# Glycolysis

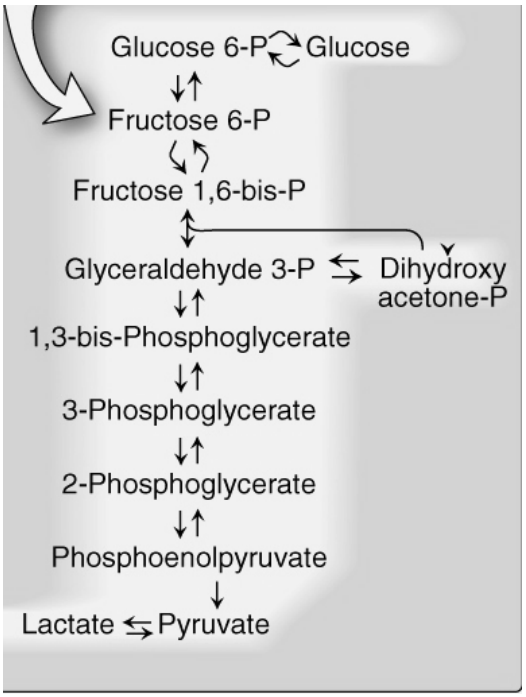
## Introduction to Metabolism

## Regulation of Metabolism

## Overview of Glycolysis

## Reactions of Glycolysis

Suggested Reading: Lippincott's Illustrated reviews: Biochemistry

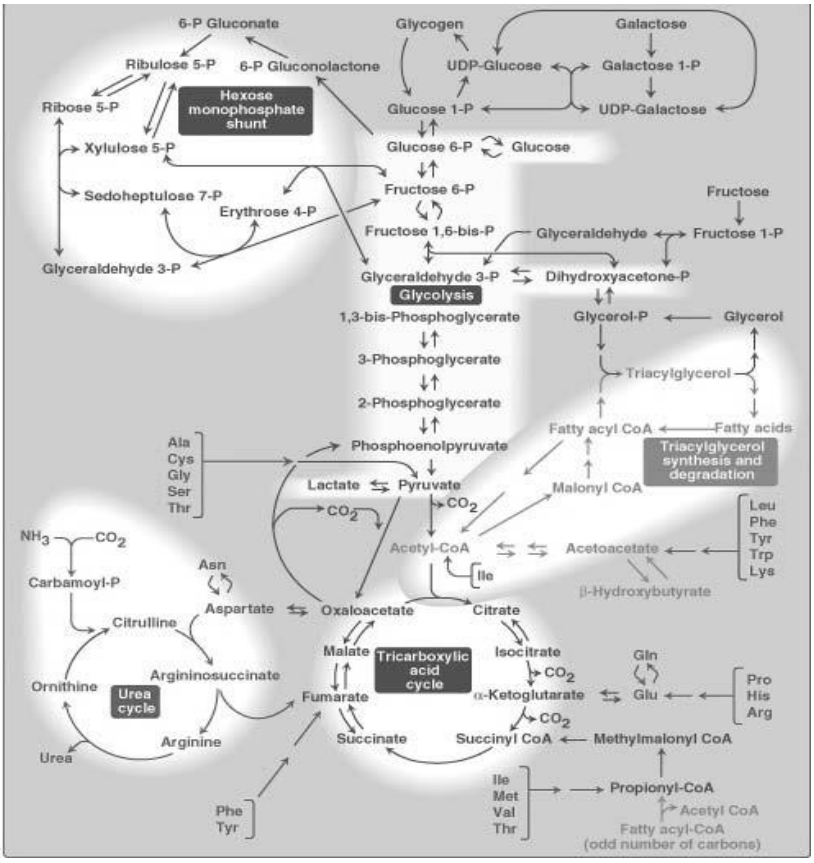



Glycolysis, an example of metabolic pathway

The product of one reaction is the substrate of the next reaction

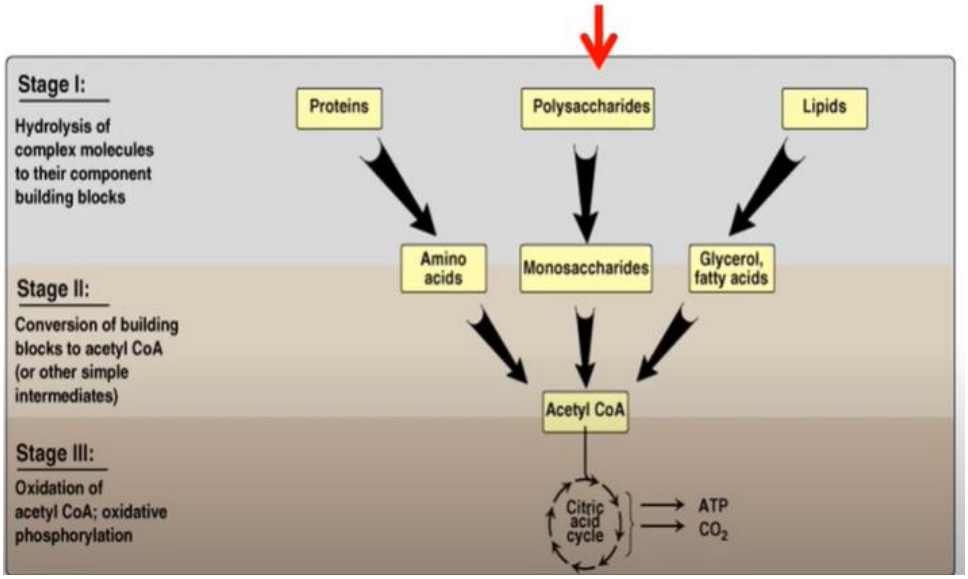
## What was mentioned in the lecture:

Glycolysis is an example of metabolic pathway, this is (in the figure) the metabolic pathway of glycolysis, As you can see, it starts from glucose that is going to be converted into glucose 6-phosphate, then fructose 6-phosphate…. down all the way to pyruvate, and pyruvate can be in equilibrium with lactate, So this is a metabolic pathway, It is a central pathway, It is a pathway that it is suggested to spend time on it and studying it very well, It is important to understand this pathway because it is a classical pathway of metabolism, a Pathway is a sequence of consecutive reactions where the product of one reaction is the substrate of the following reaction, For example, glucose is converted to glucose 6-phosphate in one reaction, in the second reaction glucose 6-phosphate is the substrate, it will be converted to fructose 6-phosphate then in the next reaction, fructose 6-phosphate will the substance, So the product of a reaction is the reactant of the next step, The total number of reactions in this pathway is 10 reactions.



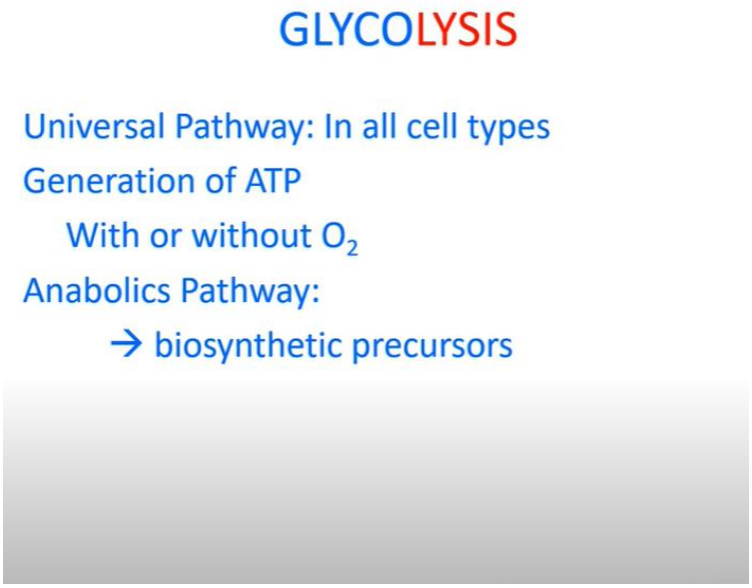

## What was mentioned in the lecture:

metabolism actually consists from many metabolic pathways, this slide is a complicated slide, but If you look carefully through it, there are some shaded areas, each shaded area is a pathway, the metabolism consists of all the metabolic pathways that occur in the cell or in the organism, these pathway intersect to form a network of chemical reactions, but this chemical reactions do not occur in isolation from each other, they are affected by each other and they are regulated So as the metabolic pathway that is active is the one that is useful for the organism or for the cell.




## What was mentioned in the lecture:

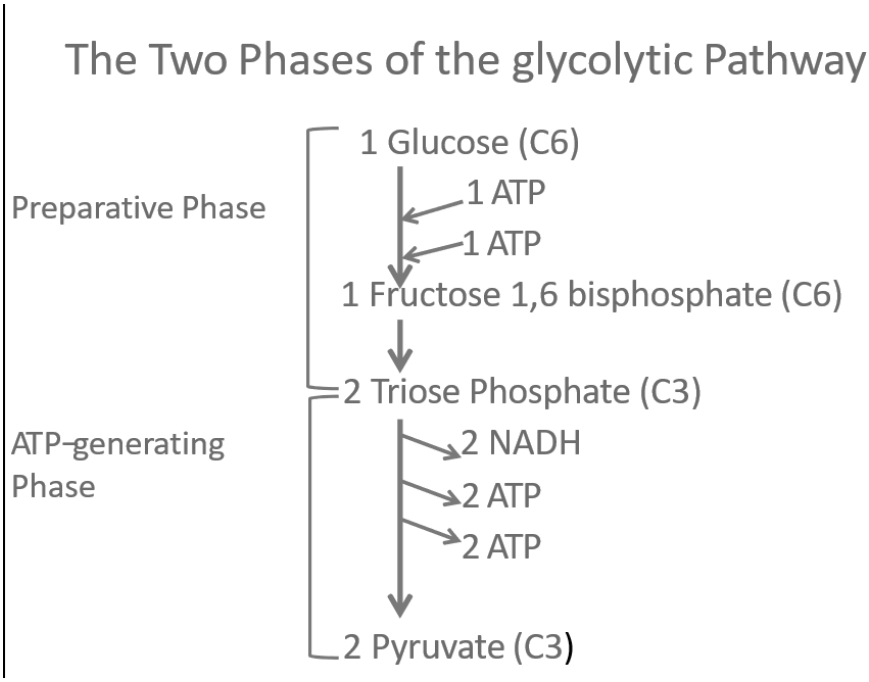
utilizing nutrients from the food to produce energy occur in three stages, in stage one, the nutrients in the food are converted into small molecules (building blocks), in our case, carbohydrates, polysaccharides or disaccharides are converted into monosaccharides in stage one, in stage two, monosaccharides in our case are converted into a smaller molecule (acetyl-CoA) that is used in the third stage for getting energy in the citric acid cycle, form now on, We'll discuss conversion of the monosaccharides into the acetyl CoA and other smaller molecules.




## What was mentioned in the lecture:

Glycolysis is defined as a universal pathway, it is found in all cell types, the sequence of reactions of glycolysis is the same in all cell types, whether you are talking about liver cells, white blood cells, bacteria, plant cells, etcetera, glycolysis is found in all kinds of cells, the first role of the glycolysis is generation of energy in the form of ATP, ATP is required by the living cells for various functions, and glycolysis is one of the most important pathways for the generation of energy in the form of ATP, being universal, glycolysis

can occur with or without $O_2$, glycolysis can also be viewed as an anabolic pathway, because some intermediates that are produced during glycolysis are used for the biosynthesis.




## What was mentioned in the lecture:

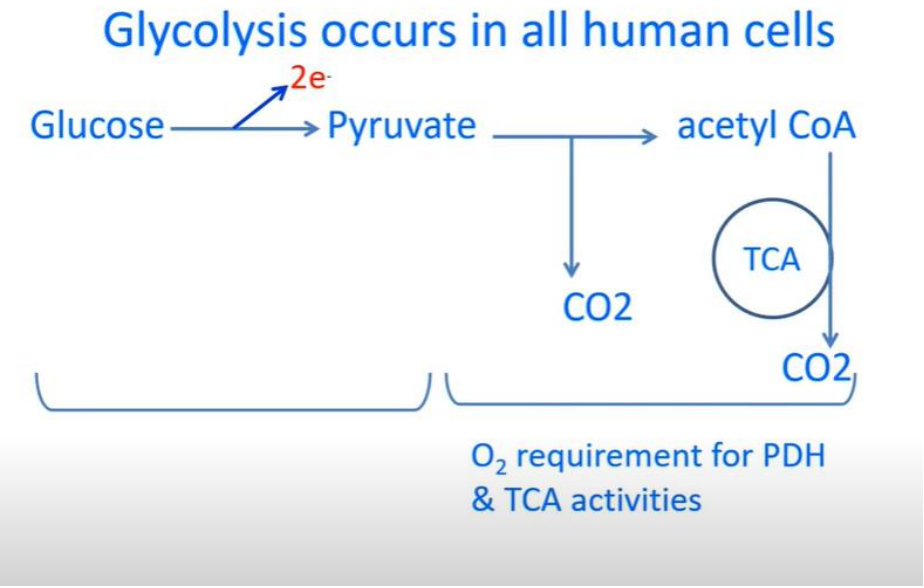
This is a general overview of glycolysis, we start with glucose that is formed from six carbons, and you can divide the pathway into two phases, the first phase is called the preparative phase, in which the glucose is phosphorylated then converted to fructose which is then cleaved into two smaller molecules each with three carbons, notice that in the preparative phase, ATP is consumed, two ATPs are used when glucose is converted to fructose 1,6-bisphosphate even though the idea of the glycolysis is to produce ATP, but in the preparative phase ATP is consumed rather than being produced, in the second phase, the products of the cleavage of fructose 1,6-bisphosphate are converted into pyruvate, a small- molecule ketoacid, the second phase is the ATP generating phase, this is where the ATP is produced, in this ATP-generating phase, four ATPs are produced, while in the first phase, two ATPs are consumed, So the net yield is two ATPs produced Whenever glucose is converted to two molecules of pyruvate, to make it easy for you to understand: the glucose is the beginning, pyruvate is the end product, and all the intermediates of glycolysis are attached to one or two phosphates, all the intermediate have six-carbon or three-carbon molecules, there's no five-carbon, four-carbon or two-carbon molecules, glycolysis occur in the cytoplasm of both prokaryotes and eukaryotes.

# Types of Glycolytic Reactions

- Phosphoryl transfer
- Isomerization
- Cleavage
- Oxidation reduction
- Phosphoryl shift
- dehydration

## What was mentioned in the lecture:

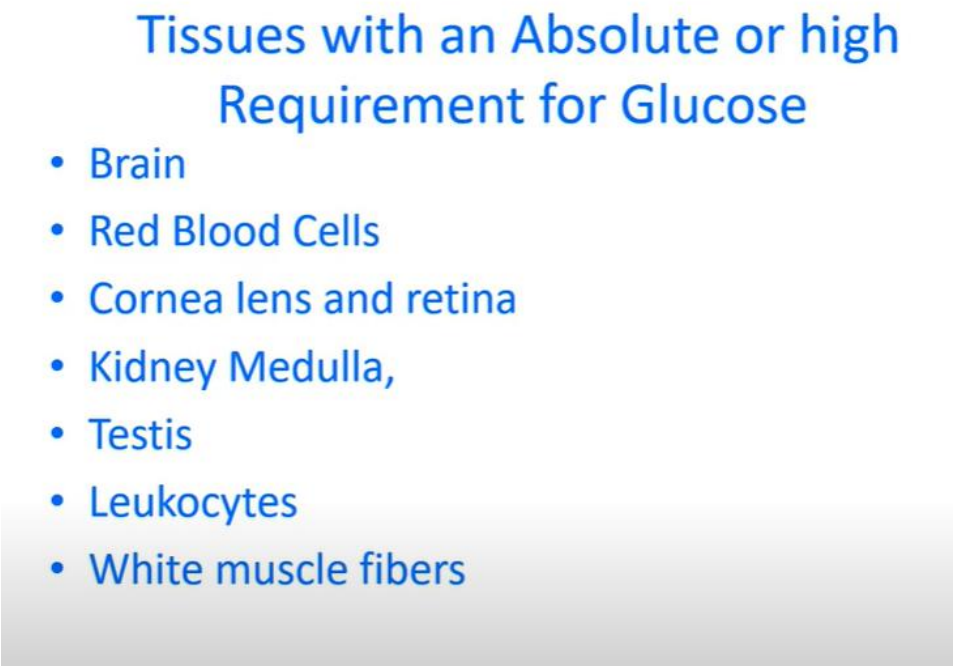
there are six types of glycolytic reactions, the first type is Phosphoryl transfer where phosphate group is transferred from ATP to produce ADP or transferred to ADP to produce ATP, so it is transfer of phosphate group from one molecule to another, from ATP to the intermediate or from the intermediate to ADP producing ATP, the second type is isomerization, isomerization will change the structure of the molecule without changing the number of carbons, hydrogens, oxygens or phosphorus, but converting one isomer to the other, isomerization is catalyzed by enzymes usually known as isomerases, the third type is cleavage where a C-C (carbon-carbon) bond is cleaved and two molecules of trioses are produced, in this case the bond that connects carbon #3 to carbon #4 is cleaved, the fourth type is oxidation-reduction reaction, Then phosphoryl shift, in which a phosphate group is transferred within the same molecule from one carbon to the other, and lastly, dehydration where water is removed from the intermediate, So knowing these six reaction types, help you to understand how the glycolysis occur in details.




## What was mentioned in the lecture:

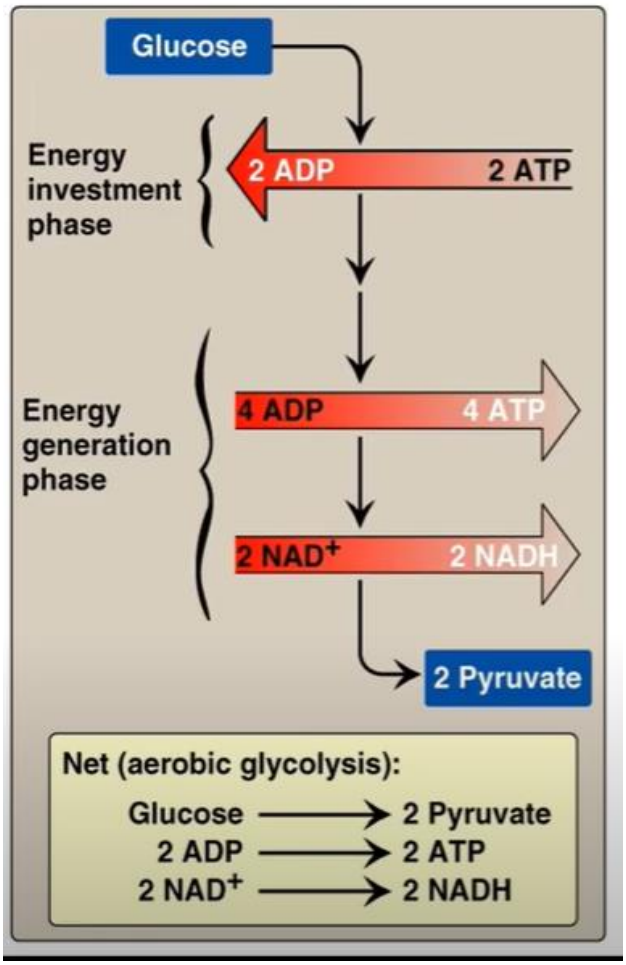
glycolysis occur in all human cells, whether liver cells, red blood cells that do not contain mitochondria or nuclei, etc. in all cells glucose is converted firstly to pyruvate, what differs is the fate of pyruvate, under aerobic conditions, pyruvate can continue to be converted to acetyl CoA by decarboxylation, this happens under aerobic conditions where oxygen is available and can be utilized, but if oxygen is not available, pyruvate is reduced to lactate, oxygen is not required for glycolysis, these are called anaerobic conditions

(without oxygen), anaerobic conditions,  glycolysis occur in all of human cells and its function is to produce ATP.



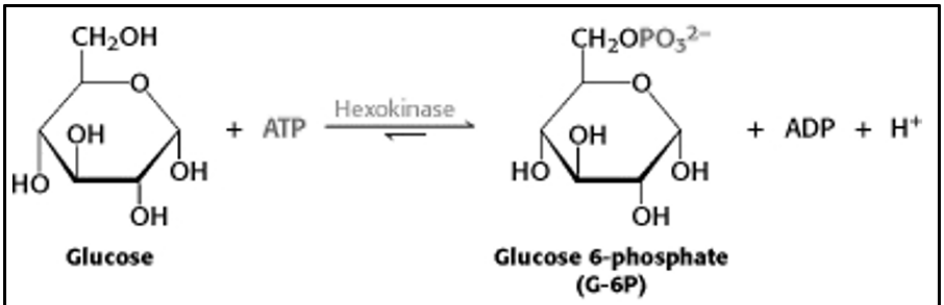

## What was mentioned in the lecture:

those are examples on tissues that require a high amount or an absolute amount of glucose (therefore they depend on glycolysis), brain requires glucose for production of energy and for biosynthesis using glycolysis's intermediates, it is absolutely dependent on glucose, red blood cells absolutely require glucose and not anything else, Brain can sometimes use ketone bodies as a source of energy, but red the blood cells require glucose and only glucose, simply because they do not contain mitochondria, therefore, any metabolic pathway that occurs in the mitochondria (like TCA cycle or electron transport chain) cannot occur in the red blood cells, among other glucose dependent tissues are the cornea قرنيّة, lens عدسة  and the retina شبكية of the eye, these require glucose because simply The cornea and the lens do not blood vessels (they must be not vascularized to be translucent), so oxygen cannot reach easily to these tissues, kidney medulla, testis, leukocytes also are glucose dependent, white muscle fibers depend on glycolysis for energy production, in contrast with red muscle fibers which use aerobic metabolism, ( remember from the histology course with Dr. Heba, Muscle fibers can be white or red and the white muscle fibers usually depends more on the glycolysis.




## What was mentioned in the lecture:

this figure also summarizes glycolysis, firstly we have an energy investment phase in which two molecules of ATP are converted to ADP, but in the energy generation phase four molecules of ATP are produced and 2 NADH molecules are produced by reduction of two $NAD^+$ molecules, and the end product is two molecules of pyruvate, that is the case with aerobic glycolysis, in anaerobic glycolysis, the end product is lactate which is the reduced from of pyruvate, and we'll not have any net yield of NADH, because they'll be used to reduce pyruvate to lactate and therefore will be oxidized again to $NAD^+$.




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

## What was mentioned in the lecture:

Now let's start with the first reaction of glycolysis, the first reaction of glycolysis is addition of a phosphate group to glucose converting it to glucose 6-phosphate (a glucose molecule whose carbon #6 is attached to a phosphate group), the bond that connects the phosphate group with glucose' carbon #6 is a phosphoester bond, The source of phosphate in this reaction cannot be an inorganic phosphate because this reaction is exergonic, therefore, the phosphate group should come from a high energy phosphate donor which is ATP, ATP donates a phosphate to glucose to produce glucose 6-phosphate, and ATP in this case is converted to ADP. What do we call the enzyme that catalyzes this reaction? It is hexokinase (remember: any enzyme that transfers phosphate groups is called kinase preceded by the name of the substrate, if it is glucose, then glucokinase or hexokinase, if glycerol, then glycerol kinase, if a protein, then protein kinase and so on ), this reaction is generally irreversible ( as you can see in the figure, the forward arrow is longer than the backward arrow), what is the importance of this first step? without a phosphate, glucose can diffuse easily through the membrane, for example, glucose can enter and leave the liver based on the concentration of glucose in the blood, if the concentration of glucose in the blood is decreased, Glucose can get out from the liver cells, but if glucose in the blood is high, it can enter into the liver cells, but once glucose is phosphorylated, it cannot leave the cells anymore, why? Notice that the phosphate group has two negative charges and it is bulky, glucose is already very polar molecule, but it becomes even more polar with two negative charges, It cannot penetrate the plasma membrane even through the GLUT, so it becomes trapped within the cell, and once it is phosphorylated, that has to go through the metabolism. there is more than one pathway that involves glucose 6-phosphate. (the purpose of this reaction is to prevent glucose to leak out of cell through GLUT which is specific for glucose but not glucose 6-phosphate, this reaction is required in the beginning of any pathway that utilizes glucose)

The enzyme is called hexokinase, there are many isoforms of hexokinase, four isoforms have been identified which are hexokinase I, II, III, and IV, hexokinases I, II and II are very similar, but IV is different, it is known as glucokinase, what are the differences between them? Always remember that f you have isoforms (isozymes) of the same enzyme, then usually each one has different function, they catalyze the same reaction, but they are regulated in different ways, they have different kinetics (different $K_m$ and

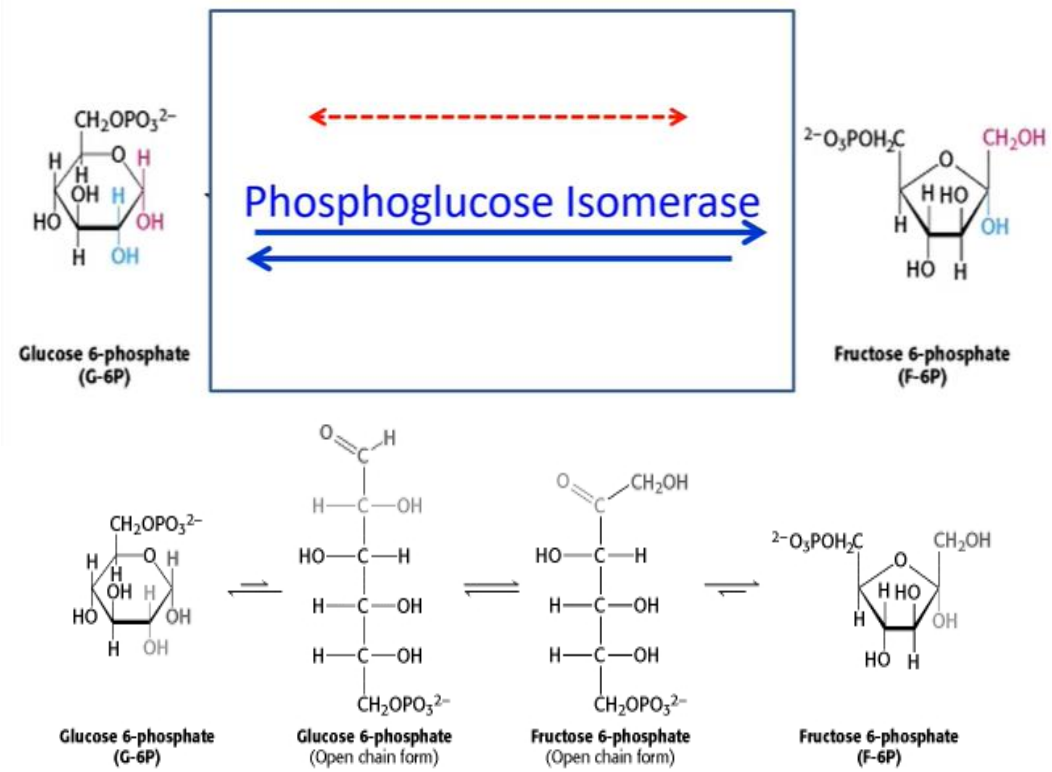
different $V_{MAX}$) and they usually have different distribution between tissues because each tissue has different or specific physiological function.

Regarding specificity, hexokinase (as the name implies hexokinase = hexose kinase) can use glucose, fructose, mannose or galactose, these four sugars can be phosphorylated by hexokinase, Whereas for glucokinase, the substrate is only glucose, so the specificity is different, Hexokinase has wide specificity whereas the glucokinase has very narrow specificity, they also differ in their $K_m$, $K_m$ refers to the affinity of the enzyme towards its substrate, ( how strong the binding or how easy the binding of the substrate to the enzyme) $K_m$ for hexokinase is less than 0.02 millimolar, while for glucokinase $K_m$ is 10 to 20 millimolar. which one has higher affinity? it the hexokinase, because as you remember from the biochemistry course, $K_m$ equals the rate of the disassociation of the substrate from the enzymes over the rate the binding, it also means the substrate concentration needed to reach half of the Vmax, therefore more $K_m$ means less affinity and vice versa. In another words, less $K_m$ means the less substrate concentration we need to reach half of the Vmax.

Glucokinase is found in the liver whereas hexokinase is found in all tissues, therefore the liver must carry a different function because it contains a different isoform with lower affinity but high specificity, hexokinase cannot be induced (activated), while glucokinase can be activated by insulin and glucose, insulin stimulates synthesis of glucokinase by liver cells, glucokinase functions only if the concentration of glucose is high, while hexokinase can function at any concentration of glucose, let's link these things with each other.

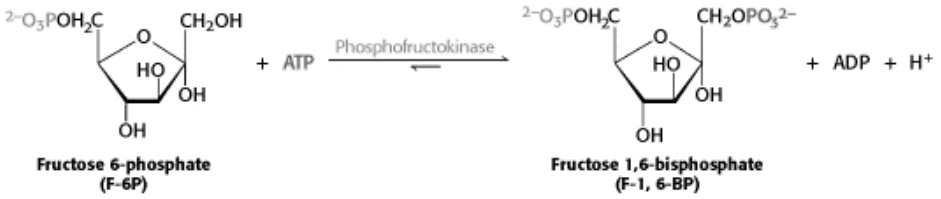
glucose is taken by the liver when the glucose concentration is high in order to store it as glycogen, or -after glycogen- to convert it into fatty acids, carbohydrates can be converted into fatty acid if eaten excessively, so the liver functions to uptake glucose when it is abundant, Whereas other tissues need glucose , even if the concentration for glucose is low, for example, the brain should be able to take the glucose even if the concentration of glucose is very low, it has absolute dependence on glucose, Similarly, RBCs require glucose at any concentration, but the liver takes glucose only if the concentration of the glucose is high, in this case, it will take it converted to glycogen or fatty acids, this is the idea behind having two different isoforms.

This reaction is irreversible, It has large negative $\Delta G$, therefore it should be highly regulated, if glucose is converted to glucose 6-phosphate, it cannot go back, it is the regulated by its own product, glucose 6-phosphate inhibits the enzyme, the idea behind that is that if we leave the reaction not regulated, the concentration of glucose 6-phosphate becomes very high in the cell, but in way that exceeds the cell demands, that will consume the ATP, therefore it has to be regulated for being an irreversible reaction.



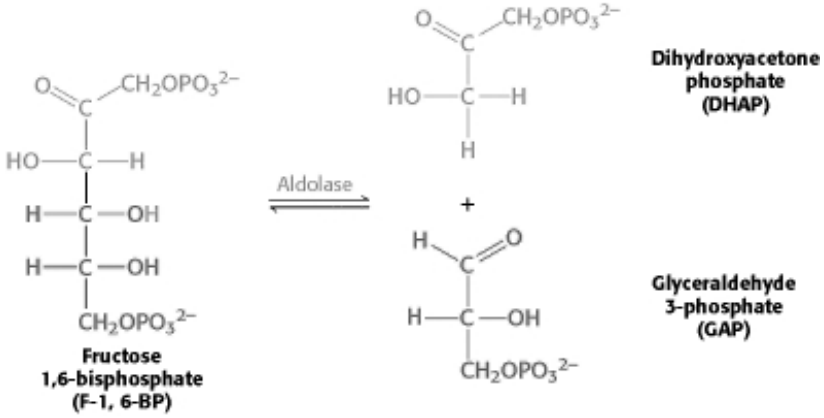

## What was mentioned in the lecture:

The next step is conversion of glucose 6-phosphate into fructose 6-phosphate, this is an isomerization reaction  the enzyme is called phosphoglucose isomerase (ant isomerization reaction is carried by an isomerase and to distinguish isomerases from each other we proceed the name with the substrate's name), note that this reaction is easily reversible, if glucose 6-phosphate concentration raises the reaction will go into the forward direction but if fructose 6-phosphate concentration is high the reaction will go into the reverse direction, to understand how this reaction occur take a look at the structures of glucose and fructose in open chain formula, what is the difference between a glucose 6- phosphate and fructose 6- phosphate? the difference is in carbon #1 and #2, in glucose carbon #1 has a carbonyl group while carbon #2 has a hydroxyl group while in fructose it is the opposite, the rest of the two molecules are the same, carbons #3, #4, #5, and #6 are not altered in this reaction, this is an easy action, it can even occur slowly in alkaline conditions without catalysts or enzymes. (in the lab)



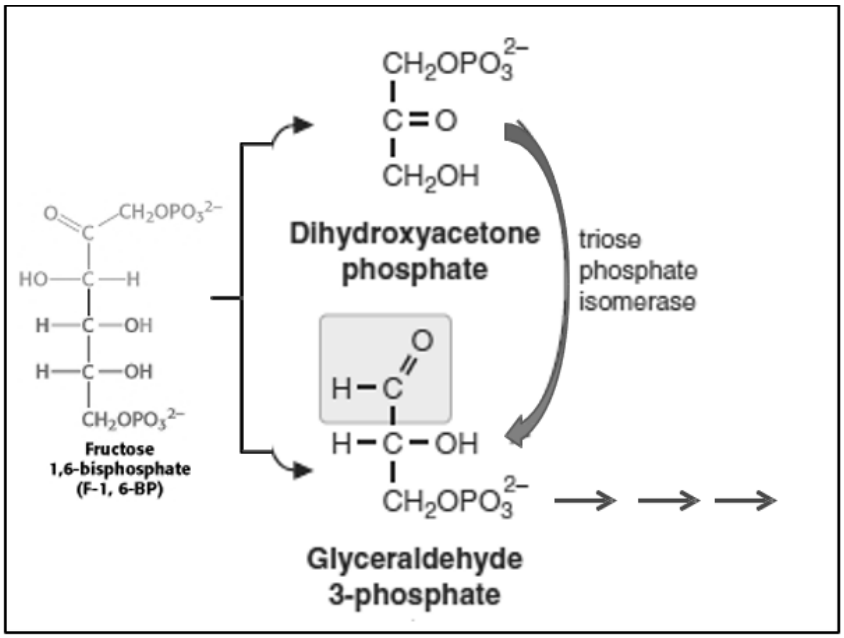

## What was mentioned in the lecture:

The next reaction is addition of a phosphate group to fructose 6-phosphate converting it to fructose 1,6-bisphosphate, again here, the source of phosphate group is ATP and the enzyme is a kinase, named after the substrate phosphofructokinase (PFK), why do we call the product fructose 1,6-bisphosphate and not diphosphate? Diphosphate means that the two phosphates are connected to each other (like in an ATP molecule), while bisphosphates means that the phosphates are attached to two different atoms, this reaction is irreversible, once fructose 6-phosphate is phosphorylated to fructose 1,6-bisphosphate, the product can be used only for glycolysis, therefore, this step is called the committed step, because fructose 1,6-bisphosphate is committed to glycolysis and not any other pathway, Therefore, this step has to be strictly regulated, phosphofructokinase is a regulated enzyme, and by regulating this enzyme, you are almost regulating the rate of the whole process of glycolysis.



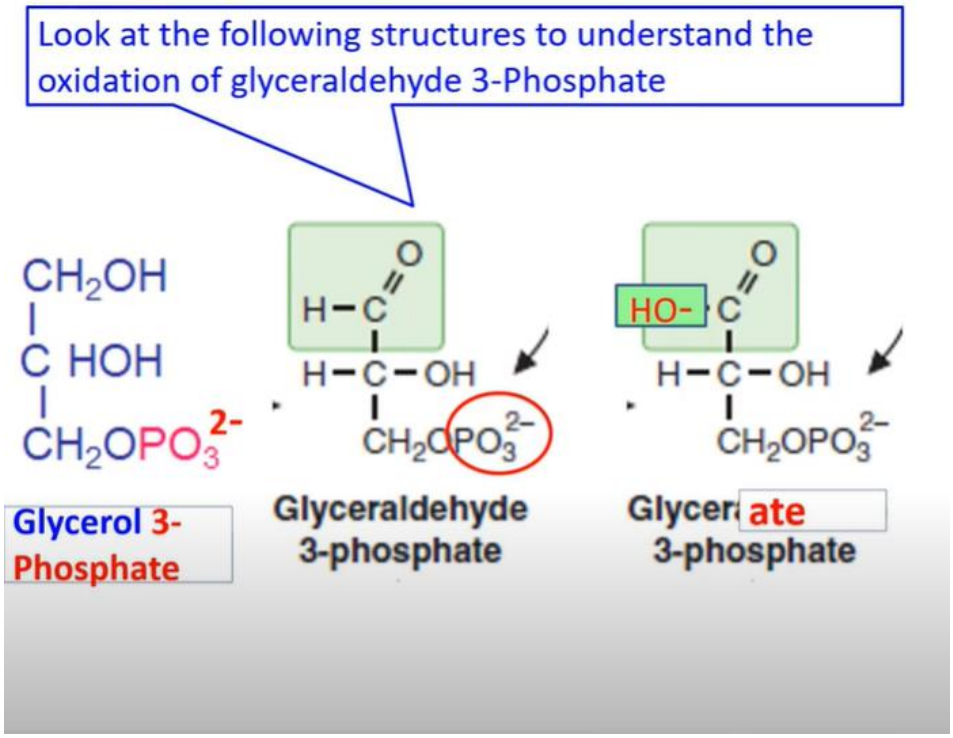

## What was mentioned in the lecture:

The next step is cleavage of the fructose 1,6-bisphosphate, this cleavage occurs between carbon #3 and carbon #4, when this bond is cleaved, that gives two small molecules, the first three carbons (carbon #1, #2 and #3) form dihydroxyacetone phosphate, while carbons #4, #5, #6 form glyceraldehyde 3-phosphate, because cleavage occurs between a carbon that bears a hydroxyl group and a carbon which has carbonyl group next to it (a β beta hydroxy compound), this is called aldol cleavage, from that, the enzyme has got its name which is aldolase, this reaction is reversible.




## What was mentioned in the lecture:

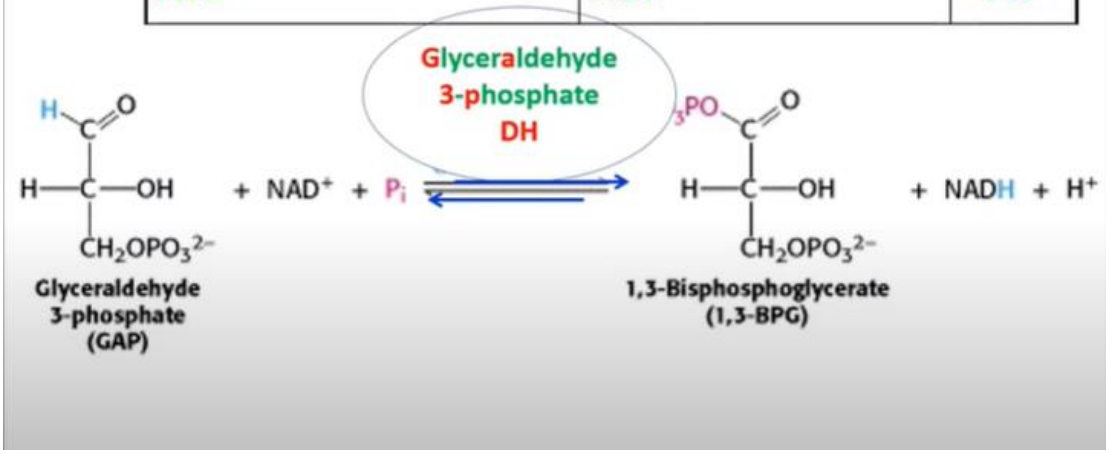
The two triose products of cleave can be converted to each other (they're interchangeable), dihydroxyacetone phosphate (the ketotriose) can be converted to the aldotriose which is glyceraldehyde 3-phosphate and vice versa, this conversion is isomerization, and it is catalyzed by an enzyme called triose phosphate isomerase ("triose phosphate" the substrate and "isomerase" the reaction), glyceraldehyde 3-phosphate is the one that's going to be directly used in the next reaction, while dihydroxyacetone phosphate will be continuously converted to glyceraldehyde 3-phosphate by the isomerase, continuous consuming of glyceraldehyde 3-phosphate by the subsequent reactions will shift the equilibrium of the isomerization reaction toward producing more glyceraldehyde 3-phosphate (Le Châtelier's principle), so it like the net reaction is conversion of fructose 1,6-bisphosphate to two molecules of glyceraldehyde 3-phosphate. Form now on, think of each reaction as if it was doubled.




## What was mentioned in the lecture:

This is to help you memorize and understand the structures, glyceraldehyde is an aldotriose with an aldehyde group on carbon #1 and two hydroxyl groups at carbons #2 and #3, glyceraldehyde 3-phosphate has a phosphate group on carbon #3 attached though a phosphoester bond, what is on the left is glycerol 3-phosphate which is the reduced form of glyceraldehyde 3-phosphate (remember from OXPHOS lecture: glyceraldehyde 3-phosphate and glycerol 3-phosphate are used as a mitochondrial shuttling system), remember that oxidation reactions convert hydroxyl groups to carbonyl (aldehyde or ketone) groups while reduction reactions do the opposite thing, if you had further oxidized the aldehyde group on carbon#1 of glyceraldehyde 3-phosphate, you'll end with glycerate (glyceric acid) 3-phosphate with a carboxyl group, the next reaction is oxidation of glyceraldehyde 3-phosphate.

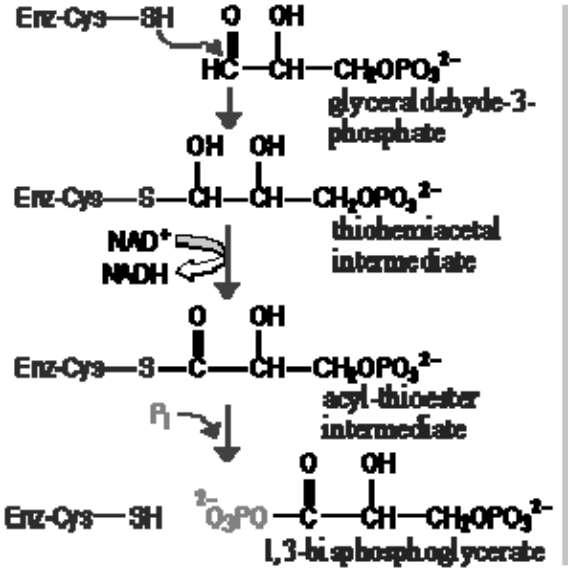
| Compound +$H_2O$ | Product + phosphate | ΔG° |
|---|---|---|
| Phosphoenol pyruvate | Pyruvate | -14.8 |
| 1,3 bisphosphoglycerate | 3 phosphoglycerate | -11.8 |
| Creatine phosphate | Creatine | - 10.3 |
| **ATP** | **ADP** | **- 7.3** |




## What was mentioned in the lecture:

This reaction is oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate, this reaction includes oxidation and adding of a phosphate group, always oxidation is accompanied with reduction, therefore, if glyceraldehyde 3-phosphate is oxidized, what is reduced? It is $NAD^+$, $NAD^+$ is reduced to NADH, always, if there is an oxidation-reduction reaction and the electron acceptor is $NAD^+$, $NADP^+$ or FAD, we call the enzyme dehydrogenase, therefore the enzyme that catalyzes this reaction is called glyceraldehyde 3-phosphate dehydrogenase, it removes hydrogen from the substrate and give it to $NAD^+$.

The table above shows $\Delta G^o$ of hydrolysis of phosphate groups in some compounds (we've seen this before in bioenergetics lecture), hydrolysis of 1,3-bisphosphoglycerate' phosphate group into 3-phosphoglycerate has a $\Delta G^O$ of -11.8 Kcal, that is a lot of energy, even more than conversion ATP to ADP, therefore, 1,3-bisphosphoglycerate can donate its phosphate group to ADP to form ATP, as we'll see in the next reaction




Mechanism of the reaction:

E and S form covalent linkage

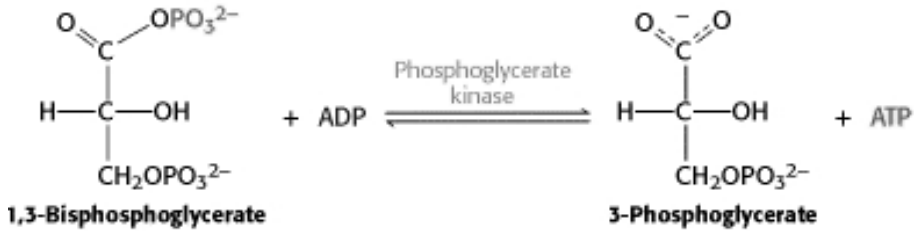
S is oxidized and NADH is formed

NADH is released

$P_i$ attacks the thioester bond releasing the product

## What was mentioned in the lecture:

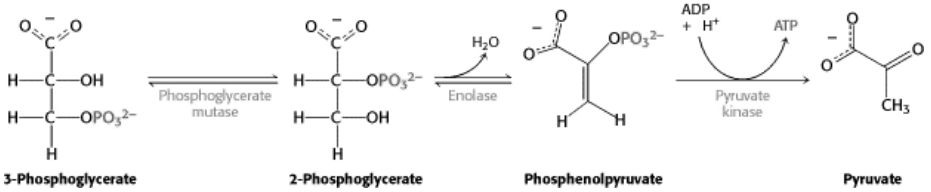
Let's talk briefly about the mechanism of the reaction of oxidation of glyceraldehyde 3-phosphate, glyceraldehyde 3-phosphate dehydrogenase has cysteine residues with thiol group in its active site, -SH groups, just like -OH groups, can react to form ester of hemiacetal groups, the thiol groups reacts with the aldehyde group of glyceraldehyde 3-phosphate forming a thiohemiacetal intermediate, then, the thiohemiacetal group is oxidized to a thioester group by oxidizing the -OH group into an =O group (see the figure), thioester groups (like ones formed by Coenzyme A), has a high energy (cleavage of thioester bonds is highly exergonic), so far, the enzyme is still covalently bound to the substrate, this covalent bond can be cleaved by addition of an inorganic phosphate (notice: the source here is not ATP, this reaction is called phosphorolysis rather than hydrolysis), then the glycerate is release as 1,3-bisphosphoglycerate and the enzymes is back to its original form, this is enzyme is very interesting, it allows only inorganic phosphates to attack the thioester bond, if it allows water to attack this bond, 3-phosphoglycerate will be released and we won't be able to synthesize ATP in the next reaction as we'll see later.




## What was mentioned in the lecture:

in this reaction, 1,3-bisphosphoglycerate will donate a phosphate group to ADP forming ATP and 3-phosphoglycerate, this reaction is reversible under cellular conditions although the hydrolysis of 1,3-bisphosphate is highly exergonic (1,3-bisphosphoglycerate has a high potential for phosphoryl transfer, the bond that connects the phosphate to carbon #1 is a high energy anhydride bond ), because the reaction is reversible (unlike the other two phosphoryl transfer reactions in this pathway (G→G6P and F6P→FBP) which are irreversible), the enzyme is called glycerate kinase (also phosphoglycerate kinase) because it can phosphorylate glycerate in the opposite direction.

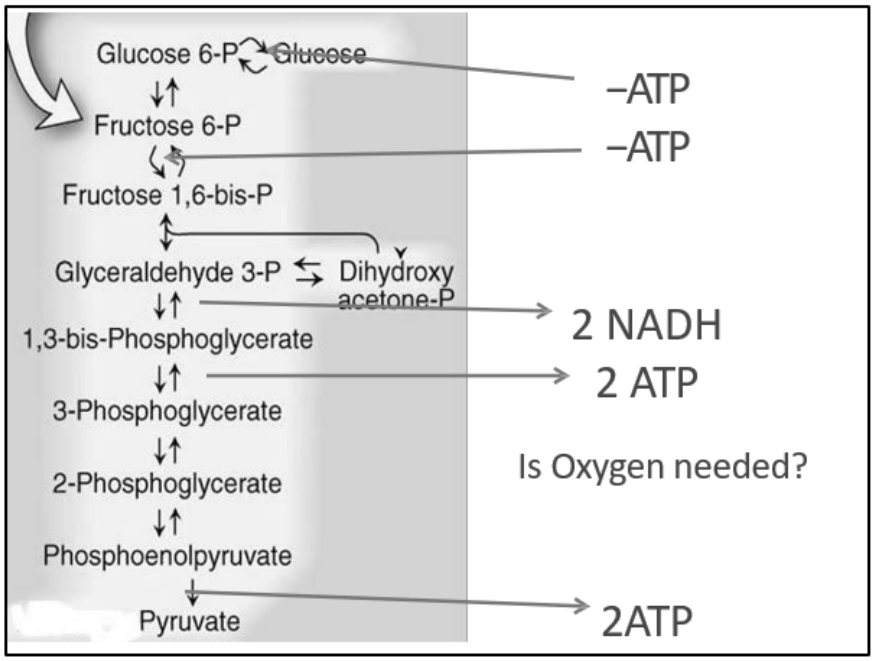
The $\Delta G^{o}$ for this rection is –4.5 kcal (see the table in page 14, 1,3-bisphosphoglycerate → 3-phosphoglycerate has a $\Delta G^{o}$ of -11.8 kcal while ADP → ATP has a $\Delta G^{o}$ of +7.3 kcal (because it is the reverse of ATP → ADP so we just change the sign) and the sum of these is -4.5 kcal)




## What was mentioned in the lecture:

Those are the remaining three reaction that will convert 3-phosphoglycarte into pyruvate, let's see how this happens, firstly, 3-phosphoglycerate is converted to 2-phosphoglycerate by a phosphoryl shift reaction in which a phosphate group is shifted from carbon#3 to carbon #2, the enzyme here is called phosphoglycerate mutase (remember: mutases are isomerases), in the next reaction 2-phosphoglycerate is dehydrated by enolase which is a lyase, resulting in formation of a double bond between carbon #2 and carbon #3, the product is called phosphoenolpyruvate, phosphoenolpyruvate is a high energy compound, and in the last reaction, phosphoenolpyruvate acts as a donor for the phosphate group, it donates it to ADP forming ATP and becomes pyruvate, this is the last reaction and it is irreversible because phosphoenolpyruvate is a high energy molecules, the last enzyme is pyruvate kinase.

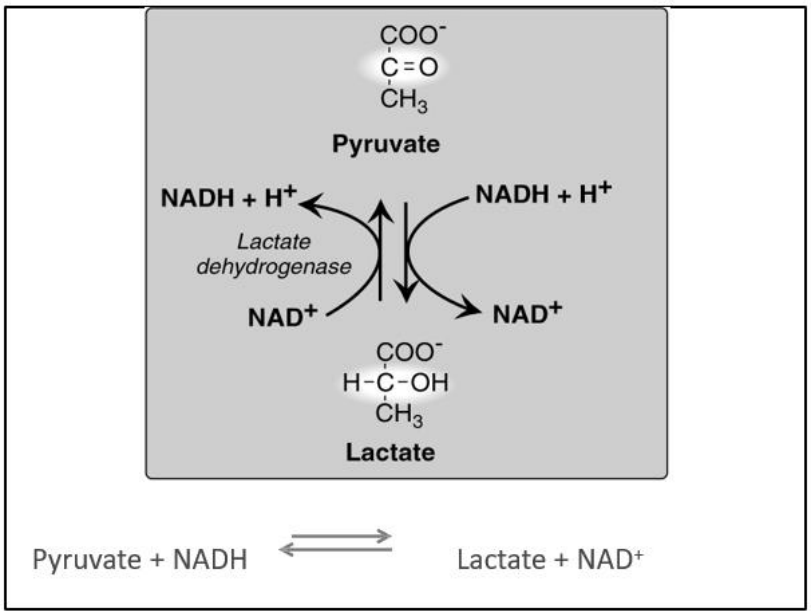
In the last reaction that is catalyzed by pyruvate kinase, a phosphate group is transferred from phosphoenolpyruvate to ADP forming pyruvate and ATP, $\Delta G^{o}$ for this reaction (we calculate by the same way as in the previous slide) is -7.5 kcal, this is a lot a energy isn't it? Therefore, this reaction is irreversible and must be strictly regulated.




## What was mentioned in the lecture:

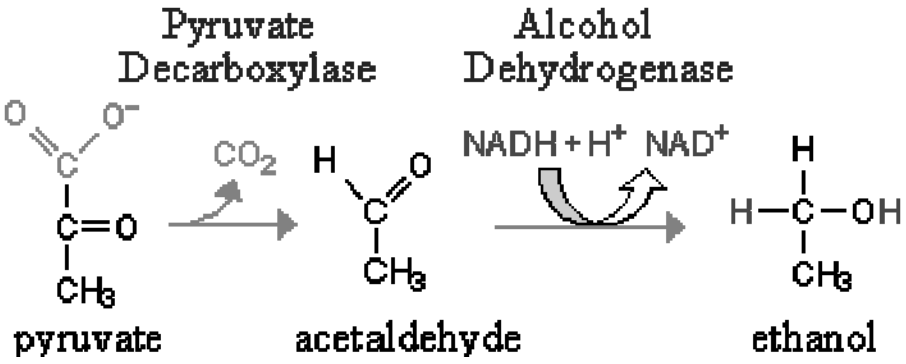
this is an overview of the whole pathway, the first step consumes one ATP, the third step consumes another ATP, in the oxidation reduction step (oxidation of glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate) two NADH molecules are produced, in the transfer of a phosphate group from 1,3-bisphosphoglycerate, two ATP molecules are produced ( because actually one glucose molecule produces two molecules of 1,3-bisphosphoglycerate), another two are formed by the transfer of a phosphate group from phosphoenolpyruvate, the net ATP production is two molecules for each glucose molecule, we have produced 4 and consumed 2, along with 2 NADH molecules that can be further oxidized in the electron transport chain in the case of aerobic conditions.

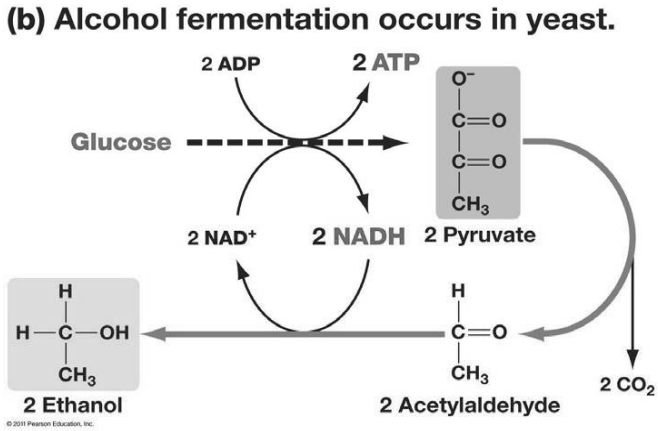
Is oxygen ($O_2$) needed in glycolysis? Yes and no, yes because NADH was produced in this process, and a cell has very limited amount of $NAD^+$ (the amount of glucose that enter the cell is much larger than that of $NAD^+$), it is coenzyme, it is like glycolysis consumes $NAD^+$, and therefore glycolysis cannot continue until we compensate the lost $NAD^+$ (for glycolysis to continue, any NADH produced must be oxidized back to $NAD^+$), and for that, we need oxygen to convert NADH to $NAD^+$ back in the electron transport chain, that is the case in aerobic conditions, where pyruvate will be converted to acetyl-CoA and enter the mitochondria to enter TCA cycle and then electron transport chain, what about anaerobic conditions?




## What was mentioned in the lecture:

When oxygen is not available or not needed (like in anaerobic conditions or if the cell is originally anaerobic), pyruvate will be reduced to lactate, NADH will donate the electrons to pyruvate converting it into lactate, and NADH will be converted into $NAD^+$. Therefore, if the end product is lactate rather than pyruvate, $NAD^+$ will be compensated and glycolysis can continue, therefore, oxygen can be not needed because NADH can be oxidized in a different way, this reaction is reversible, and it is catalyzed by lactate dehydrogenase enzyme, lactate dehydrogenase is a very important enzyme in the body, it is even used in diagnosis of myocardial infarction (remember this form the biochemistry course in the last semester), lactic acid is abundant in yoghurt.

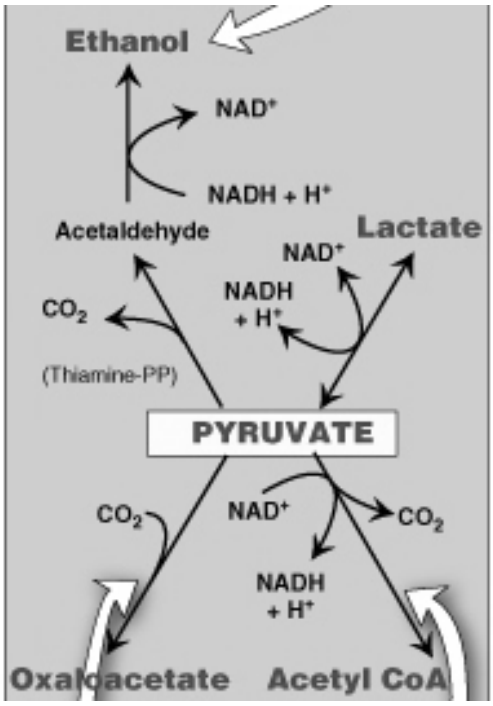



## What was mentioned in the lecture:

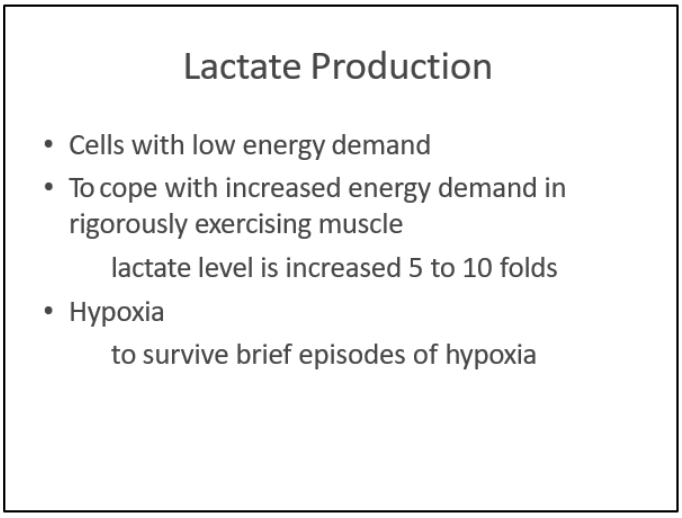
When glycolysis happens in anaerobic organisms such as certain species of bacteria and yeast, in certain yeast species, pyruvate can be decarboxylated into acetaldehyde (ethanal), which can be reduced into ethanol by NADH as a reducing agent, this is another way by which $NAD^+$ is compensated, in this case, glycolysis ends with ethanol, and oxygen is not needed, this process is called alcoholic fermentation, two enzymes are required in this process which are pyruvate decarboxylase and alcohol dehydrogenase.

In the process of alcoholic fermentation, the product of the first reaction is $CO_2$ in addition to acetaldehyde, this reaction is used in production of $CO_2$ when we make bread, when we add yeast with some sugar into the dough, it raises because of production of $CO_2$, alcoholic fermentation doesn't take place in human body, but lactic fermentation does.




## What was mentioned in the lecture:

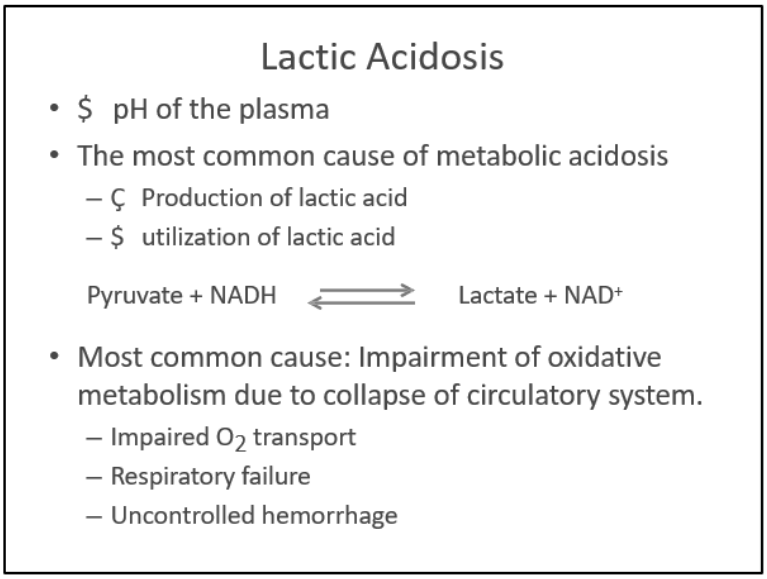
This figure depicts the different fates of pyruvate, it can be decarboxylated to acetaldehyde which can be further reduced into ethanol as occurs in bacteria and yeast, pyruvate can also be reduced to lactate, as happens is muscular tissue during exercise, lactate also occurs if the amount of oxygen is inadequate, or if cells cannot use oxygen like if they do not have mitochondria (as RBCs), but if oxygen is adequate and the cell has mitochondria, pyruvate will be converted to acetyl CoA, in this conversion, $CO_2$ is produced, another $NAD^+$ is converted to NADH, and acetyl CoA will enter the TCA cycle, pyruvate can be also carboxylated to oxaloacetate.




## What was mentioned in the lecture:

When does lactate is produced in our body? It is produced in cells with low energy demand such as RBCs, RBCs have very low energy demand because they do not strenuous functions such as contraction or synthesis, they use ATP just for the $Na^+$-$k^+$ pump to keep the plasma membrane intact, also lactate is

produced in muscle fibers to cope with increased energy demand in rigorously exercising muscle, meaning that is white muscle fiber which depend on anaerobic glycolysis for energy because it is fast, lactate will be produced in large amounts (it increase 5 to 10 folds than normal) when an overload is put on the muscle, lactate is a dead end, it cannot be used in cells which do not possess mitochondria, therefore it is released into plasma in that case, cells also produce lactate when they want to survive brief episodes of hypoxia, meaning that, if oxygen demands were decreased, maybe by a collapse in the circulation or because decrease in arterial pressure, lactate is produced, in that case, glycolysis keep taking place in those cell in order to keep making some ATP (2 ATP better than nothing), complete conversion of a molecule of glucose into $CO_2$ and $H_2O$ (by glycolysis, TCA cycle and ETC) yields about 30 ATP molecules, but conversion of glucose to two molecules of lactate yields only 2 molecules of ATP.




## What was mentioned in the lecture:

Overproduction of lactic acid and releasing it into plasma causes a condition known as lactic acidosis, lactic acidosis leads to a decrease in blood plasma's pH, lactic acidosis is considered metabolic acidosis (remember from the biochemistry course: we have two types of acidosis: respiratory and metabolic), lactic acidosis is the most common cause of acidosis, lactic acidosis can be caused by overproduction of lactic acid or decreased utilization of lactic acid, in normal conditions, lactate is carried to the liver, where it will be oxidized back to pyruvate, (remember: the concentration of substances determine whether the reaction will go in the forward direction or the reverse direction), over production of lactate in presence of oxygen to shift the equilibrium of the reaction toward production of more pyruvate, the most common cause of lactic acidosis is impairment of oxidative metabolism (like oxidative phosphorylation) due to collapse of the circulatory system, impaired $O_2$ transport, respiratory failure and uncontrolled hemorrhage الله يجيرنا , for example, people who have COVID19 face difficulties in respiration, and therefore they'' have over production of lactic acid.

## Lactic Acidosis

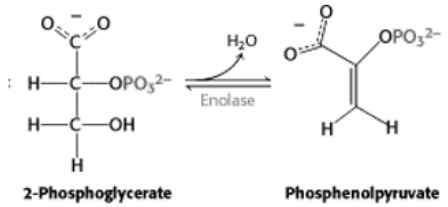
- Direct inhibition of oxidative phosphorylation
- Hypoxia in any tissue
- Alcohol intoxication ( high NADH/ NAD+ )
- $ Gluconeogenesis
- $ Pyruvate Dehydorgenase
- $ TCA cycle activity
- $ Pyruvate carboxylase

## What was mentioned in the lecture:

Lactic acidosis happens by direct inhibition of oxidative phosphorylation, hypoxia of any tissue (these two can be caused by the reasons we mentioned in the previous slide), and alcohol intoxication, the first step in metabolism of alcohol is using of $NAD^+$ to oxidize ethanol to acetaldehyde in the liver, converting $NAD^+$ to NADH, high ratio of NADH to $NAD^+$ causes impaired gluconeogenesis, because with more NADH the equilibrium of the reaction (pyruvate + NADH ⇄ $NAD^+$+ lactate) will be shifted toward producing of more lactate, that also causes decreasing in pyruvate dehydrogenase, pyruvate carboxylase and TCA cycle activities, because pyruvate is continuously being converted to lactate.

## Inorganic Inhibitors of Glycolysis
## Fluoride

- Fluoride inhibits Enolase
-



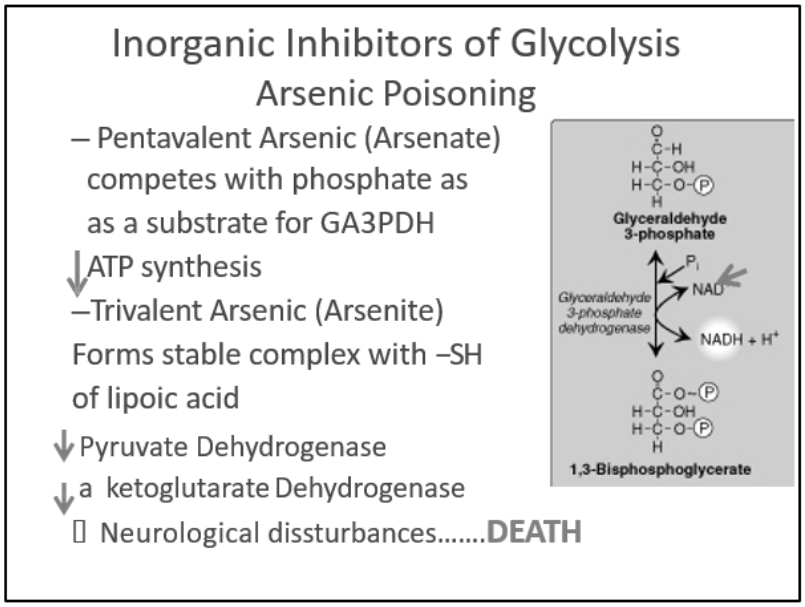

Fluoridated water ↓ bacterial enolase

Prevention of Dental Carries

## What was mentioned in the lecture:

Glycolysis can be inhibited by inorganic substances, that includes fluoride which inhibits enolase (the lyase that converts 2-phosphoglycerate into phosphoenolpyruvate), if you inhibit any enzyme in the pathway of

glycolysis, all enzymes before it also will be inhibited, fluoride is added to water and tooth paste to inhibit bacterial enolase, that will kill oral cavity's bacteria and prevent dental carries تسوّس الأسنان.




## What was mentioned in the lecture:

Other inorganic inhibitors of glycolysis include arsenic, arsenic can exist in two forms: pentavalent arsenic (arsenate ($AsO_4^{-3}$)) and trivalent arsenic (arsenite ($AsO_3^{-3}$)), arsenate is a competitive inhibitor for glyceraldehyde 3-phosphate dehydrogenase, it competes with phosphate group that is involved in phosphorolysis, producing 1-arseno-3-phosphoglycerate, then the arsenate group of this enzyme will be directly hydrolyzed. And we'll end up with 3-phosphoglycerate, so we won't get 1,3-bisphosphoglycerate and we'll not get ATP synthesis, glycolysis will become not producing any net ATP, because the step that makes ATP is lost

In the second case which is arsenite, (we've already discussed that in TCA cycle), arsenite irreversibly binds lipoic acid ( a coenzyme), decreasing the activity of pyruvate and α-ketoglutarate dehydrogenase, causing a decrease in energy production, leading to neurological disturbances and then death.

## Pyruvate Kinase Deficiency

- The most common among glycolytic enzyme deficiencies
- RBC's 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

### What was mentioned in the lecture:

Pyruvate kinase is the last enzyme of glycolysis, the deficiency of this enzyme affects glycolysis, pyruvate kinase deficiency is very rare, but is relatively common compared to other deficiencies of glycolytic enzymes, RBCs are the most affected cell type in this case, because there are unable to synthesize this enzyme (they do not posses nuclei, they cannot carry translation, but in the liver for example, the cell can synthesize more pyruvate kinase), deficiency of this enzyme cause mild to severe chronic hemolytic anemia, because RBCs' ability to produce ATP is now decreased, therefore, $Na^+$-$K^+$ pump function is affected and the osmotic pressure in the membrane of RBCs is disrupted causing hemolysis (premature death of RBCs), also abnormal function of $Na^+$-$K^+$ pump will cause abnormality in the flexible shape of RBCs, therefore, they'll be unable to go through capillaries , pyruvate kinase deficiency doesn't necessarily mean that the enzyme is completely absent, but the enzyme is abnormal (maybe because problems in folding, if it was completely absent there is no life),

# Regulation of Metabolism

### What was mentioned in the slide:

- Signals from within the cell
  - Substrate availability, product inhibition, allosteric
  - Rapid response, moment to moment
- Communication between cells (intercellular)
  - Slower response, longer range integration
- Second messenger
  - $Ca^{2+}$ / phosphatidylinositol system
  - Adenylyl cyclase system

### What was mentioned in the lecture: