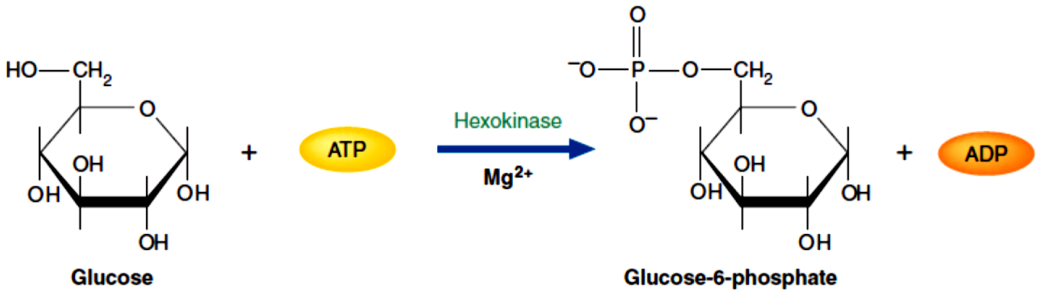
Glycolysis & the Oxidation of Pyruvate

The reactions of glycolysis constitute the main pathway of glucose utilization. All of the enzymes of glycolysis are cytosolic.

Glycolysis involves 10 enzymatic reactions:

1. The ***phosphorylation*** of glucose at position 6 forming glucose-6-phosphate (G-6-P), by **hexokinase**.



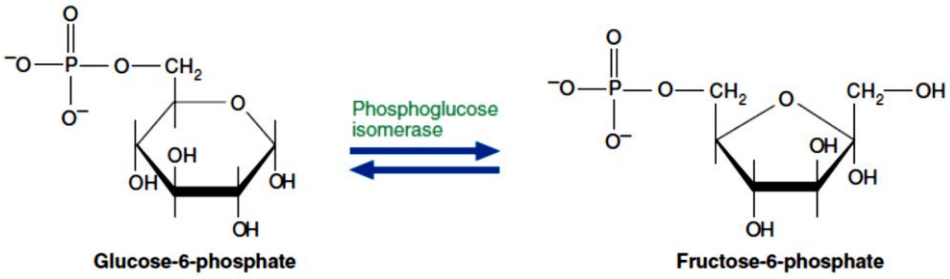
Under physiological conditions, this reaction is **irreversible**. Hexokinase has a high affinity (**low K_m**) for glucose, and it is saturated under normal conditions. Hexokinase is **inhibited allosterically** by its product, glucose-6-phosphate. In tissues other than the liver (and pancreatic β -islet cells), *the availability of glucose for glycolysis is controlled by transport into the cell, which in turn is regulated by* **insulin**.

Hexokinase has a high affinity (low *K*m) for glucose, and in the liver it is saturated under normal conditions, and so acts at a constant rate to provide G-6-p to meet the liver's needs. Liver cells also contain an isoenzyme of hexokinase, **glucokinase,** which has a *K*m very much higher than the normal intracellular concentration of glucose. The function of glucokinase in the liver is to remove glucose from the hepatic portal blood following a meal, so regulating the concentration of glucose available to peripheral tissues. This provides more G-6-p than is required for glycolysis; it is used for glycogen synthesis and lipogenesis.

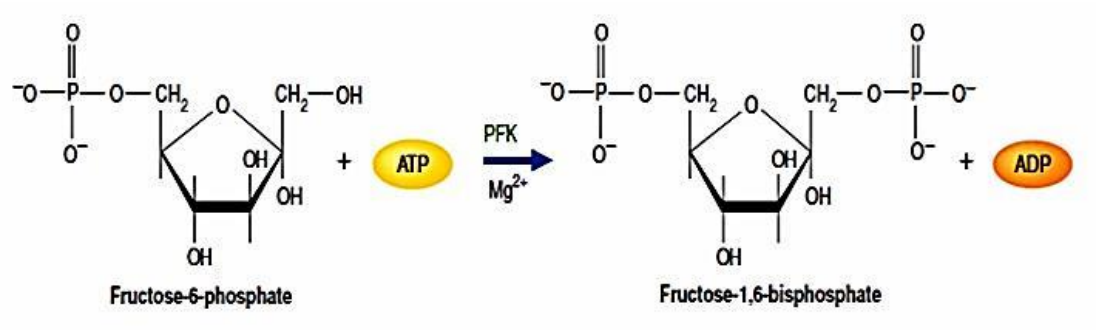
Glucokinase is also found in pancreatic β-islet cells, where it functions to detect high concentrations of glucose. As more glucose is phosphorylated by glucokinase, there is increased glycolysis, leading to increased formation of ATP. This leads to closure of an ATP-potassium channel, causing membrane depolarization and opening of a voltage gated calcium channel. The resultant influx of calcium ions leads to fusion of the insulin secretory granules with the cell membrane, and the release of insulin.

G-6-p is an important compound at the junction of several metabolic pathways: glycolysis, gluconeogenesis, the pentose phosphate pathway, glycogenesis, and glycogenolysis.

2. The ***isomerization*** of G-6-P (aldose) to fructose-6-phosphate (F-6-P) (ketose) by **Phosphohexose isomerase**.

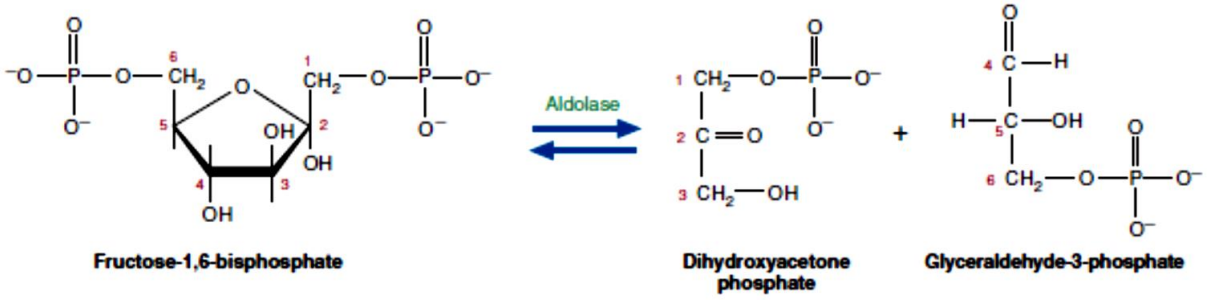


3. The ***phosphorylation*** of F-6-P to fructose 1,6 bisphosphate (F-1,6-BP) by **phosphofructokinase**.

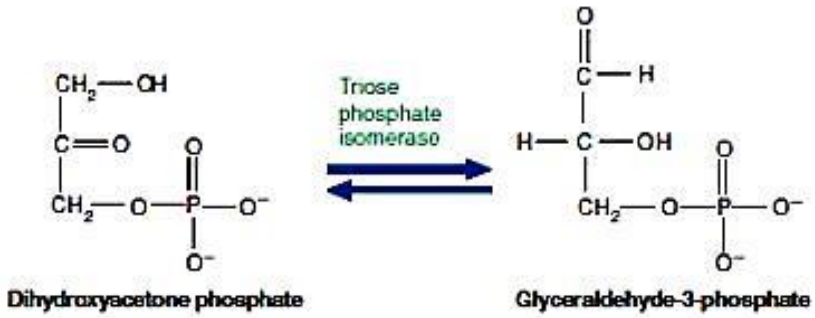


The phosphofructokinase reaction is **irreversible** under physiological conditions. Phosphofructokinase has a major role in regulating the rate of glycolysis.

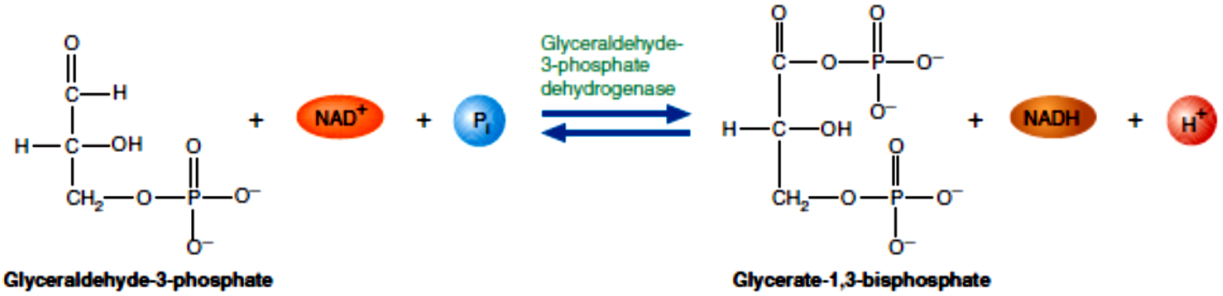
4. The ***cleavage*** of F-1,6-BP by **aldolase**, this yields two three-carbon molecules: glyceraldehyde-3-phosphate (GA-3-P) and dihydroxyacetone phosphate (DHAP).



5. The ***isomerization*** of DHAP to a second molecule of GA-3-P by **triose phosphate isomerase**.



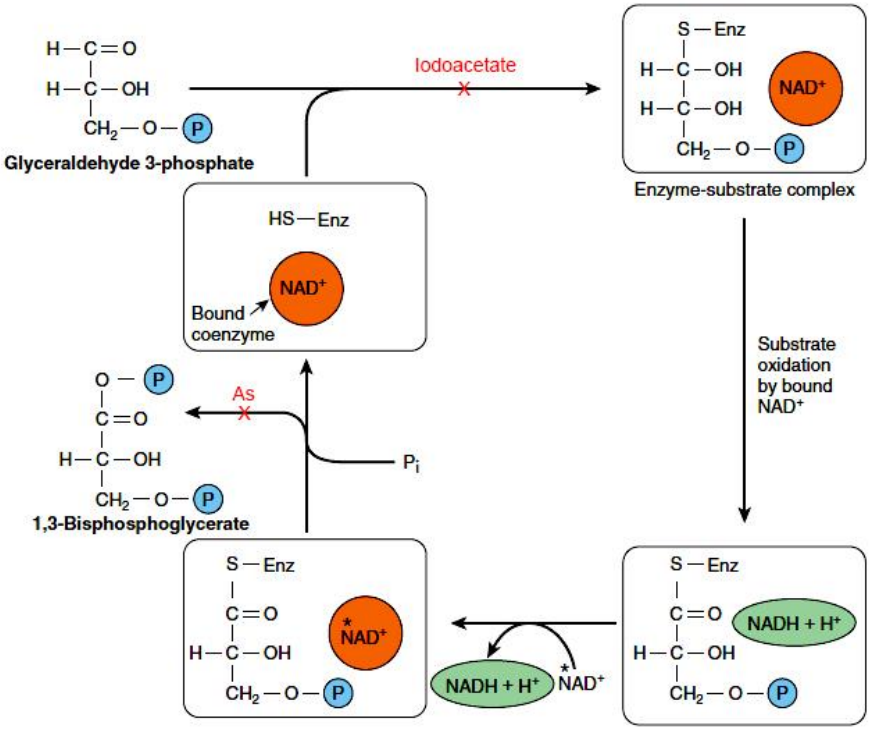
6. The ***dehydrogenation*** and concomitant ***phosphorylation*** of GA-3-P to glycerate-1,3-bisphosphate (1,3-BPGA) by **glyceraldehyde-3-phosphate dehydrogenase**.



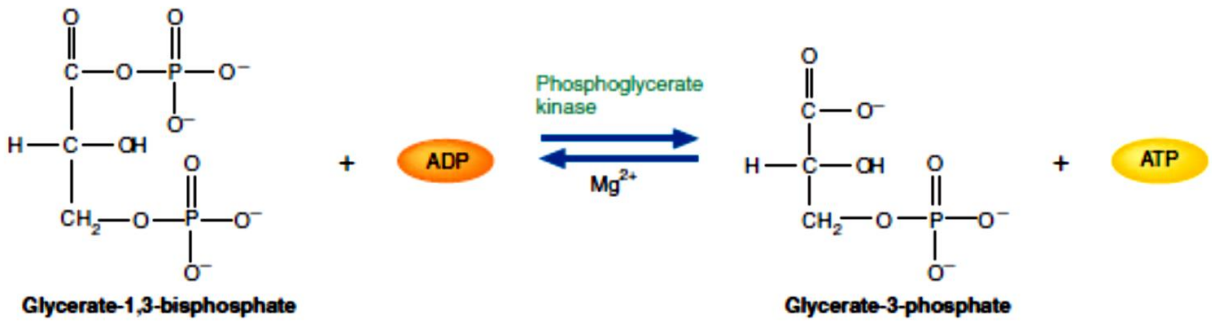
The substrate GA-3-P initially combines with an -SH group present at the active site of the enzyme, forming a thiohemiacetal that is oxidized to a thiol ester; the hydrogens removed in this oxidation are transferred to NAD^+. The thiol ester then undergoes phosphorolysis; inorganic phosphate (Pi) is added, forming 1,3-bisphospho-glycerate , and the free -SH group.

The toxicity of arsenic is the result of competition of arsenate with inorganic phosphate (Pi) in this reaction to give 1-arseno-3-phosphoglycerate, which undergoes spontaneous hydrolysis to 3-phosphoglycerate without forming ATP. The enzyme is also inhibited by the -SH poison iodoacetate.

The oxidation of glyceraldehyde 3-phosphate GA-3-P and the site of action of the site of action of toxicant are shown in the following figure.

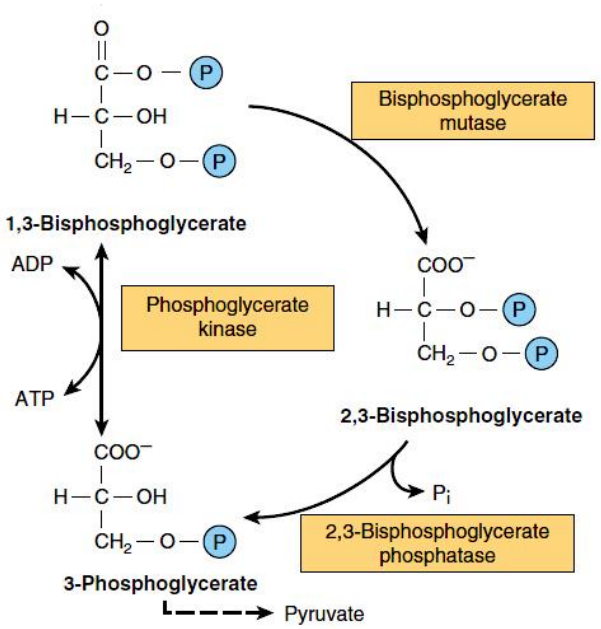


7. The ***phosphoryl group transfer*** from **1,3-BPGA** to ADP by **phosphoglycerate kinase**, which yields ATP and 3-phosphoglycerate (3-PGA).



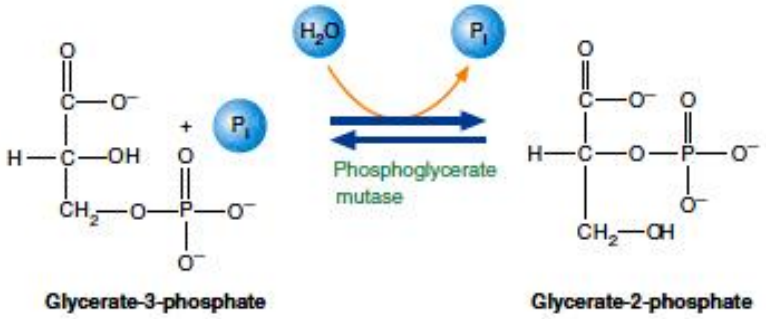
Since two molecules of triose phosphate are formed per molecule of glucose undergoing glycolysis, two molecules of ATP are formed in this reaction per molecule of glucose undergoing glycolysis.

In erythrocytes, the reaction catalyzed by **phosphoglycerate kinase** may be bypassed to some extent by the reaction of **bisphosphoglycerate mutase,** which catalyzes the conversion of 1,3-bisphosphoglycerate to 2,3-bisphosphoglycerate, followed by hydrolysis to 3-phosphoglycerate and Pi, catalyzed by **2,3-bisphosphoglycerate phosphatase;** as in the following figure.

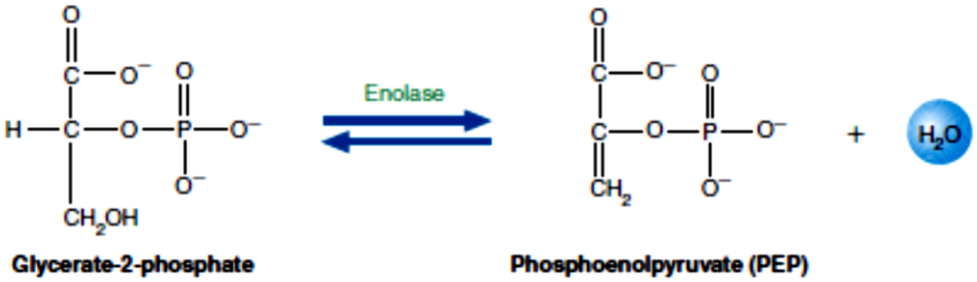


This pathway involves no net yield of ATP from glycolysis, but provides 2,3-bisphosphoglycerate, which binds to hemoglobin, decreasing its affinity for oxygen, so making oxygen more readily available to tissues.

8. The ***isomerization*** of 3-PGA to 2-PGA by **phosphoglycerate mutase**.

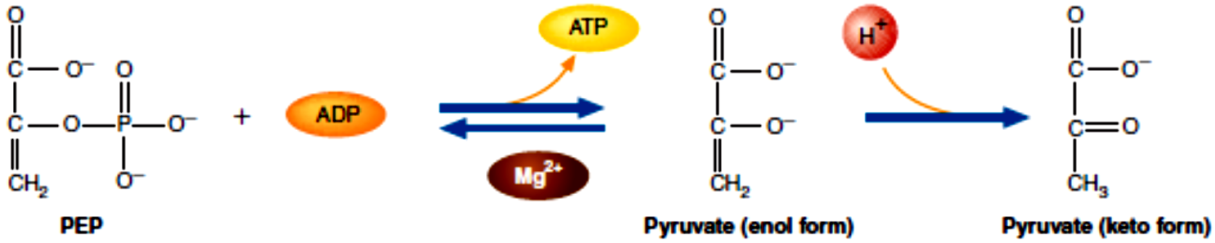


9. The ***dehydration*** of 2-PGA to phosphoenolpyruvate (PEP) by **enolase**.



Enolase is inhibited by fluoride, and when blood samples are taken for measurement of glucose, glycolysis is inhibited by taking the sample into tubes containing fluoride.

10. The ***transfer of the phosphoryl group*** from PEP to ADP by **pyruvate kinase**, to yield a second molecule of ATP.



The reaction of pyruvate kinase is essentially **irreversible** under physiological conditions,

NOTE:

The entire glycolysis pathway can be separated into two phases:

- The Preparatory phase (Reactions 1-5) – in which ATP is consumed (in reactions 1 and 3); hence is also known as the investment phase.
- The Pay Off phase (Reactions 6-10)– in which ATP is produced (in reactions 7 and 10).

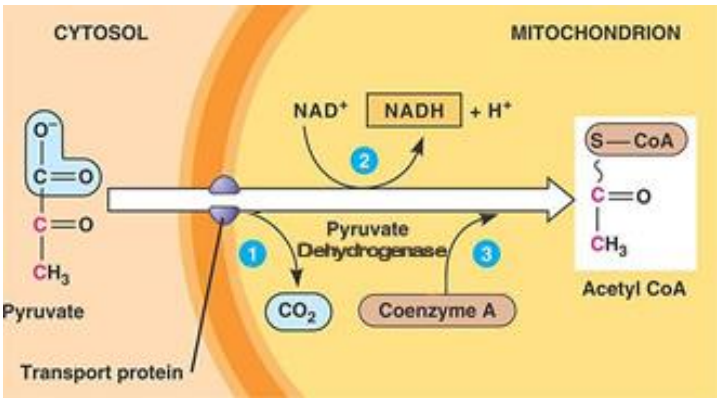
<u>The overall process of glycolysis is:</u>

Glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 Pyruvate + 2 NADH + 2 H^+ + 2 ATP

The Fates of Pyruvate

In terms of energy, the result of glycolysis is the production of two ATPs and two NADHs per molecule of glucose.
Two molecules of ATP are *expended in the initial phosphorylation steps (1 and 3)*. ATP is *gained in steps 7 and 10 (these two steps are called substrate-level phosphorylation steps)*. Since all of the steps from 6 to 10 occur twice per molecule of glucose, the net balance is a gain of two moles of ATP per mole of glucose – a very modest number indeed (considering that the overall yield in complete oxidative degradation is **around 30 moles of ATP**).
Pyruvate, the other product of glycolysis, is still an energy-rich molecule, which can yield a substantial amount of ATP. Whether or not further energy can be produced, however, depends on the ***availability of oxygen*** and the ***cell type***.

Under **aerobic** conditions, most cells in the body convert pyruvate into **acetyl-CoA** by ***pyruvate dehydrogenase complex***, which requires thiamin diphosphate (Vitamin B_1) as a coenzyme.



Note: this reaction occurs in the mitochondria; pyruvate enters the mitochondria via a proton symporter.

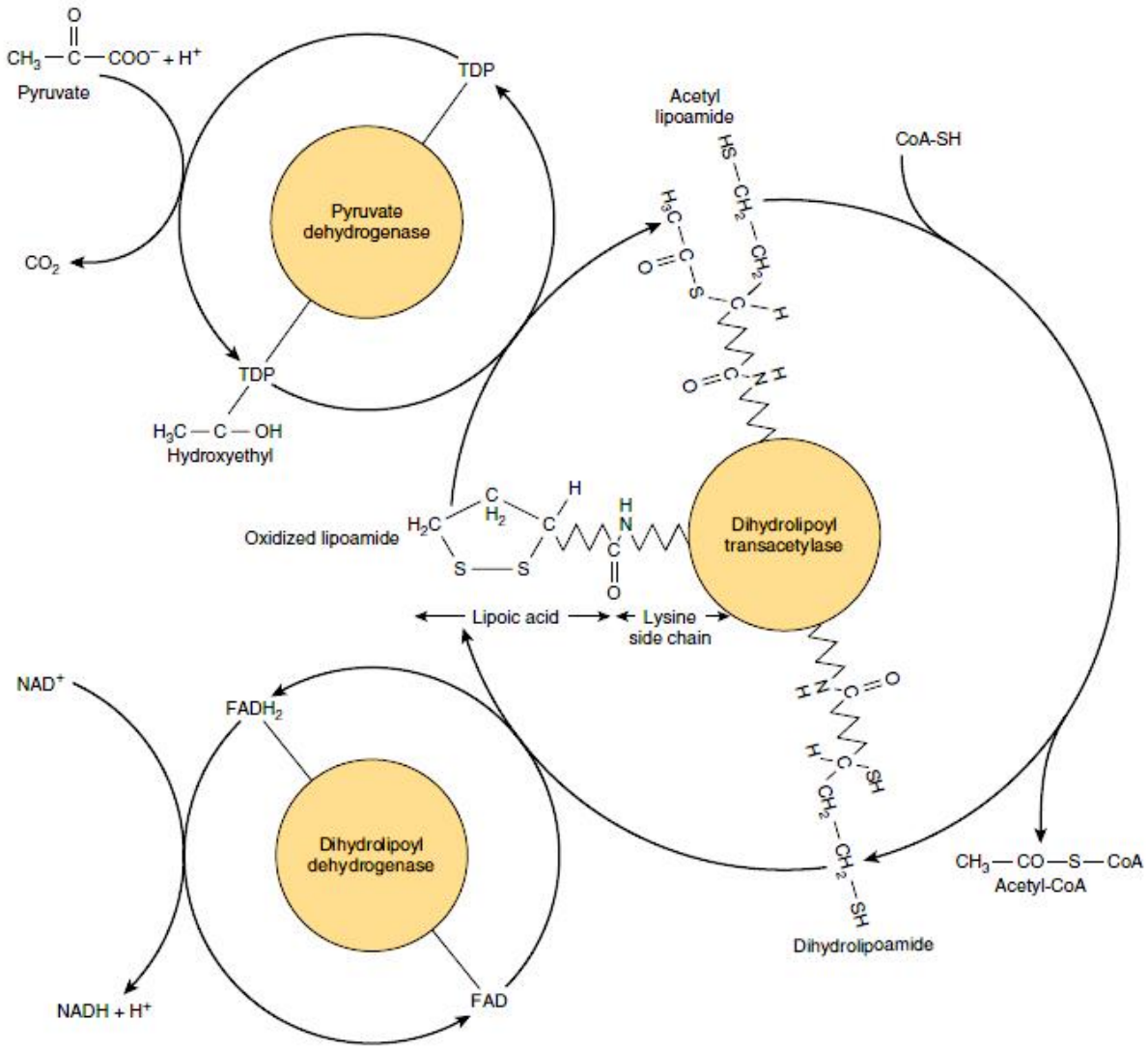
Vitamin B_1 deficiency is associated with impairment of glucose metabolism, with significant (and potentially life-threatening) lactic and pyruvic acidosis.

Pyruvate, formed in the cytosol, is transported into the mitochondrion by a proton symporter. Inside the mitochondrion, it is oxidatively decarboxylated to acetyl-CoA by a multienzyme complex that is associated with the inner mitochondrial membrane.
This **pyruvate dehydrogenase complex** is analogous to the α-ketoglutarate dehydrogenase complex of the citric acid cycle.

pyruvate dehydrogenase complex is composed of three subunits with different enzymatic actions; that are:

1. **Pyruvate dehydrogenase** which act to decarboxylate pyruvate and forming a hydroxyethyl derivative of the thiazole ring of enzyme-bound **thiamin diphosphate.**
   Thiamin is vitamin B1 and in deficiency, glucose metabolism is impaired, and there is significant (and potentially life-threatening) lactic and pyruvic acidosis.
2. **Dihydrolipoyl transacetylase** that contain oxidized lipoamide as a prosthetic group. **Thiamin diphosphate** reacts with the oxidized lipoamide to form acetyl lipoamide, which in turn react with coenzyme A to form acetyl-CoA and reduced lipoamide.
   Note: this subunit forms a long flexible arm, allowing the lipoic acid prosthetic group to rotate sequentially between the active sites of each of the enzymes.
3. **Dihydrolipoyl dehydrogenase** which is a flavoprotein; i.e. containing FAD as prosthetic group.
   Dihydrolipoyl dehydrogenase reoxidzes the reduced lipoamide by FAD, which is reduced into $FADH_2$. $FADH_2$ in turn is reoxized by NAD^+ into FAD and forming NADH. The reducing equivalents is transfered to the respiratory chain.

The following figure is schematic representation of the pyruvate dehydrogenase complex structure and actions.

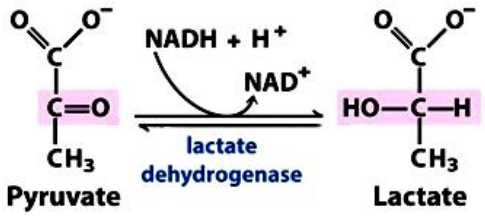


The overall reaction is:

Pyruvate + NAD^+ + CoA → Acetyl-CoA + NADH + H^+ + CO_2

The pyruvate dehydrogenase complex consists of a number of polypeptide chains of each of the three component enzymes, and the intermediates do not dissociate, but are channeled from one enzyme site to the next. This increases the rate of reaction and prevents side reactions, increasing overall efficiency.

Under **anaerobic** condition pyruvate is reduced by ***lactate dehydrogenase*** to **lactate**

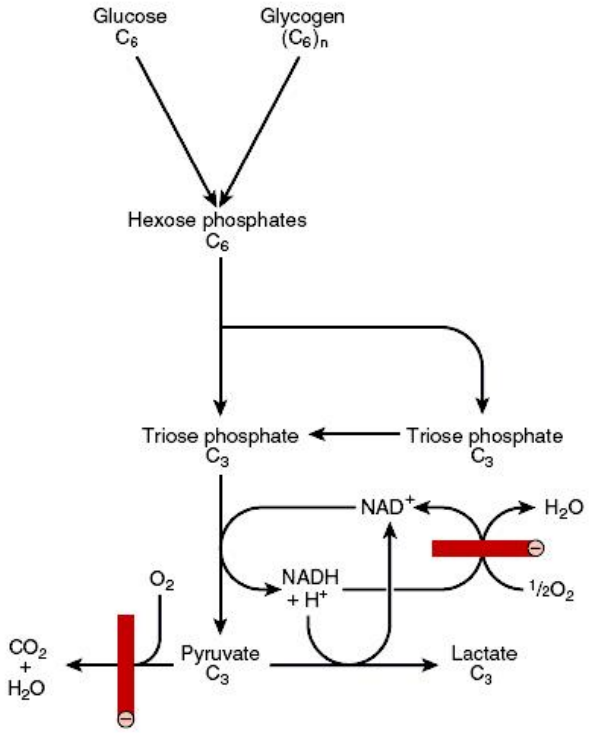


Note: this reaction occurs in the cytosol

This is true of skeletal muscle, where the rate of work output, and hence the need for ATP formation, may exceed the rate at which oxygen can be taken up and utilized.

Glycolysis in erythrocytes always terminates in lactate, because the subsequent reactions of pyruvate oxidation are mitochondrial, and erythrocytes lack mitochondria.

To make oxygen-free (anaerobic) glycolysis feasible, we have to solve one problem. In the glyceraldehyde-3-phosphate dehydrogenase reaction, a molecule of NAD^+ is consumed and converted to NADH. Under aerobic conditions, that is when oxygen is available; NADH is reverted to NAD^+ in the respiratory chain. However, under anaerobic conditions, we need another means to regenerate NAD^+. This problem is overcomed by the reduction of pyruvate to lactate.



The maximal level of exertion of skeletal muscles cannot be kept up for long. One will soon experience exhaustion or tiredness and pain. Exhaustion is due to the depletion of ATP and of glucose, and inhibition of glycolysis due to lactate accumulation which produces acidosis that inhibits the glycolytic enzymes (most importantly PFK); while pain is due to the accumulation of lactate in the tissues and the blood.

Other tissues that normally derive much of their energy from glycolysis and produce lactate include brain, gastrointestinal tract, renal medulla, retina, and skin. Lactate production is also increased in septic shock, and many cancers also produce lactate. The liver (mostly), kidneys, and heart normally take up lactate and oxidize it.

Glycolysis and Pyruvate dehydrogenase Regulation

Although most of the reactions of glycolysis are freely reversible, ***three*** are markedly exergonic and must therefore be considered to be physiologically irreversible.

These reactions, catalyzed by **hexokinase**, **phosphofructokinase**, and **pyruvate kinase**, are the major sites of regulation of glycolysis.

Phosphofructokinase is the most important control element in the mammalian glycolytic pathway. It is significantly inhibited at normal intracellular concentrations of ATP; this inhibition this inhibition can be rapidly relieved by 5′AMP that is formed as ADP begins to accumulate, signaling the need for an increased rate of glycolysis. It is also inhibited by citrate.

Fructose and glucose metabolism converge at the level of the triose-phosphates, and bypasses the main regulatory steps, so resulting in formation of more pyruvate and acetyl-CoA than is required for ATP formation. In the liver and adipose tissue, this leads to increased lipogenesis, and a high intake of fructose may be a factor in the development of obesity.

Pyruvate dehydrogenase is inhibited ***allosterically*** by its products, acetyl-CoA, and NADH. It is also regulated by ***phosphorylation*** (catalyzed by a kinase), resulting in decreased activity and by dephosphorylation (catalyzed by a phosphatase) that causes an increase in activity. The kinase is activated by increases in the [ATP]/[ADP], [acetyl-CoA]/[CoA], and [NADH]/[NAD^+] ratios.

CLINICAL ASPECTS

Because of the dependence of the brain on glucose as a fuel, these metabolic defects commonly cause neurological disturbances.

The exercise capacity of patients with muscle phosphofructokinase deficiency is low, particularly if they are on high-carbohydrate diets. By providing lipid as an alternative fuel, work capacity is improved, when blood free fatty acid and ketone bodies are increased.

Inherited pyruvate kinase and aldolase deficiency in erythrocytes cause hemolytic anemia.

Arsenic and mercuric ions bind with and inhibit pyruvate dehydrogenase, as does a dietary deficiency of thiamin, allowing pyruvate to accumulate. Many alcoholics are thiamin deficient (both because of a poor diet and because alcohol inhibits thiamin absorption), and may develop potentially fatal pyruvic and lactic acidosis. Patients with inherited pyruvate dehydrogenase deficiency, also present with lactic acidosis, particularly after a glucose load.