Chemistry of Cells and Tissues
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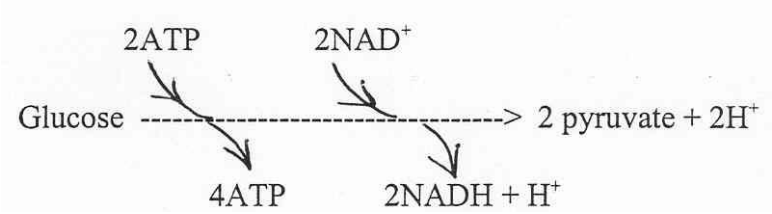
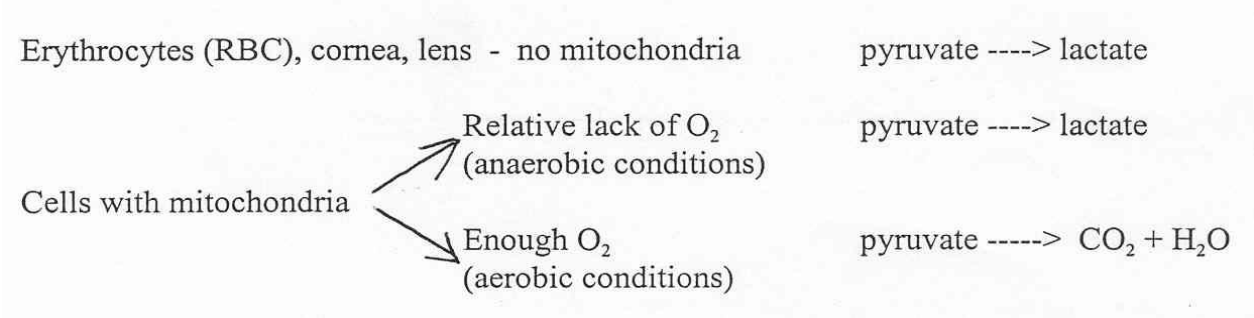
GLYCOLYSIS

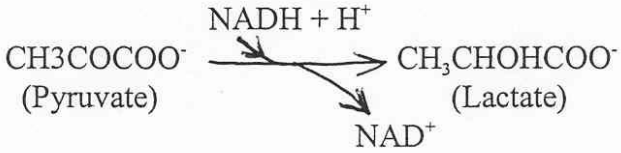
Glycolysis is a set of reactions that converts glucose to pyruvate or lactate. This is the first metabolic pathway to be elucidated and hence is considered as a paradigm of metabolic pathways. Glycolysis is also called Embden-Meyerhoff pathway. The complete set of reactions occurs in the cytoplasm of virtually every animal cell. The entire process occurs without molecular oxygen.



Glycolysis consumes 2 ATP and generates 4ATP. Thus, the process results in the generation of 2 **net** ATP. The process also generates 2 NADH. What happens to pyruvate depends upon the presence or absence of mitochondria in the cell or upon the availability of oxygen in mitochondria – containing cells.



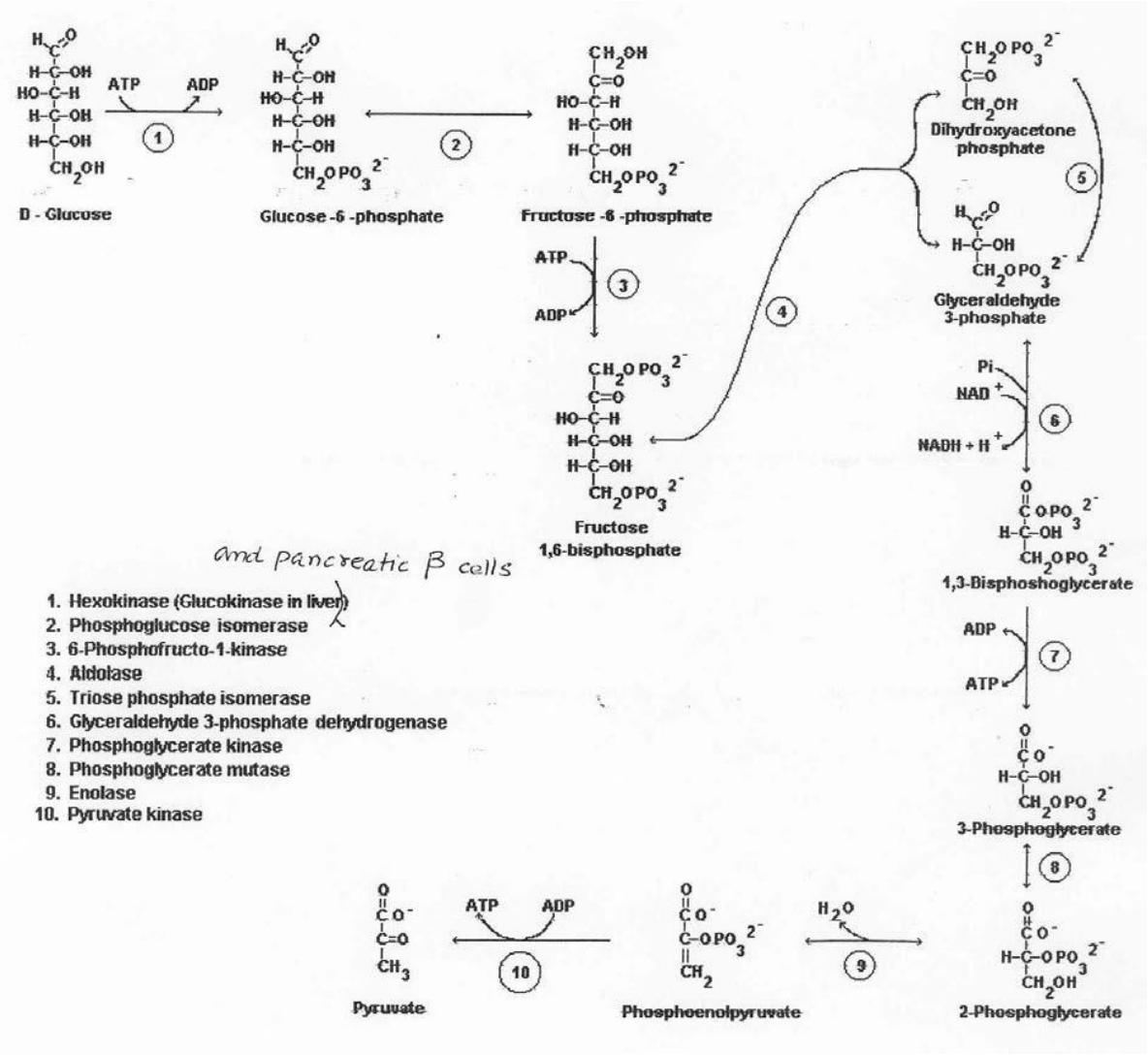
For the glycolytic pathway to continue, NAD^+ has to be regenerated. In erythrocytes (no mitochondria) and in mitochondria – possessing cells under anaerobic conditions, NAD^+ is regenerated from NADH during the conversion of pyruvate to lactate.



In mitochondria – possessing cells under aerobic conditions, NAD^+ is regenerated by either malate – aspartate shuttle or α-glycerophosphate shuttle, which transfer the reducing equivalents from NADH into mitochondria for electron transport chain, thus regenerating NAD^+ in the cytoplasm.

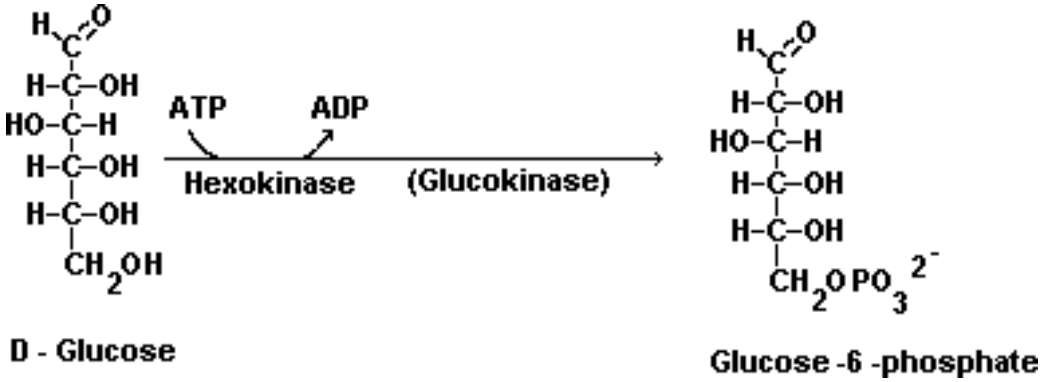
Reactions

Glycolysis consists of two phases. In the first phase, glucose is broken down to two molecules of glyceraldehyde-3-phosphate in a series of five reactions. In the second phase, another series of five reactions convert these two molecules of glyceraldehyde-3-phosphate into two molecules of pyruvate. Phase I consumes 2 ATP and Phase II generates 4 ATP. The net ATP production in the entire process is 2.



Individual Reactions

Reaction # 1: **Hexokinase/Glucokinase**



a. This is the first intracellular reaction of glycolysis (remember all reactions are in the cytoplasm).
b. Requires an ATP (Mg). This is one of the investment reactions.
c. The phosphorylation of glucose traps the glucose inside the cell.
d. The reaction is considered irreversible.
e. Hexokinase has a K_m for glucose of less than 0.1 mM (high affinity). It is also inhibited by the product glucose-6-phosphate
f. Liver hepatocytes and pancreatic β cells contain another enzyme **Glucokinase**.
   Glucokinase has a K_m for glucose of about 10 mM (low affinity).
   Glucokinase is not inhibited by its product glucose-6-phosphate.
   Glucokinase is induced by insulin.
   The levels of glucokinase in the liver of untreated Type 1 diabetics are lower than normal.
   Hexokinase and glucokinase are isoenzymes. Therefore, irrespective of the isoenzyme catalyzing the reaction, the Keq, ΔG, and ΔG^o for the reaction remain the same.

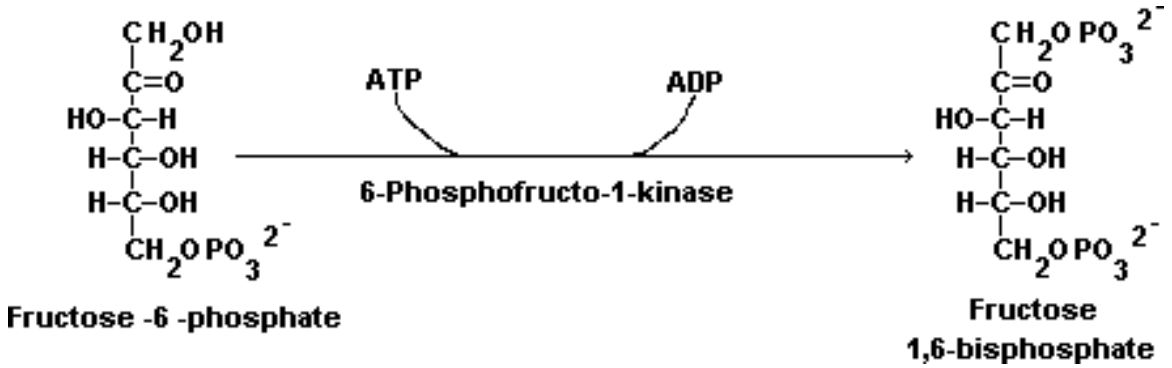
Reaction #2: **Phosphoglucose isomerase**.

Glucose -6 -phosphate

Fructose -6 -phosphate

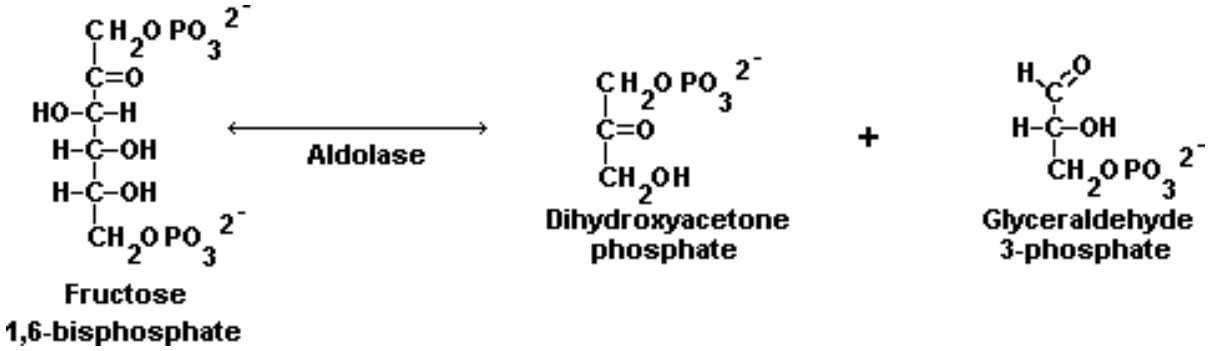
a. This reaction is readily reversible (not a controlling step) and functions in both glycolysis and gluconeogenesis.
b. Conversion of an aldose to a ketose.

Reaction #3: **6-Phosphofructo-1-kinase (PFK-1)** or **Phosphofructokinase-1**.

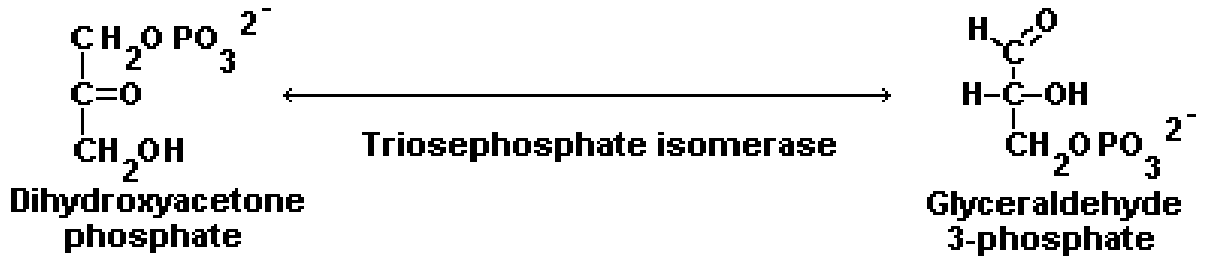


a. Reaction is the rate-limiting step of glycolysis.
b. It is irreversible, and the committed step. It is an allosteric enzyme and also a major regulatory enzyme.
c. We have invested our second ATP molecule.

Reaction #4: **Aldolase**.



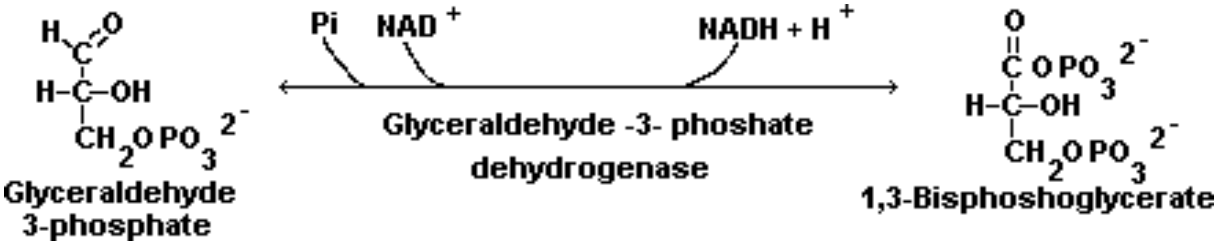
a. We now have two phosphorylated trioses.
b. Only glyceraldehyde-3-phosphate is used in glycolysis. Therefore, dihydroxyacetone phosphate has to be converted into glyceraldehyde-3-phosphate. This occurs in the next step.



Reaction #5: **Triose phosphate isomerase.**

a. Catalyzes the interconversion of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate.
b. Because of the interconversion, one glucose molecule can be converted to two glyceraldehyde-3-phosphate molecules.

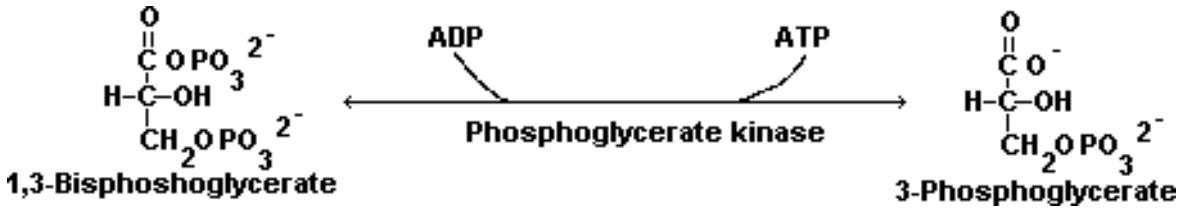
Reaction # 6: **Glyceraldehyde-3-phosphate dehydrogenase**



a. The enzyme oxidizes the number one carbon aldehyde and then adds a phosphate group. We have an acid anhydride in the product 1,3-bisphophoglycerate. Remember from bioenergetics that acid anhydrides are high-energy bonds.
b. The phosphate on the number 3 carbon is **not** an high-energy bond

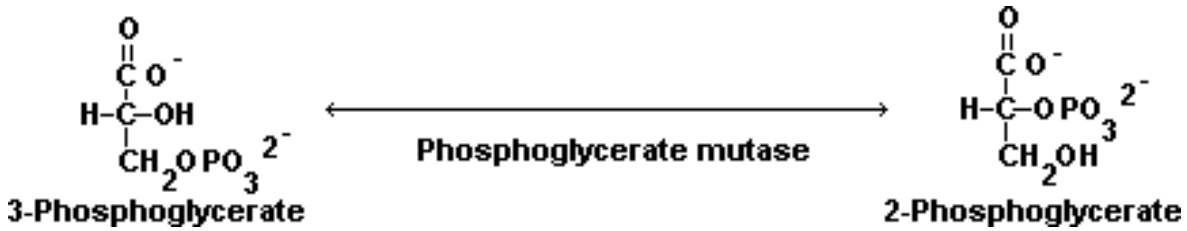
c. We have used an NAD^+ for the oxidation reaction. The cell has limited amounts of NAD^+, so somewhere along the line we have to regenerate it or glycolysis will stop.
d. This reaction is a target for Arsenate (AsO_4^{3-}). The arsenate resembles inorganic phosphate (Pi). In the presence of arsenate, the product of the reaction is 1-arseno-3-phosphoglycerate. This product is unstable and decomposes into arsenate and 3-phosphoglycerate with no ATP formation. After this step, glycolysis continues.
e. The enzyme contains an essential thiol (cysteine-SH) group at the active site. Iodoacetic acid (ICH_2COOH) is also an inhibitor of this reaction. It reacts with the active site SH group and inhibits the enzyme.

Reaction #7: **Phosphoglycerate kinase**

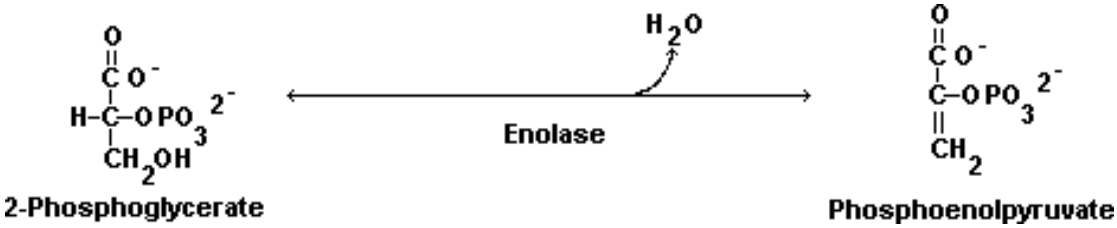


a. This is the first step of energy production.
b. This is referred to as **substrate-level phosphorylation** as opposed to oxidative phosphorylation that occurs in mitochondrial ATP production.
d. We have recovered both ATP that were invested. Remember that each glucose gives 2 phosphoglycerate molecules.

Reaction # 8: **Phosphoglycerate mutase**.

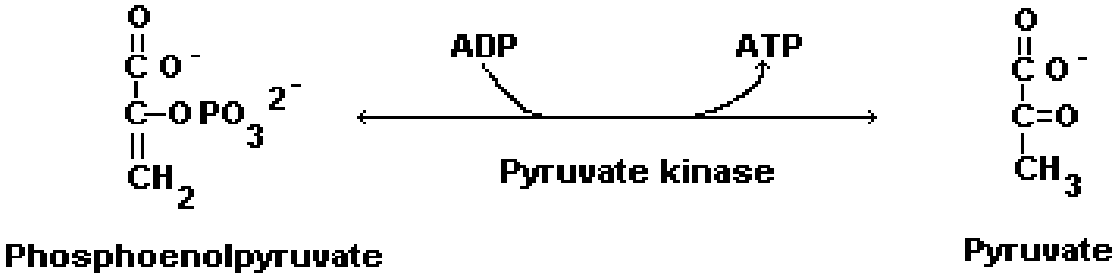


Reaction#9: **Enolase**



a. Catalyzes the dehydration of 2-phosphoglycerate to form phosphoenolpyruvate (PEP).
c. Recall from the bioenergetics lecture that PEP contains an high-energy bond.
d. This reaction is inhibited by Fluoride.

Reaction # 10: **Pyruvate Kinase**



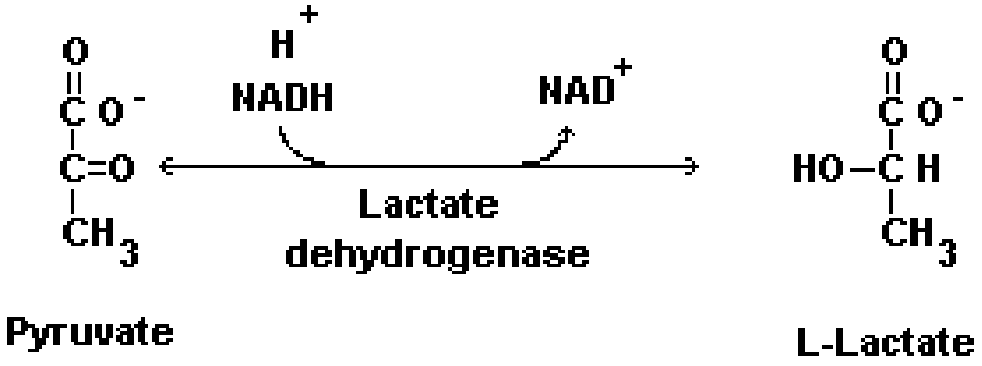
a. Catalyzes the transfer of the phosphate from PEP to ADP to generate ATP and

pyruvate.

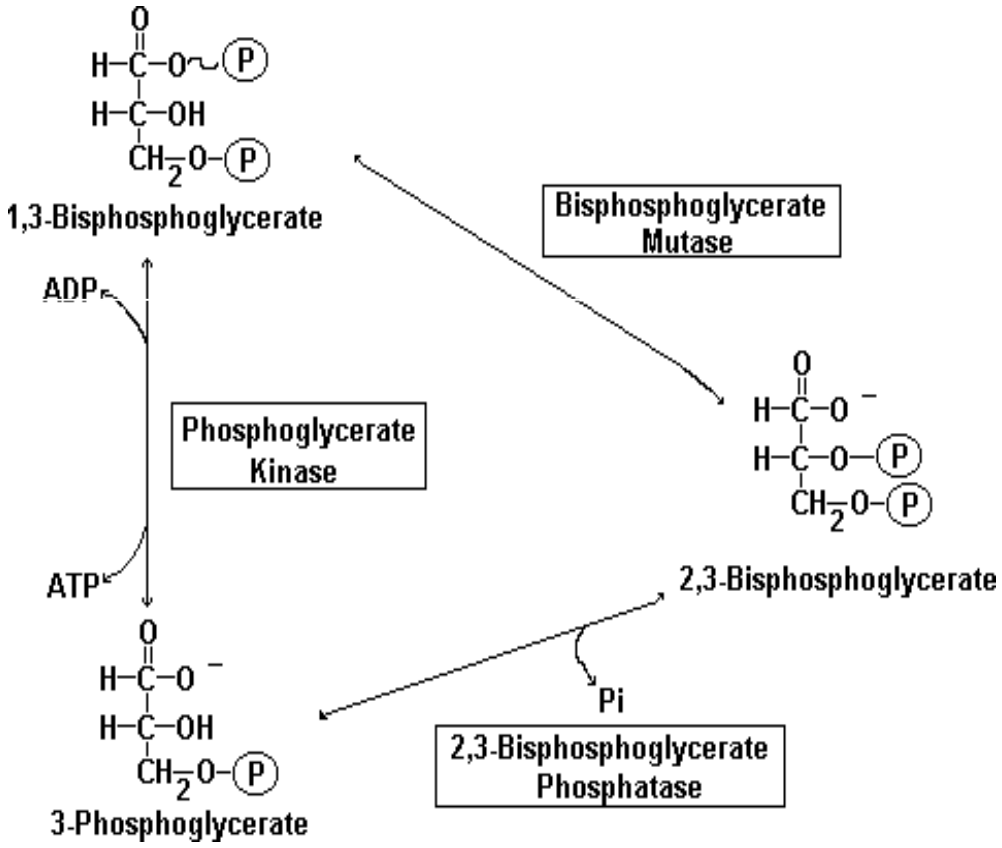
b. Again, this is **substrate-level phosphorylation**.
c. This reaction completes that part of glycolysis that is common to both anaerobic and aerobic metabolism.
d. Under aerobic conditions and the presence of mitochondria, pyruvate can enter the citric acid cycle. NAD^+ is regenerated by malate/aspartate shuttle or by α-glycerophosphate shuttle
e. Under anaerobic conditions or in the absence of mitochondria, pyruvate is reduced to lactate via the following reaction and regenerate NAD^+.

Anerobic Glycolysis

When pyruvate cannot be oxidized within mitochondria for some reason (e.g., hypoxia, genetic defects in pyruvate dehydrogenase or citric acid cycle enzymes, genetic defects in electron transport chain), pyruvate is reduced to lactate by lactate dehydrogenase.



Synthesis of 2, 3-bisphosphoglycerate



a. All cells contain a catalytic amount of 2,3-BPG.
b. Red blood cells contain a high concentration of 2,3-BPG (~ 4mM). It facilitates the release of oxygen from hemoglobin.
c. ~20% of glucose goes through this shunt in RBC.
d. No net ATP is generated if 1,3-BPG is converted to 3-PG via 2,3-BPG. If the entire glycolytic pathway in RBC occurs via the formation of 2,3-BPG, glycolysis will yield no net ATP because the 2 ATP produced by pyruvate kinase will be equal to 2 ATP consumed in Phase I of glycolysis.
e. 2, 3-BPG levels in erythrocytes increase in high altitude where partial pressure of oxygen is low.
f. Loss-of-function mutations in pyruvate kinase increase the levels of 2,3-BPG in RBC, thus shifting the sigmoidal curve of oxygen binding to hemoglobin to the right. If loss-of-function mutations occurs in any of the enzymes upstream of the step involved in the formation of 1,3-BPG, the levels of 2,3-BPG in RBC will be lower than normal, thus shifting the curve to the left.

Control of Glycolysis

It is obvious that glycolysis must be controlled.

Enzymatic control can be exercised by three different common methods.

a. Allosteric effectors. The transient binding of molecules to the enzyme to change the conformation. Effect is observed in milliseconds.
b. Covalent modification. Generally phosphorylation. Effect in seconds.
a. Transcription of enzyme. Effect observed in hours.

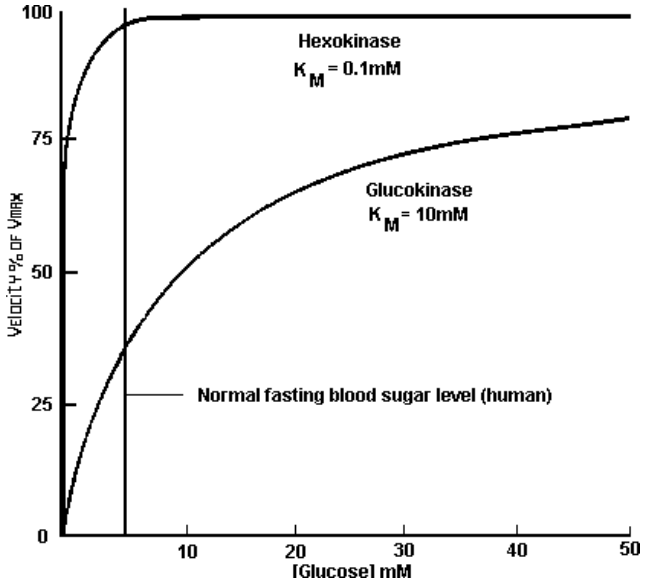
There are only three major points of control of glycolysis.

Three points of control

Hexokinase and glucokinase: Reaction # 1

Hexokinase is found in most tissues.

1. It has a low K_m for glucose (<0.1mM).
   a. Allows for the phosphorylation of glucose when blood levels of glucose are low.
   b. This permits tissues that rely on glycolysis extensively to carry out the phosphorylation.
   c. Hexokinase is strongly inhibited by its product, glucose-6-Phosphate (G6P).
2. Liver parenchymal cells (hepatocytes) contain an isozyme of hexokinase, glucokinase.
   a. High K_m for glucose (10 mM).



   b. Concentration in cell reflects blood concentration.
   c. Liver uses glucose at a significant rate only when blood glucose is elevated.

3. Glucokinase is not inhibited by G6P.
4. It is inhibited by fructose-6-phosphate (F6P) [which is generated by reaction # 2] and stimulated by fructose-1-phosphate (F1P).
    a. Regulation occurs via an inhibitory protein.
        1). F6P stimulates the binding of the inhibitory protein.
        2). F1P stimulates the dissociation of the inhibitory protein.
5. Glucokinase is inducible in liver by insulin. cAMP decreases glucokinase levels in liver.
6. Glucokinase is also present in pancreatic β cells where it plays an essential role in glucose-induced insulin secretion. Because of the low affinity, this enzyme serves as a sensor of blood glucose level to co-ordinate insulin secretion appropriately.

6-Phosphofructo-1-kinase (PFK-1) reaction # 3

1. This is the rate-limiting step of glycolysis.
2. First committed step of glycolysis.
3. Subject to the greatest degree of regulation by allosteric effectors.
4. Positive effectors:

    a. Pi and AMP are positive effectors of PFK-1.
    b. AMP is a negative effector of F1,6BPase, which catalyzes the reverse reaction, converting F1, 6BP into F6P and Pi.
    c. Fructose 2,6-bisphosphate. This is a positive effector of PFK-1 and a negative effector of F1,6BPase.
5. Negative effectors.
    a. **ATP:** ATP is an allosteric inhibitor of PFK-1.
    b. **Citrate:** Glycolysis and citric acid cycle are coupled via PFK-1 because citrate, an intermediate in citric acid cycle, is an allosteric inhibitor of PFK-1. When the citric acid cycle is saturated with high levels of citrate, citrate leaves mitochondria via a transporter (tricarboxylate transporter) and inhibits PFK-1. This prevents generation of pyruvate, which feeds into citric acid cycle.
    d. Pasteur effect. Oxygen inhibits glycolysis.
        The consumption of glucose in the presence of oxygen generates much more ATP. The inhibition is probably due to the inhibition of PFK-1 by ATP.

C. Pyruvate kinase: reaction # 10

1. Inhibited by high concentrations of ATP.
2. Isoenzyme found in liver is activated by fructose 1,6-bisphosphate.
3. Liver enzyme is subject to phosphorylation.
    a. Active in the dephosphorylated state.
    b. Inactive in the phosphorylated state.

Inactivation by phosphorylation is a function of cAMP-dependent protein kinase in liver.

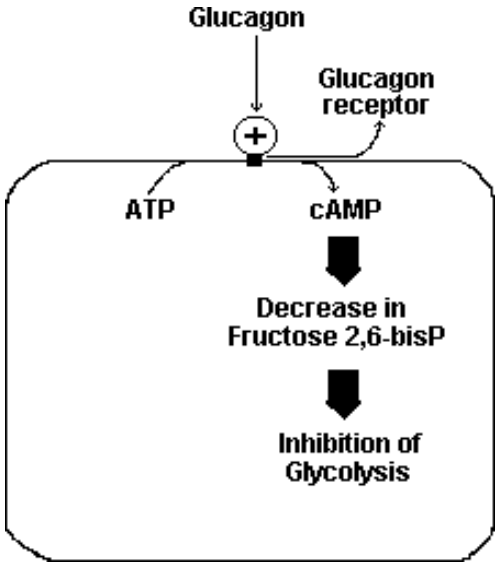
4. Enzyme is inducible by high carbohydrate concentration and also high insulin levels.

Control of 6-Phosphofructo-1-kinase (PFK-1) by Fructose 2,6-bisphosphate [Regulation by cAMP]

A. Let's talk about its effect in liver.

1. Fructose 2,6-bisphosphate behaves like AMP.
   a. Positive effector of 6-phosphofructo-1-kinase (PFK-1). The positive effect involves an increase in the affinity of PFK-1 for F6P and also a decrease in the inhibitory effect of ATP.
   b. Negative allosteric effector of fructose 1,6-bisphosphatase.
2. This compound is not a product of glycolysis.
   a. In fact, its only known function is as a regulator.
   b. It is found in all cells.

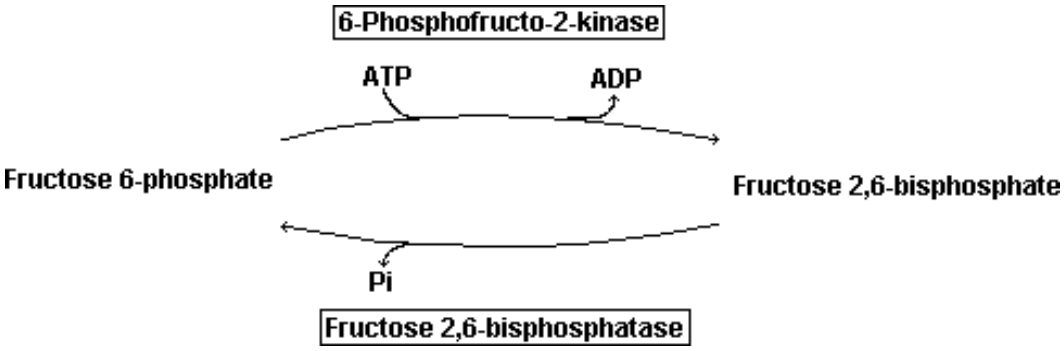
B. Hormonal control of fructose 2,6-bisphosphate in **liver.**



1. Glucagon (a 29-aa hormonal peptide) is released by the α-cells of the pancreas and interacts with a glucagon receptor on the surface of the liver plasma membrane.
2. The binding of glucagon to the receptor is "sensed' by adenylate cyclase (enzyme located on the inner surface of the plasma membrane).
   a. Adenylate cyclase catalyzes the conversion of cytosolic ATP to cAMP and PPi.
3. cAMP ultimately causes a decrease in the levels of fructose 2,6-bisphosphate.
   a. cAMP acts as a second messenger.
   b. Note, cAMP acts to inhibit glycolysis in liver, but in most other cells cAMP stimulates glycolysis. The difference is that liver is the primary organ, which controls blood glucose levels. Liver can make glucose (gluconeogenesis) as well as degrade glucose (glycolysis) as needed. cAMP stimulates gluconeogenesis in

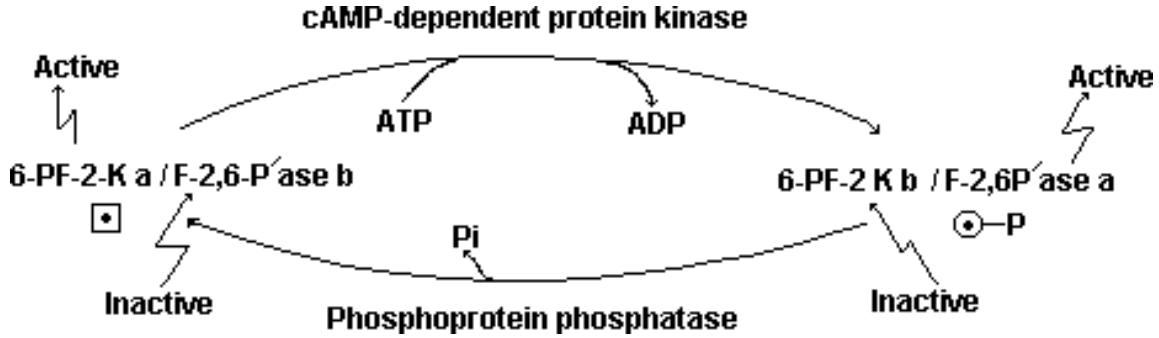
liver and inhibits glycolysis. cAMP-induced decrease in fructose 2, 6-bisphosphate levels is at least partly responsible for these effects. In some cells, cAMP actually stimulates the synthesis of fructose 2,6-bisphosphate (e.g., heart muscle). In skeletal muscle, cAMP does not affect the synthesis of fructose-2, 6-bisphosphate.

C. Synthesis of Fructose 2,6-bisphosphate



1. Fructose 2,6-bisphosphate is synthesized from fructose 6-phosphate (an intermediate in glycolysis) via the enzyme 6-phosphofructo 2-kinase (PFK-2). This is **not** the same as 6-phosphofructo 1-kinase (PFK-1).
2. Fructose 2,6-bisphosphate is destroyed in cells by the enzyme fructose 2,6-bisphosphatase.
3. Both of the above enzyme activities are located on the same protein. It is a bifunctional enzyme and is referred to as 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase.
4. cAMP is responsible for the regulation of fructose 2,6-bisphosphate levels in liver.

D. The PKF-2/F2, 6BPase is regulated by protein kinase A and phosphoprotein phosphatase as follows.



1. Phosphorylation causes inactivation of the active site responsible for the synthesis of

fructose 2,6-bisphosphate and activation of the site responsible for the hydrolysis.

2. Dephosphorylation has the opposite effect.
3. Increased levels of glucagon cause an increase in cAMP levels.
    a. This second messenger activates cAMP dependent protein kinase.
    b. This phosphorylates 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase.
    c. The synthesis of fructose 2,6-bisphosphate is inhibited and its degradation is promoted.
    d. A decrease in fructose 2,6-bisphosphate makes 6-phosphofructo-1-kinase less effective and fructose 1,6-bisphosphatase more effective.
    e. Glycolysis is inhibited.
4. Insulin opposes the action of glucagon.
   Glucagon and insulin clearly act in opposition to one another, and the insulin/glucagon ratio of blood determines the intracellular levels of fructose 2,6-bisphosphate and therefore the rate of glycolysis.
5. Different tissues express different isoforms of the bifunctional enzyme PFK-2K/F-2, 6BPase, encoded by different genes. The liver isoform possesses sites for protein kinase A-dependent phosphorylation. cAMP inactivates PFK-2 and activates F-2, 6-BPase and thus reduces F-2, 6-BP levels. The heart isoform has phosphorylation sites for protein kinase A, but at different part of the molecule. cAMP activates PFK-2 and has no effect on F-2, 6-BPase and hence increases F-2, 6-BP levels. The skeletal muscle isoform does not possess phosphorylation sites for protein kinase A and hence has no effect on F-2, 6-BP levels.

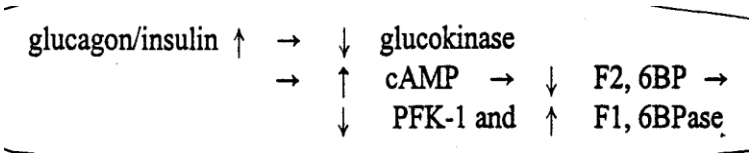
Clinical considerations:

A. Lactic acidosis

1. Characterized by high blood levels of lactate (generally greater than 5 mM, while normal levels are usually less than 1.2 mM). Blood pH can be less than 7.1 in severe cases.
2. Most tissues can convert lactate to CO_2 and H_2O through the TCA cycle.
    a. If the oxygen supply is inadequate, cells must rely on glycolysis for ATP production.
    b. A decrease in ATP enhances glycolysis at the level of PFK-1 and produces more pyruvate and hence lactate.
3. Accumulation of plasma lactate may be secondary to tissue hypoxia (circulatory insufficiency due to shock, heart failure), severe anemia, mitochondrial enzyme defects and inhibitors of oxygen transport (carbon monoxide, cyanide), liver disease, and ethanol.
4. Bicarbonate is usually administered to control acidosis associated with lactate overproduction. Dichloroacetate is used as a drug to treat lactic acidosis because it activates pyruvate dehydrogenase in mitochondria, which facilitates the conversion of pyruvate to acetyl CoA so that lactate production is decreased.
5. Strenuous exercise leads to anerobic glycolysis. This causes overproduction of lactate.

B. Diabetes

In diabetes, insulin activity is low (type 1 – no insulin; type 2 – insulin resistance). Consequently, glucagon levels increase. Thus, glucagon/insulin ratio is higher in diabetes. There is no change in glucose uptake in liver because this tissue does not express GLUT4, the insulin-responsive facilitative glucose transporter. In skeletal muscle and adipocytes, glucose uptake decreases because of the absence of insulin-dependent recruitment of GLUT4 into the plasma membrane. In liver, glucose is not metabolized effectively because:



This also results in an increase in gluconeogenesis, producing more glucose from gluconeogenic precursors alanine and glycerol. These precursers come from muscle and adipocytes as a result of increased protein and aminoacid breakdown and lipolysis to provide energy because of the decreased utility of glucose as the energy source.

C. Glucokinase deficiency: Mutations in glucokinase gene leading to inactivation of the enzyme are associated with a form of non-insulin-dependent diabetes mellitus (type 2) called Maturity Onset diabetes of the Young (MODY). Complete absence of the enzyme activity will lead to type 1 diabetes because of the lack of insulin secretion in response to blood glucose. This condition is associated with severe hyperglycemia. Mutations in the gene leading to increased activity of the enzyme will cause hyperinsulinemic hypoglycemia.

D. Pyruvate kinase deficiency and hemolytic anemia

1. Genetic defect causing a 5-20% reduction in red cell pyruvate kinase levels.
   a. It's rare but the most common genetic disease associated with the glycolytic pathway.
2. Results in markedly lower ATP concentrations in erythrocytes.
3. Cells swell and lyse.
4. Reticulocytes are unaffected because these "immature" red cells contain mitochondria and are able to generate ATP through oxidative phosphorylation.
5. The levels of 2, 3-bisphosphoglycerate are high in erythrocytes.
6. No Heinz bodies
7. The 2nd most common genetic cause of hemolytic anemia, only next to glucose 6-phosphate dehydrogenase deficiency.

E. Other glycolytic enzyme defects

Deficiencies in the activities of phosphofructokinase, phopshoglycerate kinase, phosphoglycerate mutase, and lactate dehydrogenase represent important genetic defects in glycolysis. All of these have certain common clinical features: exercise intolerance, myoglobinuria, hemolytic anemia, absence of lactate increase in forearm exercise test, and increased glycogen deposition in the liver and skeletal muscle.