12

Sheet Slides

Done By

Rawan almujaibel

Contributed in the Scientific Correction

Ayman Musleh

Contributed in the Grammatical Correction

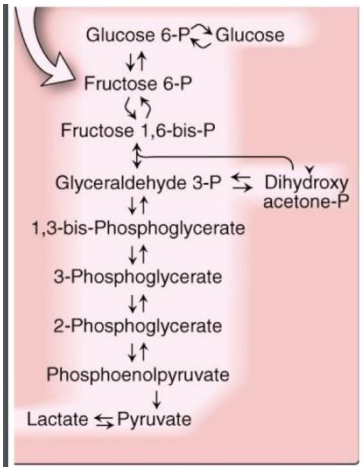
Ayman Musleh

Doctor

Dr. Nayef

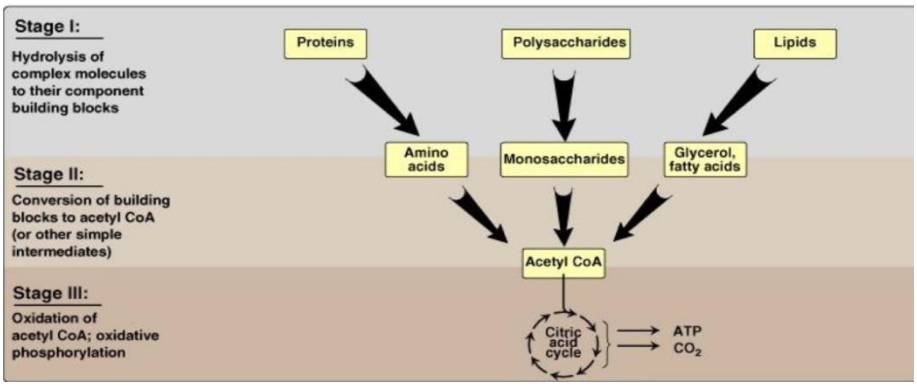
In the previous lecture we talked about digestion and absorption of carbohydrates. In this lecture we will be talking about glycolysis.

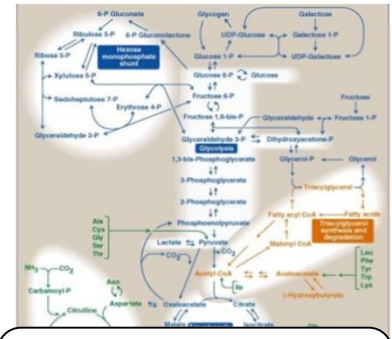
Glycolysis



This picture shows the metabolic pathway of the glycolysis from glucose to pyruvate or lactate. The product of one reaction is the substrate of the next reaction.

As you can see here in the 2nd picture, it shows the glycolysis pathway that is in between (central position) of the whole pathways of all macromolecules.



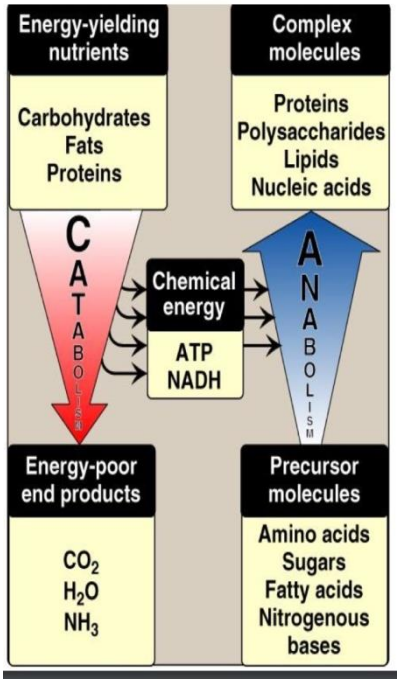


Metabolic pathways intersect to form network of chemical reactions

At first, we have to go through some concepts:

General Regulatory Aspects in Metabolism:

Metabolic pathways involve the building up (Anabolism) and breaking down of molecules(Catabolism).



1. Catabolic pathway: is serve to capture chemical energy in the form of *ATP* from *degradation* of energy-rich fuel molecules (Carbohydrates, Fats, and Proteins), to produce energy-poor end products, it is *a convergent process* (wide variety of molecules → transformed into a few common end products).

2. Anabolic: is a diverge process in which a few biosynthesis precursors (such as amino acids, sugars, fatty acids, nitrogenous bases) form a wide variety of polymeric, or complex products such as proteins, polysaccharides, Lipids, and Nucleic acids. Anabolic reactions require energy (are endergonic), which is generally provided by the hydrolysis of

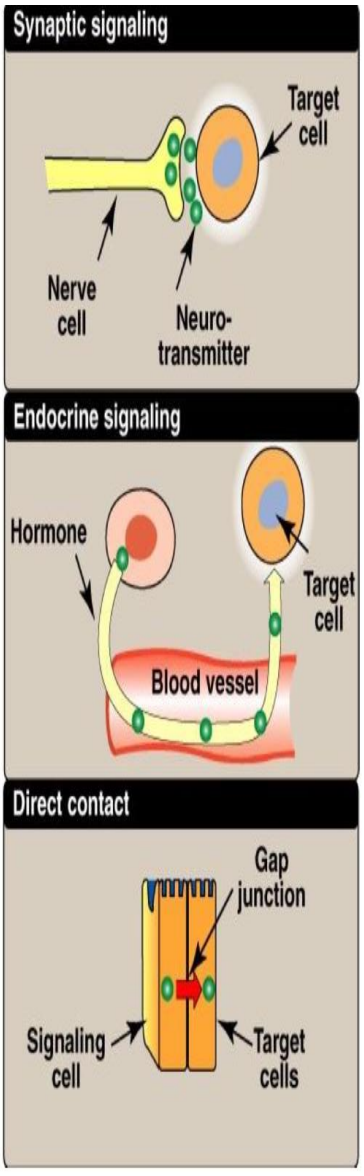
ATP to adenosine diphosphate (ADP) and inorganic phosphate (Pi). Anabolic reactions often involve chemical reductions in which the reducing power is most frequently provided by the electron donor (NADPH).

Regulation of Metabolism

Cell is under a very intelligent regulation; the production of energy or the synthesis of end products meets the needs of the cell, and this is controlled by some sort of regulatory signals. There are different types of regulatory signals in the body:

1. Signals within the cell (intracellular signaling):

The rate of a metabolic pathway can respond to regulatory signals that arise from the cell. They are considered the fastest signals (happens within seconds and they are very rapid). For example, the rate of a pathway may be influenced by the:



A. **Availability of substrates**

B. **Product inhibition**

C. Alterations in the levels of **allosteric activators or inhibitors**.

These intracellular signals typically elicit rapid responses and are important for the moment-to-moment regulation of metabolism.

2. Communication between cells (intercellular signaling):

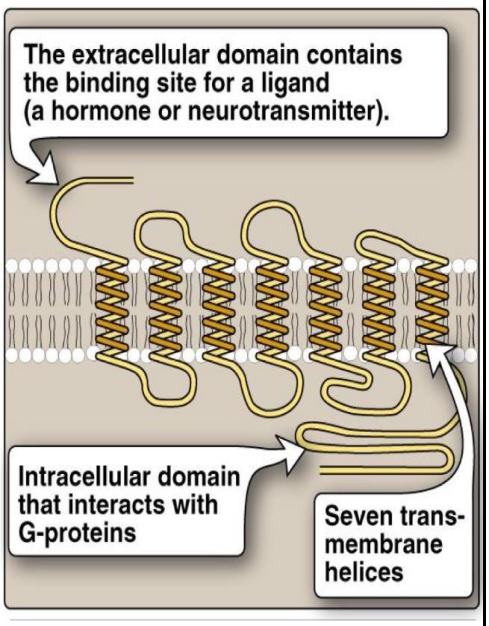
The ability to respond to intercellular signals is essential for the development and survival of organisms. Signaling between cells provides for long-range integration of metabolism and usually results in a response. For example, endocrine glands secrete hormones (neurotransmitters) that travel in the blood and go to one cell or more target cells. For energy metabolism, the most important route of communication is the chemical signaling between cells by bloodborne hormones or by neurotransmitter.

intercellular signaling can occur between adjacent and non-adjacent cells.

3. Second messenger systems:

So named because they intervene between original messenger (the neurotransmitter or hormone) and the ultimate effect on the cell. They are part of the cascade of events that converts (transduces) neurotransmitter or hormone binding into a cellular response. Two of the most widely recognized second messenger systems are:

- **Ca2+ / phosphatidylinositol system**.
- **Adenylyl cyclase system.**



Both involve in the binding of ligands to specific G protein-coupled receptors (GPCR) on the cell membrane.

As you can see in the picture, (GPCR) receptor has 3 domains:

1. An extracellular domain that contains the binding site for a ligand which is a hormone or neurotransmitter.

2. trans-membrane (seven helices) domain.

3. intracellular domain that interacts with G-protein.

Neurotransmitters/hormones bind on the extracellular domain, and then specific signals are transmitted trough out the trans-membrane domain. And finally, the intracellular domain interacts with a specific GPCR.

The commonly used mechanism for these 2 systems is:

The communication that happens between the cells, for example, neurotransmitters are secreted from nerve cells (sympathetic signaling), or hormones are secreted from an endocrine gland into the blood to the target cells (endocrine signaling), or even from direct contact by neighboring cells with gap junction between themselves that allows material to exchange between cells.

In the Carbohydrates metabolism we encounter more with **Adenylyl cyclase system.** Ca2+/ phosphatidylinositol system will be discussed later as it is used in the Lipid and Carbohydrates metabolism as well**.**

- **Adenylyl Cyclase system:**The recognition of a chemical signal by some G-protein family, such as *β*- and *α_2-adrenergic receptor* receptors, triggers either an increase or decrease in the activity of adenylyl cyclase (AC). How? This occurs in two steps:

1. The activation of Guanosine triphosphate-dependent regulatory proteins.

2. The activation of cAMP dependent protein kinases.

- ***Guanosine triphosphate-dependent regulatory proteins:***

1- G-proteins are composed of (α, β, and γ subunits). In the inactive form of G-protein, the α subunit is bound to GDP.

2- Ligand binding causes a conformational change in the receptor, triggering replacement of this GDP with GTP.

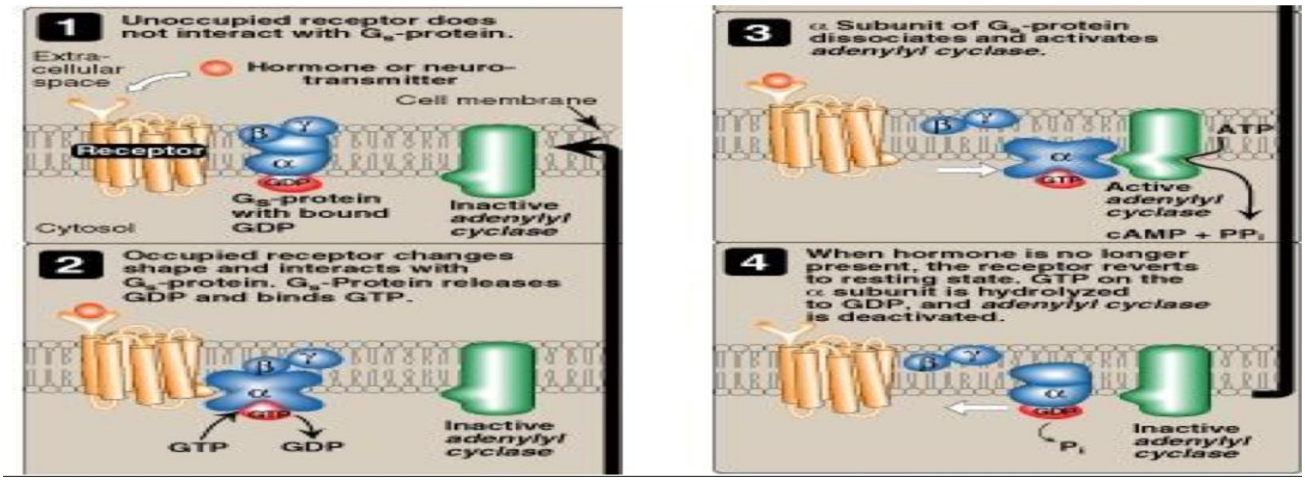
3- The GTP-bound form of the α subunit dissociates from the $\beta\gamma$ subunits and moves to the inactive adenylyl cyclase (AC) affecting the enzyme activity in cell membrane.

4- Then, by this binding of Gα proteins to AC, the cAMP is produced using the ATP and as the ligand is still binding to the receptor the production of cAMP is continued.

(The α subunit has GTPase activity which converts GTP to GDP rapidly).

5- When the ligand is no longer present, the receptor reverts to the resting state, GTP on the α subunit is hydrolyzed to GDP, the α *subunit* rejoins $\beta\gamma$ subunits, and adenylyl cyclase (AC) is deactivated.

(So we have to know that cAMP is the second messenger for hormones or neurotransmitters)



NOTE from the DR:

There is an enzyme hydrolyzes cAMP to 5'-AMP which is called phospho-di-esterase. The 5'-AMP functions are different from cAMP functions. **There are different compounds of (caffeine) that are present in the coffee which help us to stay awake. Have you ever asked yourself how does that happen?

These compounds in the coffee inhibit the phosphor-di-esterase and prolong the action of the cAMP.

The activation of cAMP dependent protein kinases (PKA):

1. cAMP activates PKA by binding with its two regulatory subunits, causing the release of its two catalytically active subunits.

2. These subunits transfer phosphate from ATP to specific serine or threonine residues of protein substrates >>> *In other words, they phosphorylate proteins*.

The phosphorylated proteins may:

1. Act directly on the cell's ion channels.

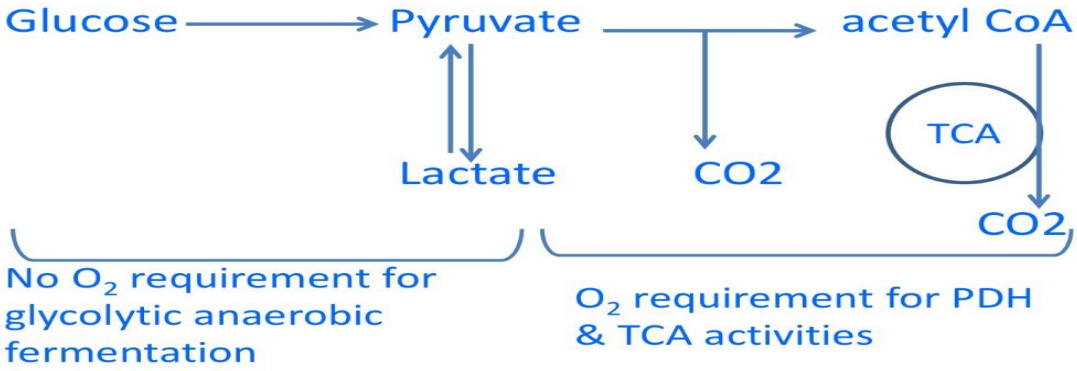
2. If enzymes, they may become activated or inhibited.

3. They also may bind to certain promoters.

When we have enough amounts of phosphorylated proteins, the system will be reversed to get balanced, by an enzyme *called protein phosphatase*.

Glycolysis

The glycolytic pathway: is a pathway used by all tissues for the oxidation of glucose to provide energy (as ATP) and intermediates for other metabolic pathways. In other words, it is a universal pathway used by all cell types and by all organisms (aerobic and anaerobic) in order to generate ATP and provides the precursors for anabolic pathways; for example, some amino acids that are produced from its intermediates.



Glycolysis can be divided into 2 main phases:

1. Preparative phase (energy-investment phase): in which phosphorylated forms of intermediates are synthesized using 2 ATP molecules.

2. ATP generating phase (energy-generation phase): This phase includes *2 NADH molecules and 4 ATP molecules production*. *Forming a net of 2 molecules of ATP by substrate-level phosphorylation.*

Aerobic glycolysis: is a series of ten reactions in which the *pyruvate* is the end product, it occurs in cells with mitochondria and an adequate supply of O2.

Aerobic glycolysis sets the stage for the *oxidative decarboxylation* of pyruvate to acetyl CoA, a major fuel of the TCA cycle.

Anaerobic glycolysis: is the conversion of glucose to lactate, in which the pyruvate is reduced to lactate as NADH is oxidized to NAD+. it occurs without the participation of O2.

Anaerobic glycolysis allows the production of ATP in tissues that lack mitochondria or in the cells deprived of sufficient O2.

There are lots of tissues with an absolute of high requirement for glucose:

Such as: Brain, Red blood cells, Cornea lens and retina, Kidney, Medulla, Testis, Leukocytes and White muscles fibers.

Glycolysis reaction:

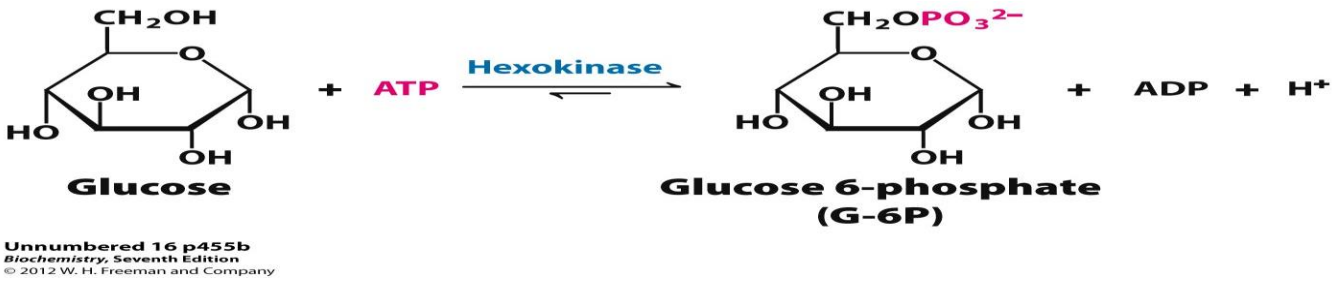
1. Glucose phosphorylation: Glucose → Glucose 6-phosphate (consuming 1ATP)

Phosphorylated sugar molecules do not readily penetrate cell membranes because there are no specific transmembrane carriers for these compounds and because they are too polar to diffuse through the lipid core of membranes. Therefore, the irreversible phosphorylation of glucose effectively traps the sugar as cytosolic glucose-6-phosphate and commits it to further metabolism in the cell.

The phosphorylation is catalyzed by four isozymes (I-IV) of the enzyme Hexokinase in all mammal tissues.

In most tissues, Hexokinases (I-III) catalyze the reaction

In the liver tissue, Hexokinase IV (Glucokinase) catalyze this reaction.



Unnumbered 16 p455b
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

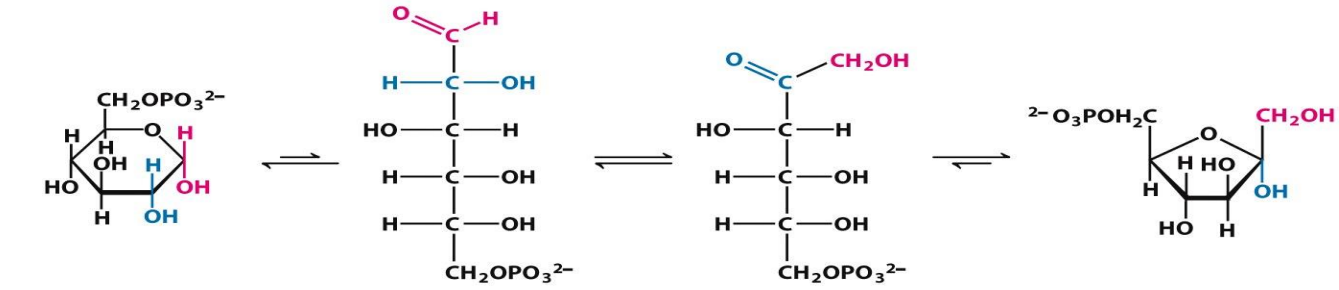
	Hexokinase	Glucokinase
Occurrence	In all tissue	In liver
Km	< 0.02 mM	10-20mM (V max is high)
Specificity	Can phosphorylate: Glucose. Fructose, Manos, Galactose.	Glucose. (other sugars are phosphorylated but in less efficiency)
Induction	Not induced	Increased insulin, glucose concetration
Function	At any glucose level in the blood. (like in the Brain which must be function all the times).	Only> 100 mg/dl.

---

2. Glucose 6-phosphate isomerization: Glucose 6-phosphate → Fructose 6-phosphate

The isomerization of *glucose 6-phosphate* to *fructose 6-phosphate* is catalyzed by the enzyme *phosphoglucose isomerase*. The reaction is readily reversible and is not a rate-limiting or regulated step.

Note: *Inter-conversion* between sugars occurs when they exist in *open-chain forms*, not in *ring (cyclic) forms.*



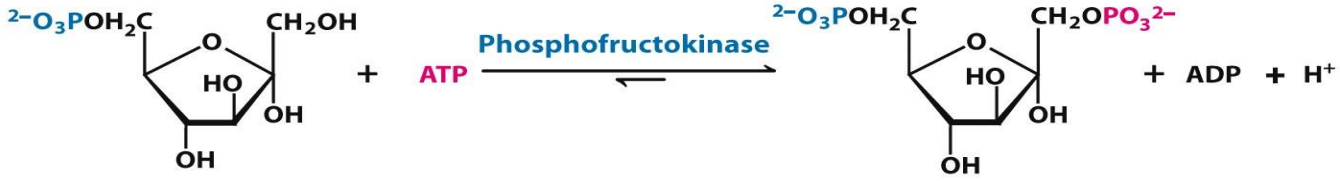
Unnumbered 16 p457
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

---

3. Fructose 6-phosphate phosphorylation: Fructose 6-phosphate → Fructose 1,6-bisphosphate. (consuming 1ATP)

It is highly irreversible phosphorylation reaction, which is catalyzed by the enzyme *phospho-fructokinase-1 (PKF-1)*.

(*The most important control point and the rate-limiting and committed step of glycolysis*. PKF-1 is controlled by the available concentrations of the substrates ATP and fructose 6-phosphate as well as by other regulatory molecules.



Fructose 6-phosphate (F-6P)

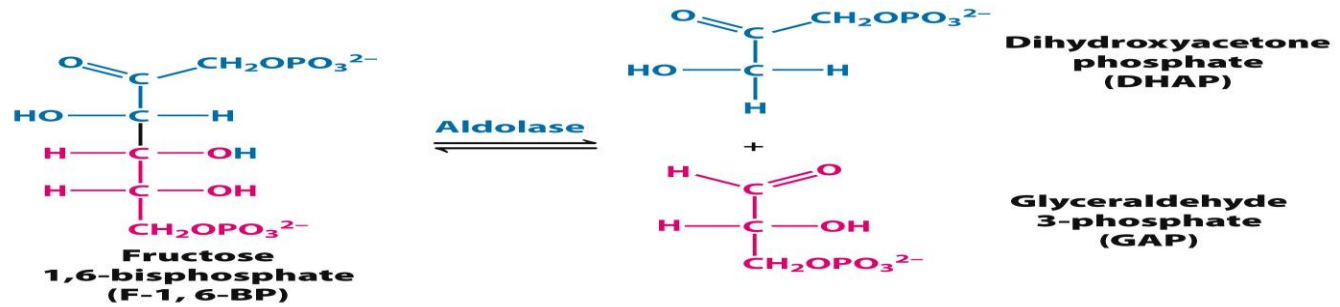
Fructose 1,6-bisphosphate (F-1, 6-BP)

Unnumbered 16 p458a
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

---

4. Fructose 1,6-bisphosphate cleavage: Fructose 1,6-bisphosphate → (DHAP) + (GAP)

The Enzyme Aldolase cleaves *fructose 1,6-bisphosphate* to dihydroxyacetone phosphate (DHAP) and glyceraldehydes 3-phosphate. The reaction is reversible and not regulated.



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

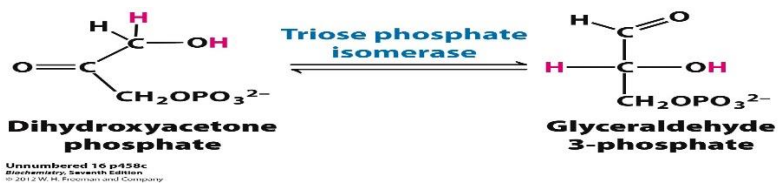
Dihydroxyacetone phosphate (DHAP)

Glyceraldehyde 3-phosphate (GAP)

Unnumbered 16 p458b
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

---

5. Dihydroxyacetone phosphate isomerization: Triose phosphate isomerase interconverts these 2 trioses (DHAP and glyceraldehyde 3-phosphate). DHAP must be isomerized to glyceraldehyde 3-phosphate for further metabolism by the glycolytic pathway. This isomerization results in the net production of two molecules of glyceraldehydes 3-phosphate from cleavage products of fructose 1,6 bisphosphate.



Dihydroxyacetone phosphate

Glyceraldehyde 3-phosphate

Unnumbered 16 p458c
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

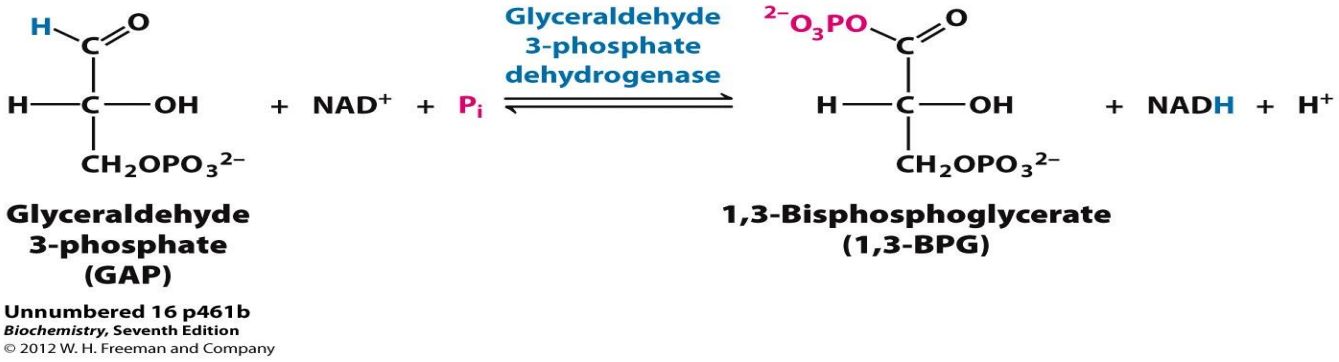
The first five steps are involved in Energy-investment phase.

Now let's complete with the last five steps which are involved in Energy-generating step phase of glycolysis:

For all reactions that follow in this phase, keep in mind that the six-carbon glucose has been split into two three-carbon units. Thus, to account for everything properly, remember that there are two of each three carbon compound in the reactions shown.

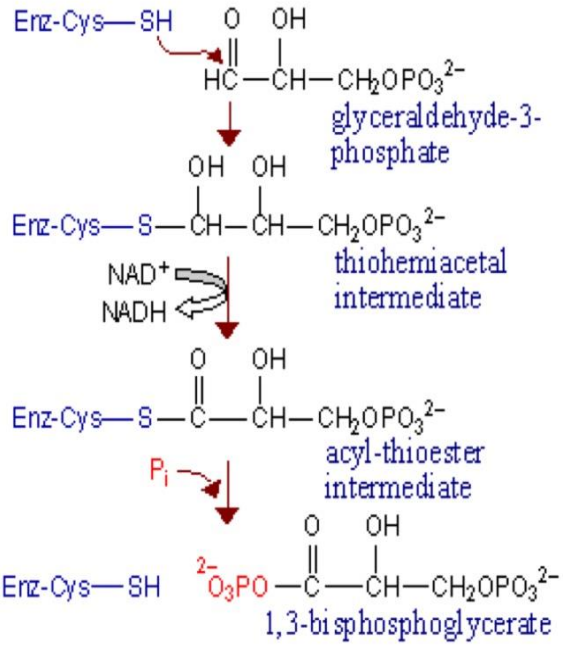
6. Glyceraldehyde 3-phosphate oxidation: GAP → 1,3-BPG (Producing 2 NADH)

The conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate by an enzyme called *glyceraldehyde 3-phophate dehydrogenase is the first oxidation-reaction of glycolysis.*



The synthesis of 1,3-bisphosphoglycerate:

The oxidation of the aldehyde group of glyceraldehyde 3-phophate to a carboxyl group is coupled to the attachment of phosphate group to the carboxyl group. This phosphate group, linked to carbon 1 of the 1,3-BPG product by a high energy bond, conserves much of the free energy produced by the oxidation of glyceraldehydes 3-phosphate. This high energy phosphate drives ATP synthesis in the next reaction of glycolysis.



Mechanism of the reaction:

1. First, *a cysteine residue* in the active site of GAPDH attacks the *carbonyl group of GAP* covalently forming an intermediate called *thiohemiacetal*.

2. NAD^+ accepts a hydride ion from GAP, forming *NADH* while GAP is simultaneously oxidized to an *acyl-thioester intermediate*

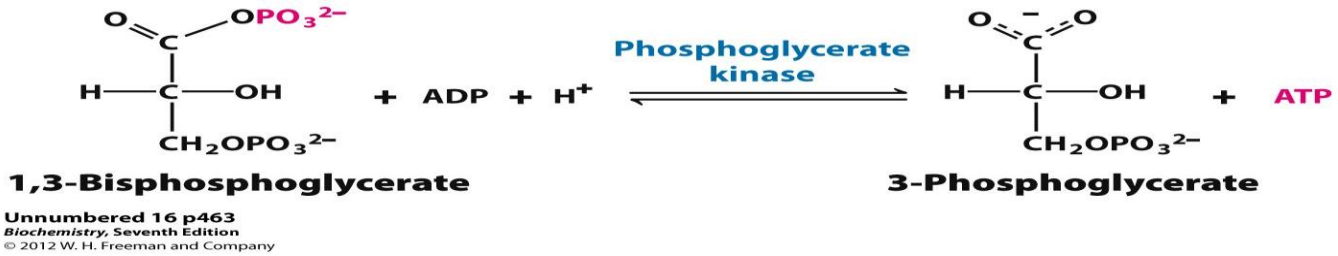
3. Pi attacks the thioester bond releasing *1,3-bisphosphoglycerate*

Note: 1,3-bisphosphoglycerate has a high energy even higher than ATP molecule.

---

7. 3-phosphoglycerate synthesis and ATP production: 1,3-BPG → 3-phosphglycerate *(Producing 2 ATP)*

When 1,3-BPG is converted to 3-phosphglycerate, the high-energy phosphate group of 1,3-BPG is used to synthesize ATP from ADP. This reaction is catalyzed by an enzyme called phosphoglycerate kinase, which, unlike most other kinases, is physiologically reversible.



---

8. Phosphate group shift: 3-phosphglycerate → 2-phosphglycerate

The phosphate group is shifted from carbon 3 to carbon 2 of phosphoglycerate by phosphoglycerate mutase, this reaction is freely reversible.

---

9. 2-phosphglycerate dehydration: 2-phosphglycerate → phosphoenolpyruvate (PEP)

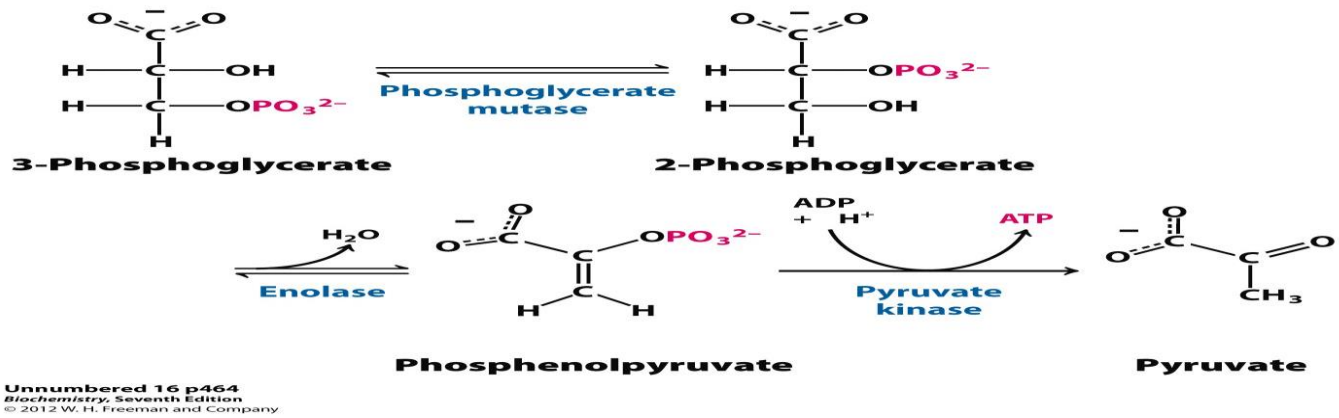
The 2-phosphoglycerate is dehydrated by enolase redistributes the energy within the substrate, forming phosphoenolpyruvate (PEP), which contains a high energy enol phosphate. The reaction is reversible, despite the high energy nature of the product.

Note: Enolase is inhibited by florid ion, so that's why in tooth baste we have florid ions to inhibit the enolase that bacteria have in order to prevent forming the blacks on teeth)

---

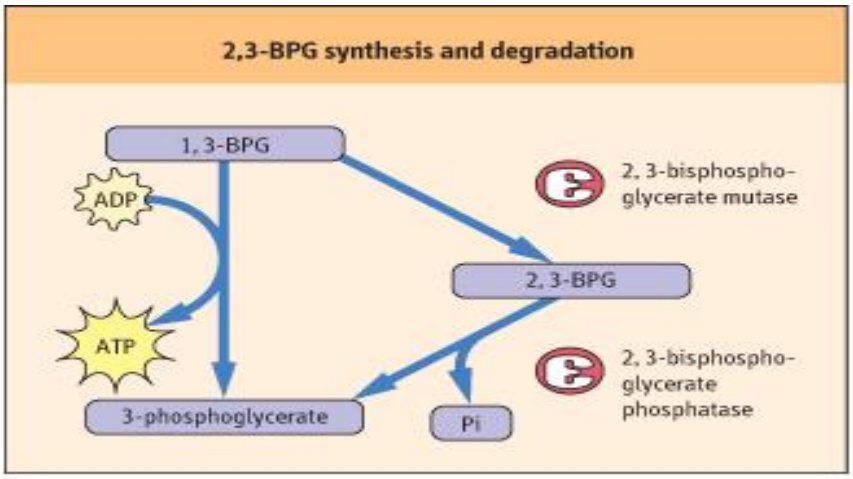
10. Pyruvate synthesis and ATP production: PEP → pyruvate *(Producing 2 ATP)*

The conversion of PEP to pyruvate, catalyzed by pyruvate kinase (PK), is the third irreversible reaction of glycolysis. The high energy enol phosphate in PEP is used to synthesize ATP from ADP and is another example of substrate-level phosphorylation.



Synthesis of 2,3 bisphosphoglycerate in RBC

Some of the 1,3-BPG is converted to 2,3-BPG by the action of bisphosphoglycerate mutase. 2,3-BPG, which is found in only trace amounts in most cells, is present at high concentration in RBC (4-5 mM) and serves to increase O2 delivery (it is important regulator of O2 delivery and transport). 2,3-PBG is hydrolyzed by a phosphatase to 3-phosphoglycerate, which is also an intermediate in glycolysis. In the RBC, glycolysis is modified by inclusion of these shunt reactions.



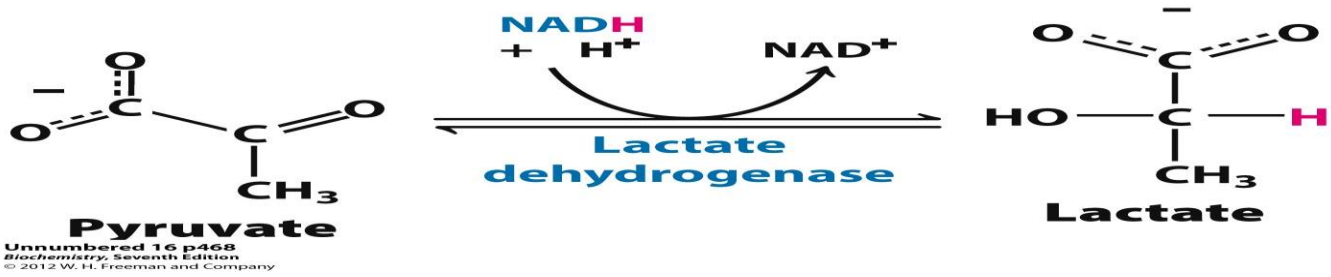
Metabolic Fates of Pyruvate:

1. Pyruvate reduction to lactate:

Lactate, formed from pyruvate by lactate dehydrogenase, is the final product of anaerobic glycolysis in eukaryotic cells. Reduction to lactate is the major fate for pyruvate in tissues that are poorly vascularized or in RBC that lack mitochondria.

Lactate formation in muscles:

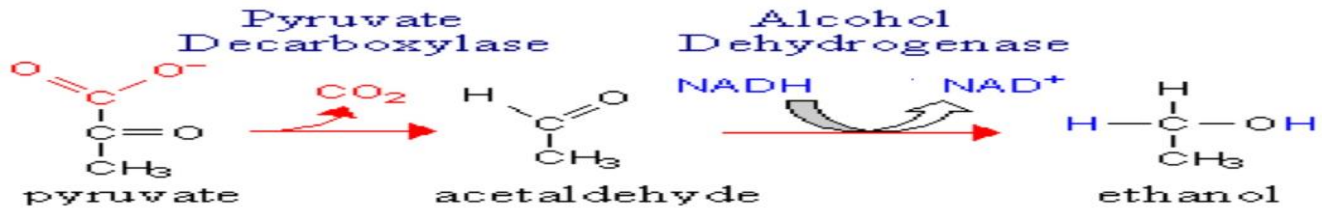
In exercising skeletal muscle, NADH production exceeds the oxidative capacity of the ETC. this results in an elevated NADH/NAD+ ratio, favoring reduction of pyruvate to lactate by LDH. Therefore, during intense exercise, lactate accumulates in muscle, causing a drop in the intracellular pH, potentially resulting in cramps. Much of this lactate eventually diffuses into the bloodstream and can be used by the liver to make glucose.

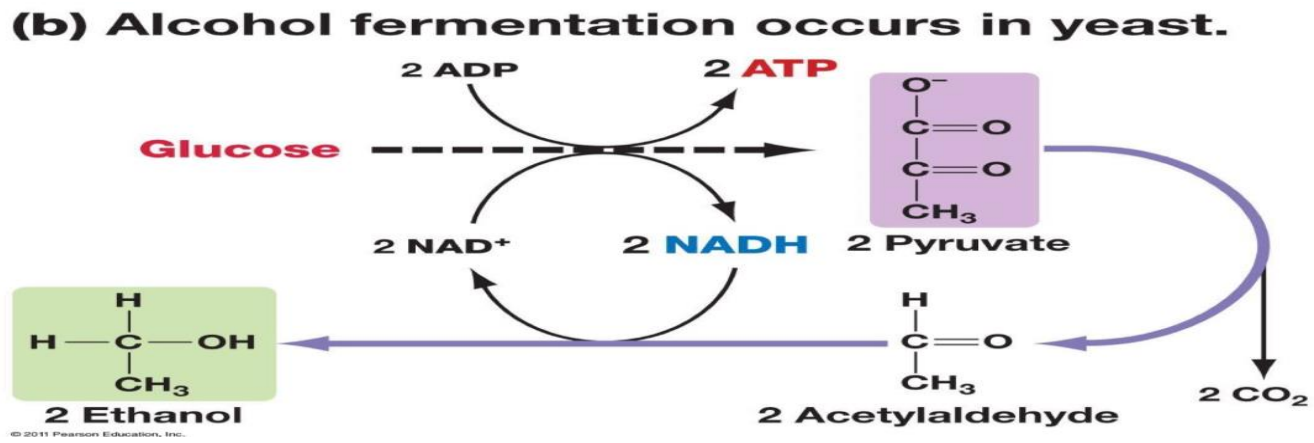


2. Alcohol fermentation (yeast and bacteria)

Here, pyruvate is converted to acetaldehyde when it undergoes decarboxylation by an enzyme called pyruvate decarboxylase. Then an acetaldehyde undergoes reduction by NADH and enzyme alcohol dehydrogenase to ethanol (alcohol), in this process NADH is oxidized to NAD+ which can be used again in glycolysis.

Note: No alcohol is produced in humans because they do not have pyruvate decarboxylase.





Read this before you sleep!!

Table 16.1 Reactions of glycolysis

Step	Reaction	Enzyme	Reaction type	$\Delta G^{\circ\prime}$ in kJ mol^{-1} (kcal mol^{-1})	ΔG in kJ mol^{-1} (kcal mol^{-1})
1	Glucose + ATP → glucose 6-phosphate + ADP + H^+	Hexokinase	Phosphoryl transfer	−16.7 (−4.0)	−33.5 (−8.0)
2	Glucose 6-phosphate ⇌ fructose 6-phosphate	Phosphoglucose isomerase	Isomerization	+1.7 (+0.4)	−2.5 (−0.6)
3	Fructose 6-phosphate + ATP → fructose 1,6-bisphosphate + ADP + H^+	Phosphofructokinase	Phosphoryl transfer	−14.2 (−3.4)	−22.2 (−5.3)
4	Fructose 1,6-bisphosphate ⇌ dihydroxyacetone phosphate + glyceraldehyde 3-ph	Aldolase	Aldol cleavage	+23.8 (+5.7)	−1.3 (−0.3)
5	Dihydroxyacetone phosphate ⇌ glyceraldehyde 3-phosphate	Triose phosphate isomerase	Isomerization	+7.5 (+1.8)	+2.5 (+0.6)
6	Glyceraldehyde 3-phosphate + P_i + NAD^+ ⇌ 1,3-bisphosphoglycerate + NAI	Glyceraldehyde 3-phosphate dehydrogenase	Phosphorylation coupled to oxidation	+6.3 (+1.5)	−1.7 (−0.4)
7	1,3-Bisphosphoglycerate + ADP ⇌ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	Phosphoryl transfer	−18.8 (−4.5)	+1.3 (+0.3)
8	3-Phosphoglycerate ⇌ 2-phosphoglycerate	Phosphoglycerate mutase	Phosphoryl shift	+4.6 (+1.1)	+0.8 (+0.2)
9	2-Phosphoglycerate ⇌ phosphoenolpyruvate + H_2O	Enolase	Dehydration	+1.7 (+0.4)	−3.3 (−0.8)
10	Phosphoenolpyruvate + ADP + H^+ → pyruvate + ATP	Pyruvate kinase	Phosphoryl transfer	−31.4 (−7.5)	−16.7 (−4.0)

Note: ΔG, the actual free-energy change, has been calculated from $\Delta G^{\circ\prime}$ and known concentrations of reactants under typical physiological conditions. Glycolysis can proceed only if the ΔG values of all reactions are negative. The smalls positive ΔG values of three of the above reactions indicate that the concentrations of metabolites in vivo in cells undergoing glycolysis are not precisely known.

Table 16.1

Biochemistry, Seventh Edition

Best of luck..