alcohol Dehydrogenase,** that converts **NAD$^+$** to **NADH**, then when NADH will be high, as a result, stopping of pyruvate hydrogenase, TCA cycle activity, and etc.

خلصنا البارت الأول وخدلك بريك وكمل
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