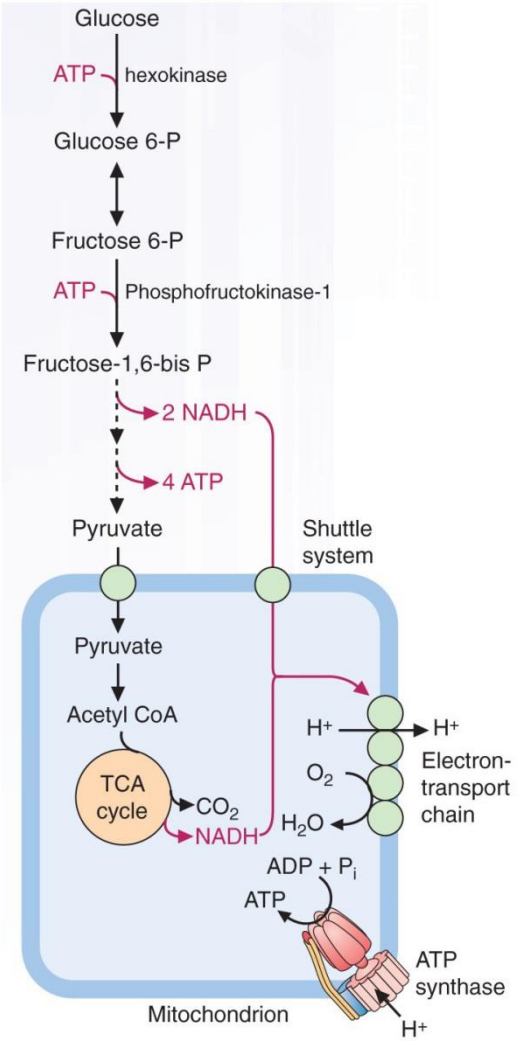
Chapter 22

Generation of Adenosine Triphosphate from Glucose, Fructose, and Galactose: Glycolysis

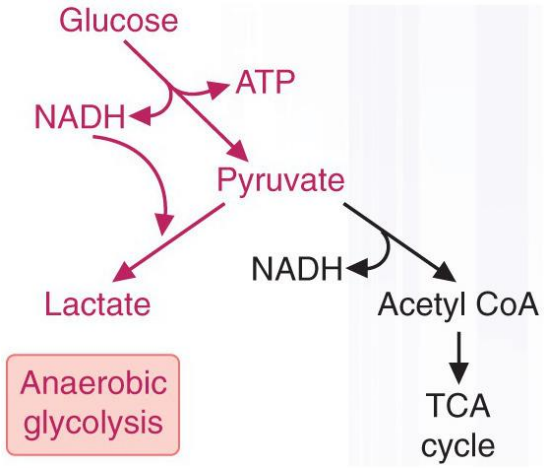
Human Biochemistry



Overview of glycolysis and the tricarboxylic acid (TCA) cycle.

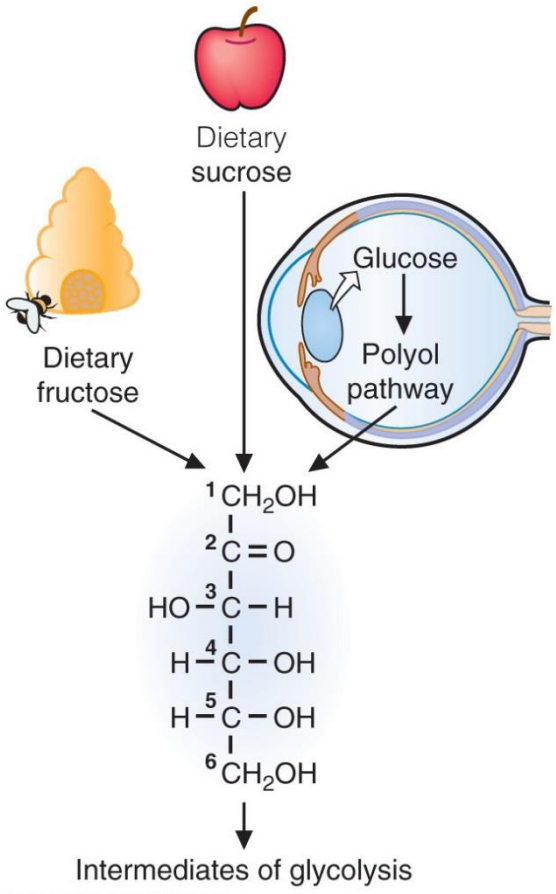
Acetyl CoA, acetyl coenzyme A; ADP, adenosine diphosphate; ATP, adenosine triphosphate; Fructose 6-P, fructose 6-phosphate; Fructose-1,6-bis P, fructose 1,6-biphosphate; Glucose 6-p, glucose 6-phosphate; NADH, reduced nicotinamide adenine dinucleotide; P_i, inorganic phosphate

- **Glycolysis** begins with the phosphorylation of glucose to glucose 6- phosphate (**glucose 6-P**) by **hexokinase** (**HK**). In subsequent steps of the pathway, one glucose 6-P molecule is oxidized to two **pyruvate** molecules with generation of two molecules of **nicotinamide adenine dinucleotide** (**NADH**).
- A net generation of two molecules of ATP occurs through direct transfer of **high energy phosphate** from intermediates of the pathway to adenosine diphosphate (ADP) (**substrate-level phosphorylation**).
- Glycolysis occurs in the **cytosol** and generates cytosolic NADH. Because NADH cannot cross the inner mitochondrial membrane, its reducing equivalents are transferred to the electron-transport chain by either the **malate–aspartate shuttle** or the **glycerol 3-phosphate shuttle**.
- Pyruvate is then oxidized completely to CO_2 by pyruvate dehydrogenase and the tricarboxylic acid (TCA) cycle. Complete **aerobic oxidation** of glucose to CO_2 can generate approximately **30 to 32 mol of ATP per mole of glucose**.
- In each cell, glycolysis is regulated to ensure that **ATP homeostasis** is maintained, without using more glucose than necessary. In most cell types, **hexokinase**, the first enzyme of glycolysis, is inhibited by glucose 6-P.
- Thus, glucose is not taken up and phosphorylated by a cell unless glucose 6-P enters a metabolic pathway, such as glycolysis or glycogen synthesis. The control of glucose 6-P entry into glycolysis occurs at phosphofructokinase-1 (**PFK-1**), the rate-limiting enzyme of the pathway.
- PFK-1 is **allosterically inhibited** by **ATP** and **allosterically activated** by adenosine monophosphate (**AMP**). AMP increases in the cytosol as ATP is hydrolyzed by energy-requiring reactions.



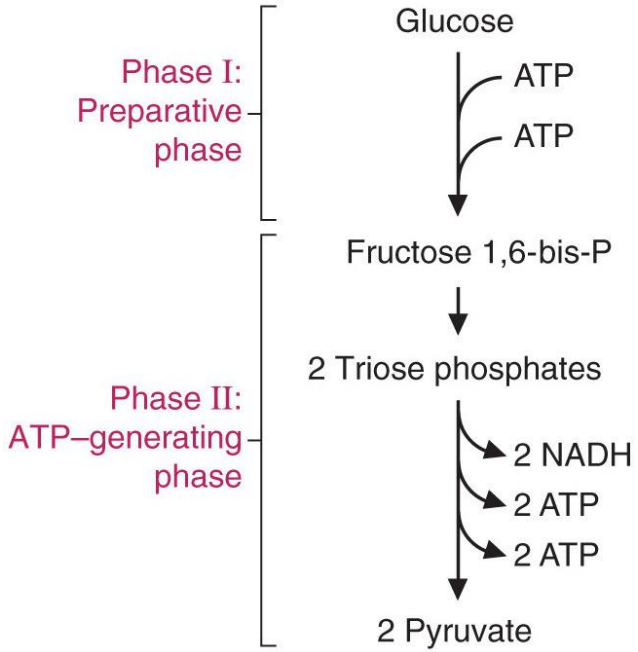
Anaerobic glycolysis (shown in *red*).
The conversion of glucose to lactate generates 2 adenosine triphosphate (ATP) from substrate-level phosphorylation. Because there is no net generation of reduced nicotinamide adenine dinucleotide (NADH), there is no need for O_2, and thus, the pathway is anaerobic. *Acetyl CoA*, acetyl coenzyme A; TCA, tricarboxylic acid

- When cells have a limited supply of oxygen (e.g., the kidney medulla), or few or no mitochondria (e.g., the red cell), or greatly increased demands for ATP (e.g., skeletal muscle during high-intensity exercise), they rely on **anaerobic glycolysis** for generation of ATP. In anaerobic glycolysis, lactate dehydrogenase oxidizes the NADH generated from glycolysis by reducing pyruvate to **lactate**.
- Because O_2 is not required to reoxidize the NADH, the pathway is referred to as anaerobic. The energy yield from anaerobic glycolysis (**2 mol ATP per mole of glucose**) is much lower than the yield from aerobic oxidation. The lactate (lactic acid) is released into the blood. Under pathologic conditions that cause hypoxia, tissues may generate enough lactic acid to cause lactic acidemia.



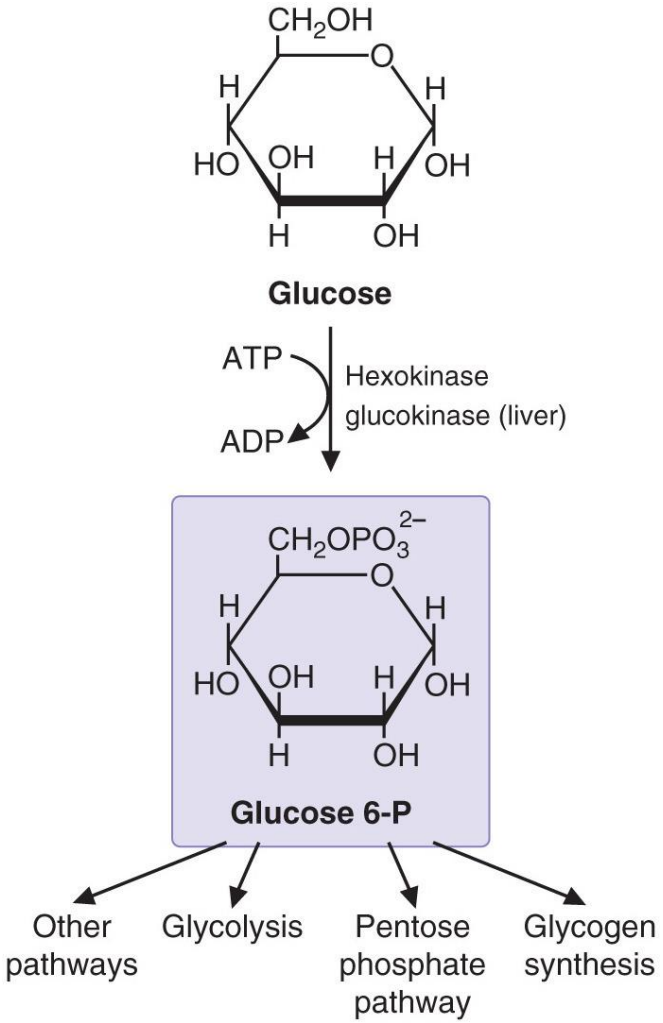
Fructose. The suar fructose is found in the diet as the free sugar in foods such as honey or as a component of the disaccharide sucrose in fruits and sweets. It also can be synthesized from glucose via the polyol pathway. In the lens of the eye, the polyol pathway contributes to the formation of cataracts. Fructose is metabolized by conversion to intermediates of glycolysis.

- **Fructose**, the second most common sugar in the adult diet, is ingested principally as the monosaccharide or as part of **sucrose**. It is metabolized principally in the liver (and to a lesser extent in the small intestine and kidney) by phosphorylation at the I-position to form **fructose I-phosphate** (fructose I-P), followed by conversion to intermediates of the glycolytic pathway.
- The major products of its metabolism in liver are, therefore, the same as for glucose (including lactate, blood glucose, and glycogen). **Essential fructosuria** (**fructokinase deficiency**) and **hereditary fructose intolerance** (a deficiency of the fructose I-P cleavage by **aldolase B**) are inherited disorders of fructose metabolism.
- Fructose synthesis from glucose in the **polyol pathway** occurs in seminal vesicles and other tissues. **Aldose reductase** converts glucose to the sugar alcohol sorbitol (a polyol), which is then oxidized to fructose. In the lens of the eye, elevated levels of **sorbitol** in diabetes mellitus may contribute to formation of **cataracts**.



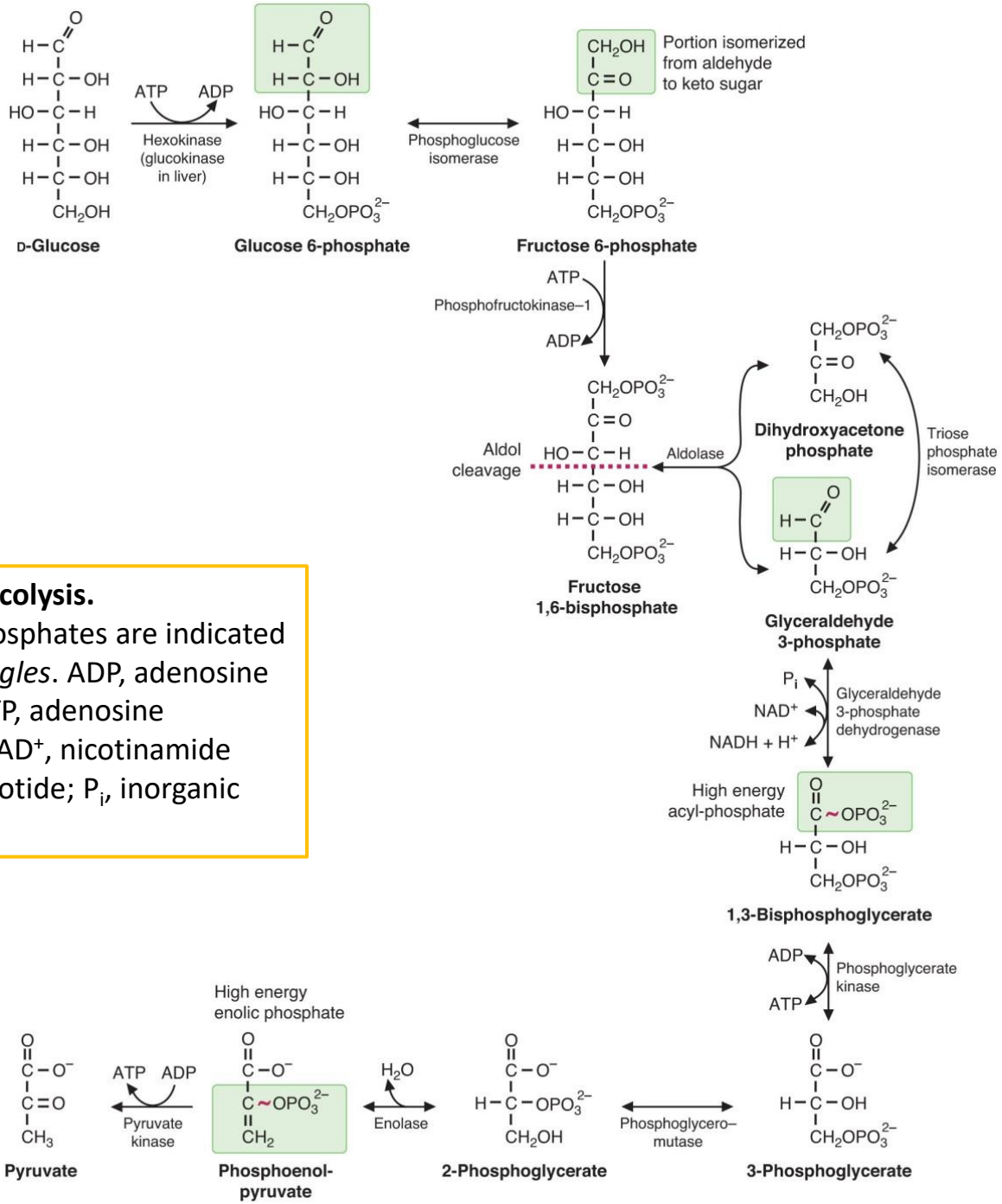
Phases of the glycolytic pathway. ATP, adenosine triphosphate; Fructose-1,6-bis P, fructose 1,6-bisphosphate; NADH, reduced nicotinamide adenine dinucleotide.

- The **glycolytic pathway**, which cleaves 1 mol of glucose to 2 mol of the three carbon compound pyruvate, consists of a preparative phase and an ATP-generating phase. In the initial preparative phase of glycolysis, glucose is phosphorylated twice by ATP and cleaved into two triose phosphates.
- The ATP expenditure in the beginning of the preparative phase is sometimes called "**priming the pump**," because this initial utilization of 2 mol of ATP per mole of glucose results in the production of 4 mol of ATP per mole of glucose in the ATP-generating phase.
- In the ATP-generating phase, glyceraldehyde 3-phosphate (glyceraldehyde 3-P; a triose phosphate) is oxidized by NAD^+ and phosphorylated using inorganic phosphate (P_i). The high-energy phosphate bond generated in this step is transferred to ADP to form ATP.
- The remaining phosphate is also rearranged to form another high-energy phosphate bond that is transferred to ADP. Because 2 mol of triose phosphate were formed, the yield from the ATP-generating phase is 4 mol of ATP and 2 mol of NADH.
- The result is a net yield of **2 mol of ATP, 2 mol of NADH, and 2 mol of pyruvate per mole of glucose.**

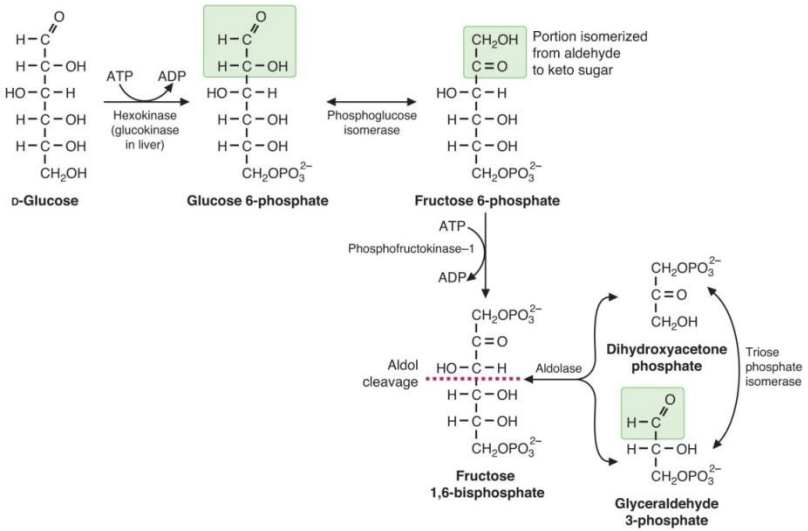


Glucose 6-phosphate (glucose 6-P) metabolism.
ADP, adenosine diphosphate; ATP, adenosine triphosphate

- Glucose metabolism begins with transfer of **a phosphate from ATP to glucose to form glucose 6-P**. Phosphorylation of glucose commits it to metabolism within the cell because glucose 6-P cannot be transported back across the plasma membrane. The phosphorylation reaction is irreversible under physiologic conditions because the reaction has a **high-negative $\Delta G^{0'}$**. Phosphorylation does not, however, commit glucose to glycolysis.
- Glucose 6-P is a branch point in carbohydrate metabolism. It is a precursor for almost every pathway that uses glucose, including **glycolysis**, the **pentose phosphate pathway**, and **glycogen synthesis**. From the opposite point of view, it also can be generated from other pathways of carbohydrate metabolism, such as **glycogenolysis**(breakdown of glycogen), the pentose phosphate pathway, and **gluconeogenesis** (the synthesis of glucose from noncarbohydrate sources).



Reactions of glycolysis.
High-energy phosphates are indicated by the *red squiggles*. ADP, adenosine diphosphate; ATP, adenosine triphosphate; NAD^+, nicotinamide adenine dinucleotide; P_i, inorganic phosphate.

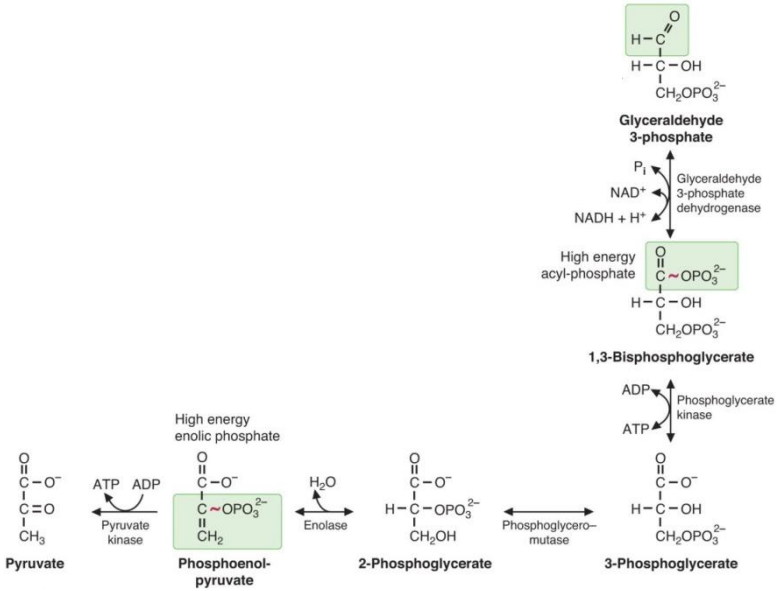


- In the remainder of the preparative phase of glycolysis, **glucose 6-P** is isomerized to fructose 6-phosphate **(fructose 6-P)**, again phosphorylated, and subsequently cleaved into two three-carbon fragments. The isomerization, which positions a keto group next to carbon 3, is essential for the subsequent cleavage of the bond between carbons 3 and 4.

- The next step of glycolysis, phosphorylation of **fructose 6-P** to fructose 1,6-bisphosphate **(fructose 1,6-bisP)** by phosphofructokinase-1 (**PFK-1**), is generally considered the first committed step of the pathway. This phosphorylation requires ATP and is thermodynamically and kinetically irreversible. Therefore, PFK-1 irrevocably commits glucose to the glycolytic pathway. PFK-1 is a regulated enzyme in cells, and its regulation controls the entry of glucose into glycolysis. Like hexokinase, it exists as tissue-specific isoenzymes whose regulatory properties match variations in the role of glycolysis in different tissues.

- **Fructose 1,6-bisP** is cleaved into two phosphorylated three-carbon compounds (**triose phosphates**) by **aldolase**. Dihydroxyacetone phosphate (**DHAP**) is isomerized to **glyceraldehyde 3-P**, which is a triose phosphate. Aldolase is named for the mechanism of the forward reaction, which is an aldol cleavage, and the mechanism of the reverse reaction, which is an aldol condensation. The enzyme exists as tissue-specific isoenzymes, which all catalyze the cleavage of fructose 1,6-bisP but differ in their specificities for fructose 1-P. The enzyme uses a lysine residue at the active site to form a covalent bond with the substrate during the course of the reaction. Inability to form this covalent linkage inactivates the enzyme.

- Thus, at this point in glycolysis, for every mole of glucose that enters the pathway, **2 mol of glyceraldehyde 3-P** are produced and continue through the pathway.



- In the next part of the glycolytic pathway, **glyceraldehyde 3-P** is oxidized and phosphorylated so that subsequent intermediates of glycolysis can donate phosphate to ADP to generate ATP.

- The first reaction in this sequence, catalyzed by **glyceraldehyde 3-P dehydrogenase**, is really the key to the pathway. This enzyme oxidizes the aldehyde group of glyceraldehyde 3-P to an enzyme-bound carboxyl group and transfers the electrons to NAD^+ to form NADH. The oxidation step is dependent on a cysteine residue at the active site of the enzyme, which forms a high-energy thioester bond during the course of the reaction. The high-energy intermediate immediately accepts an inorganic phosphate to form the high-energy acyl phosphate bond in **1,3-bisphosphoglycerate**, releasing the product from the cysteine residue on the enzyme. This high-energy phosphate bond is the start of substrate-level phosphorylation (the formation of a high-energy phosphate bond where none previously existed, without the use of oxygen).

- In the next reaction, the phosphate in this bond is transferred to ADP to form ATP by **phosphoglycerate kinase**. The energy of the acyl phosphate bond is high enough (~10 kcal/mol) so that transfer to ADP is an energetically favorable process. **3-phosphoglycerate** is also a product of this reaction.

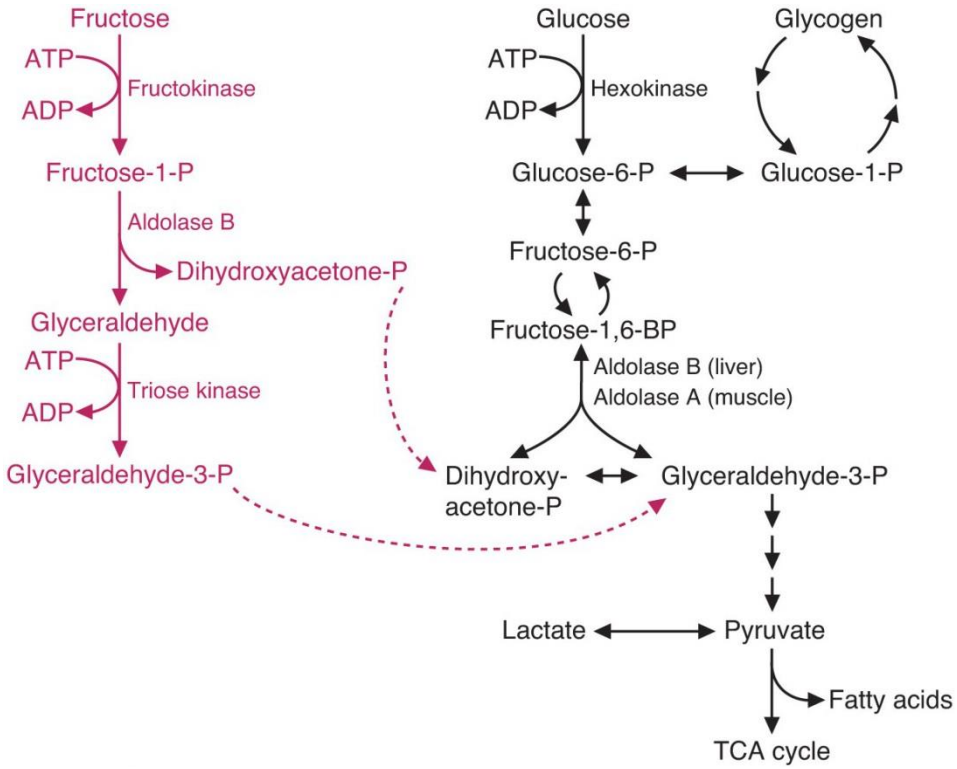
- To transfer the remaining low-energy phosphoester on **3-phosphoglycerate** to ADP, it must be converted into a high-energy bond. This conversion is accomplished by moving the phosphate to the second carbon (forming **2-phosphoglycerate**) and then removing water to form phosphoenolpyruvate (**PEP**).

- The enolphosphate bond is a high-energy bond (its hydrolysis releases approximately 14 kcal/mol of energy), so the transfer of phosphate to ADP by pyruvate kinase is energetically favorable. This final reaction converts **PEP** to **pyruvate**.

- *The overall net reaction in the glycolytic pathway is*

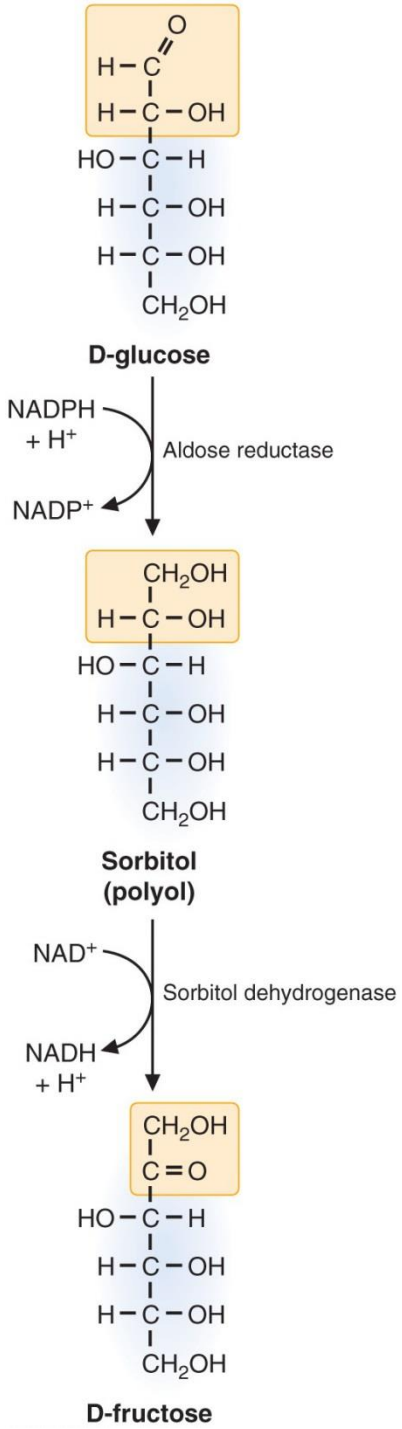
  $$Glucose + 2\ NAD^+ + 2\ P_i + 2\ ADP \rightarrow 2\ pyruvate + 2\ NADH + 4\ H^+ + 2\ ATP + 2\ H_2O$$

- *The pathway occurs with an overall negative $\Delta G^{0'}$ of approximately -22 kcal/mol. Therefore, it cannot be reversed without the expenditure of energy.*



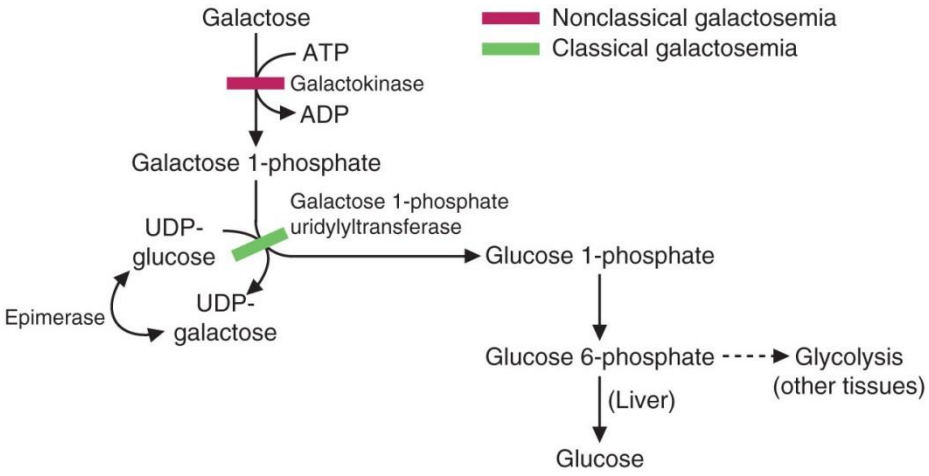
Fructose metabolism.
The pathway for the conversion of fructose to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (glyceraldehyde 3-P) is shown in *red*. These two compounds are intermediates of glycolysis and are converted in the liver principally to glucose, glycogen, or fatty acids. In the liver, aldolase B cleaves both fructose 1-phosphate (fructose 1-P) in the pathway for fructose metabolism and fructose 1,6-bisphosphate in the pathway for glycolysis.

- **Fructose** is metabolized by conversion to **glyceraldehyde 3-P** and **DHAP**, which are intermediates of glycolysis. The steps parallel those of glycolysis. The first step in the metabolism of fructose, as with glucose, is phosphorylation. **Fructokinase**, the major kinase involved, phosphorylates fructose at the 1-position. Fructokinase has high V_{max} and rapidly phosphorylates fructose as it enters the cell. The **fructose 1-P** formed is not an intermediate of glycolysis but rather is cleaved by **aldolase B** to **DHAP** (an intermediate of glycolysis) and **glyceraldehyde**. **Glyceraldehyde** is then phosphorylated to **glyceraldehyde 3-P** by **triose kinase**. **DHAP** and **glyceraldehyde 3-P** are intermediates of the glycolytic pathway and can proceed through it to **pyruvate**, the TCA cycle, and fatty acid synthesis. Alternatively, these intermediates can also be converted to glucose by **gluconeogenesis**. In other words, the fate of fructose parallels that of glucose.



- Fructose can be synthesized from glucose in the ***polyol pathway***. The polyol pathway is named for the first step of the pathway in which sugars are reduced to the sugar alcohol by the enzyme **aldose reductase**.
- Glucose is reduced to the sugar alcohol **sorbitol**, and sorbitol is then oxidized to fructose. This pathway is present in seminal vesicles, which synthesize fructose for the seminal fluid. Spermatozoa use fructose as a major fuel source while in the seminal fluid and then switch to glucose once in the female reproductive tract. Use of fructose is thought to prevent acrosomal breakdown of the plasma membrane (and consequent activation) while the spermatozoa are still in the seminal fluid.
- The polyol pathway is present in many tissues but its function in all tissues is not understood. Aldose reductase is relatively nonspecific, and its major function may be the metabolism of an aldehyde sugar other than glucose. The activity of this enzyme can lead to major problems in the **lens of the eye** where it is responsible for the production of **sorbitol** from **glucose** and **galactitol** from **galactose**. When the concentration of glucose or galactose is elevated in the blood, their respective sugar alcohols are synthesized in the lens more rapidly than they are removed, resulting in increased osmotic pressure within the lens.

The polyol pathway converts glucose to fructose.



Metabolism of galactose.
Galactose is phosphorylated to galactose 1-phosphate (galactose 1-P) by galactokinase. Galactose 1-P reacts with UDP-glucose to release glucose 1-P. Galactose, thus, can be converted to blood glucose, enter glycolysis, or enter any of the metabolic routes of glucose. In classical galactosemia, a deficiency of galactose 1-P uridylyltransferase (shown in *green*) results in the accumulation of galactose 1-P in tissues and the appearance of galactose in the blood and urine. In nonclassical galactosemia, a deficiency of galactokinase (shown in *red*) results in the accumulation of galactose.

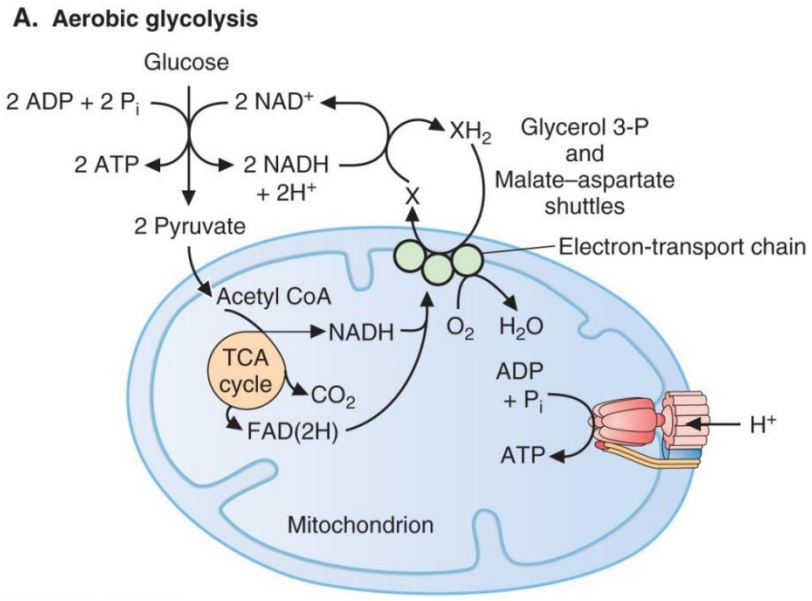
- Dietary **galactose** is metabolized principally by phosphorylation to **galactose 1-P** and then conversion to **UDP-galactose** and **glucose 1-P**. The phosphorylation of galactose, again an important first step in the pathway, is carried out by a specific kinase, **galactokinase**. The formation of UDP-galactose is accomplished by attack of the phosphate oxygen on **galactose 1-P** on the α-phosphate of UDP-glucose, releasing glucose 1-P while forming UDP-galactose. The enzyme that catalyzes this reaction is **galactose l-P uridylyltransferase**. The UDP-galactose is then converted to UDP-glucose by the reversible **UDP-glucose epimerase** (the configuration of the hydroxyl group on carbon 4 is reversed in this reaction). The net result of this sequence of reactions is that **galactose** is converted to **glucose 1-P** at the expense of one high-energy bond of adenosine triphosphate (ATP). The sum of these reactions is indicated in the following equations:

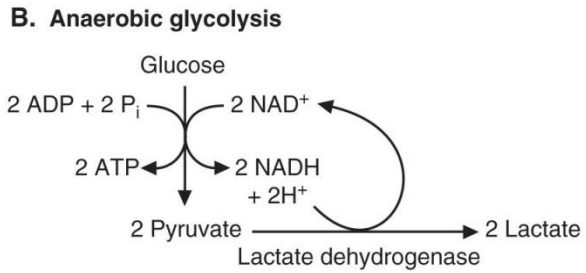
(1) Galactose + ATP $\xrightarrow{\text{Galactokinase}}$ Galactose 1-P + ADP

(2) Galactose 1-P + UDP-glucose $\xrightarrow[\text{Uridylytransferase}]{\text{Galactose 1–P}}$ UDP-galactose + glucose 1-P

(3) UDP-galactose $\xrightarrow{\text{UDP-glucose epimerase}}$ UDP-glucose

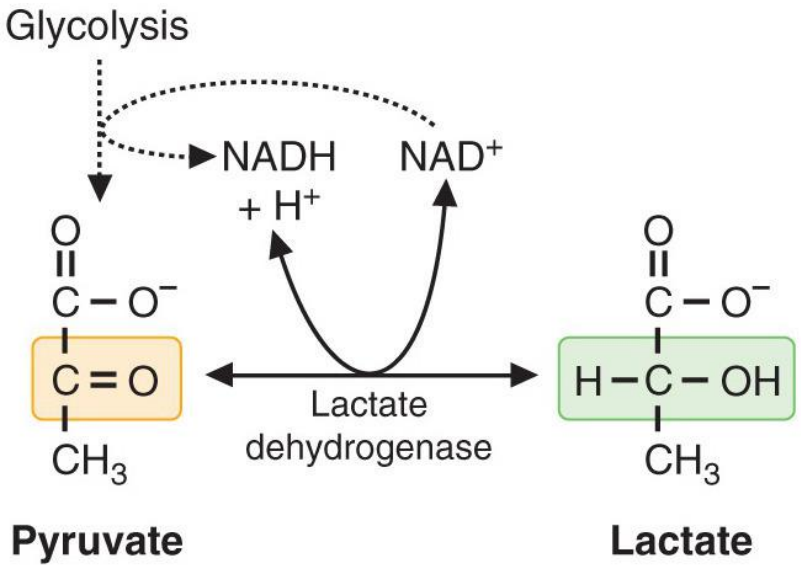
Net equation: Galactose + ATP → Glucose 1-P + ADP





Alternate fates of pyruvate.
A. The pyruvate produced by glycolysis enters mitochondria and is oxidized to CO_2 and H_2O. The reducing equivalents in NADH enter mitochondria via a shuttle system. **B.** Pyruvate is reduced to lactate in the cytosol, thereby using the reducing equivalents in NADH.

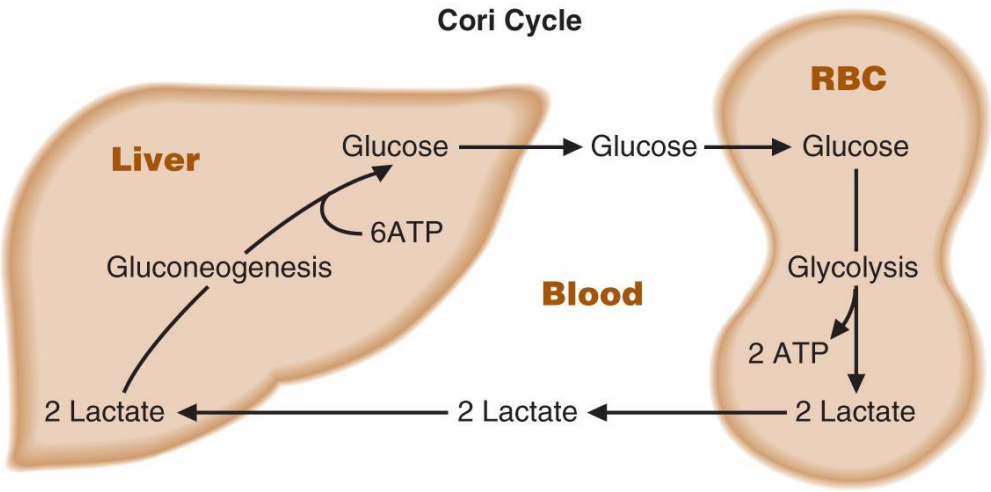
- The **NADH** produced from glycolysis must be continuously reoxidized back to **NAD^+** to provide an **electron acceptor** for the **glyceraldehyde 3-P dehydrogenase** reaction and prevent product inhibition. Without oxidation of this NADH, glycolysis cannot continue. There are two alternate routes for oxidation of cytosolic NADH.
- One route is **aerobic**, involving shuttles that transfer reducing equivalents across the mitochondrial membrane and ultimately to the **ETC** and oxygen. The other route is **anaerobic** (without the use of oxygen). In anaerobic glycolysis, NADH is reoxidized in the cytosol by lactate dehydrogenase (**LDH**), which reduces pyruvate to lactate.
- The fate of **pyruvate** depends on the route used for **NADH oxidation**. If NADH is reoxidized in a shuttle system, pyruvate can be used for other pathways, one of which is oxidation to acetyl coenzyme A (**acetyl-CoA**) and entry into the TCA cycle for complete oxidation. Alternatively, in anaerobic glycolysis, pyruvate is reduced to **lactate** and diverted away from other potential pathways. Thus, the use of the shuttle systems allows for more ATP to be generated than by anaerobic glycolysis, by both oxidizing the cytoplasmically derived NADH in the ETC and by allowing pyruvate to be oxidized completely to CO_2.
- The reason that shuttles are required for the oxidation of cytosolic NADH by the ETC is that the inner mitochondrial membrane is **impermeable** to NADH, and no transport protein exists that can translocate NADH across this membrane directly.



Lactate dehydrogenase reaction.
Pyruvate, which may be produced by glycolysis, is reduced to lactate. The reaction, which occurs in the cytosol, requires NADH and is catalyzed by lactate dehydrogenase. This reaction is readily reversible.

- When the oxidative capacity of a cell is limited (e.g., such as in the red blood cell, which has no mitochondria), the pyruvate and NADH produced from glycolysis cannot be oxidized aerobically. The NADH is therefore oxidized to NAD^+ in the cytosol by reduction of pyruvate to lactate. This reaction is catalyzed by LDH. The **net reaction for anaerobic glycolysis** is

$$Glucose + 2\ ADP + 2\ P_i \rightarrow 2\ lactate + 2\ ATP + 2\ H_2O + 2\ H^+$$

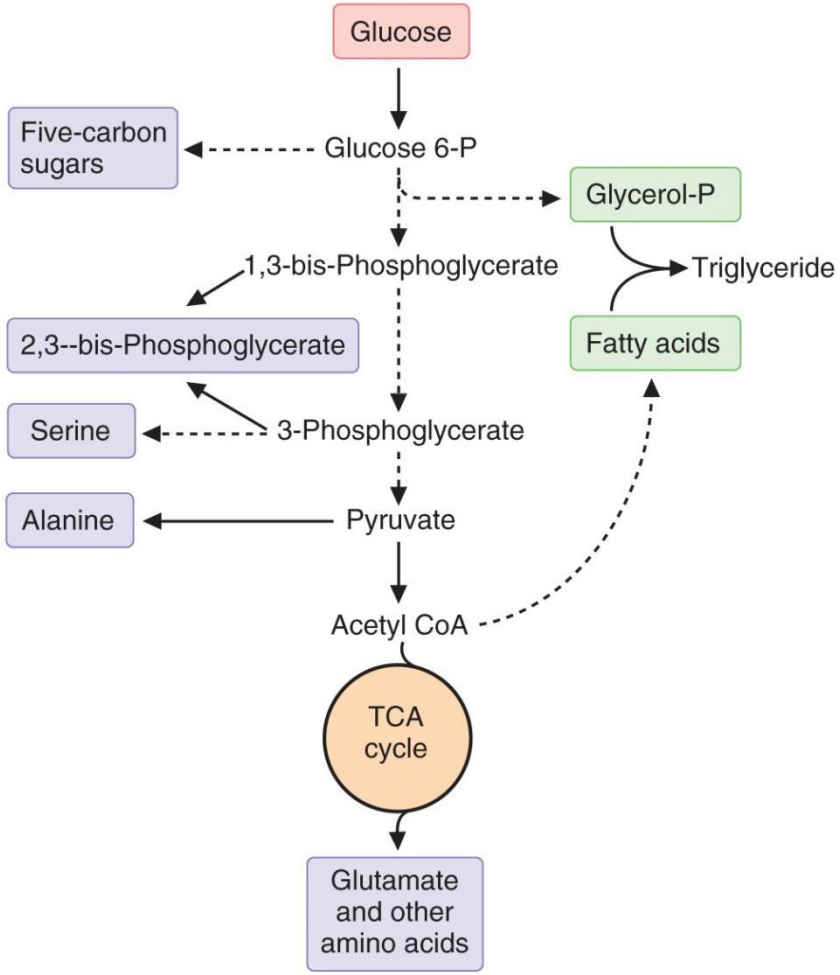


The Cori cycle.
Glucose, produced in the liver by gluconeogenesis, is converted to lactate by glycolysis in muscles, red blood cells (RBC), and many other cells. Lactate returns to the liver and is reconverted to glucose by gluconeogenesis.

- **Lactate** released from cells that undergo anaerobic glycolysis is taken up by other tissues (primarily the liver, heart, and skeletal muscle) and oxidized back to **pyruvate**. In the liver, the pyruvate is used to synthesize glucose (**gluconeogenesis**), which is returned to the blood. The **cycling of lactate** and glucose between peripheral tissues and liver is called the ***Cori cycle***.
- In many other tissues, lactate is oxidized to pyruvate, which is then oxidized to CO_2 in the TCA cycle. Although the equilibrium of the LDH reaction favors lactate production, flux occurs in the opposite direction if NADH is being rapidly oxidized in the ETC (or is being used for gluconeogenesis):

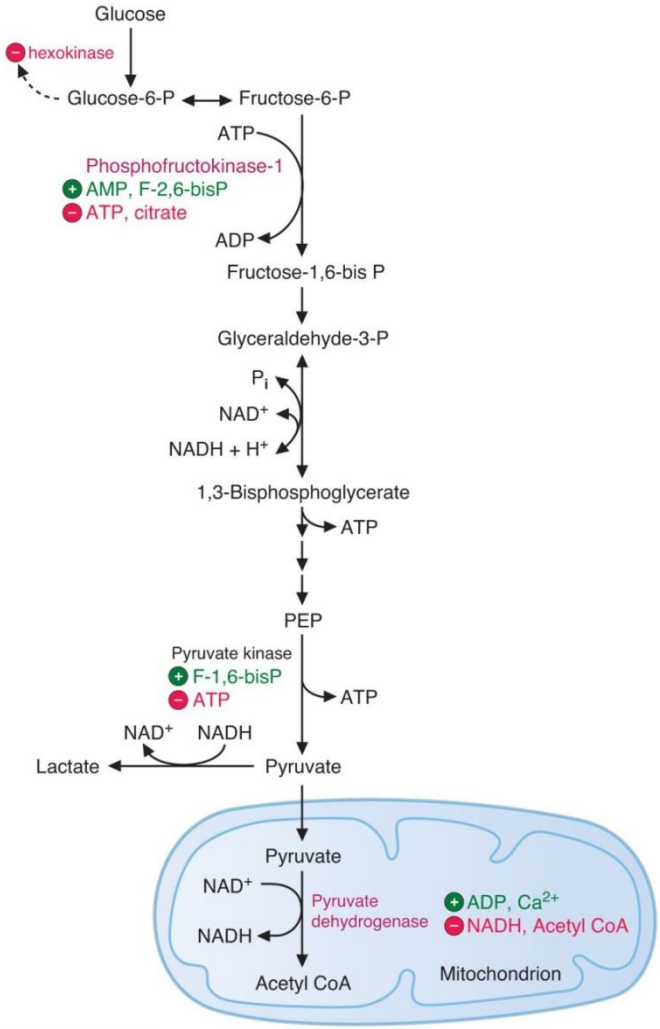
$$\text{Lactate} + \text{NAD} \rightarrow \text{pyruvate} + \text{NADH} + \text{H}^+$$

- The heart, with its huge mitochondrial content and oxidative capacity, is able to use lactate released from other tissues as a fuel. During exercise such as bicycle riding, lactate released into the blood from skeletal muscles in the leg might be used by resting skeletal muscles in the arm. In the brain, glial cells and astrocytes produce lactate, which is used by neurons or released into the blood.



Biosynthetic functions of glycolysis. Compounds formed from intermediates of glycolysis are shown in the *boxes*. These pathways are discussed in later chapters. *Dotted lines* indicate that more than one step is required for the conversion shown in the figure.

- **Glycolysis**, in addition to providing ATP, generates **precursors for biosynthetic pathways**. Intermediates of the pathway can be converted to **ribose 5-phosphate**, the sugar incorporated into nucleotides such as ATP. Other sugars, such as **UDP-glucose**, **mannose**, and **sialic acid**, are also formed from intermediates of glycolysis. Serine is synthesized from **3-phosphoglycerate**, and **alanine** from pyruvate. The backbone of triacylglycerols, **glycerol 3-P**, is derived from DHAP in the glycolytic pathway.
- The liver is the major site of biosynthetic reactions in the body. In addition to those pathways mentioned previously, the liver synthesizes **fatty acids** from the pyruvate generated by glycolysis. It also synthesizes **glucose** from lactate, glycerol 3-P, and amino acids in the gluconeogenic pathway, which is basically a **reversal of glycolysis**. Consequently, in liver, many of the glycolytic enzymes exist as isoenzymes with properties suited for these functions.



Major sites of regulation in the glycolytic pathway.
Hexokinase and phosphofructokinase-1 are the major regulatory enzymes in skeletal muscle. The activity of pyruvate dehydrogenase in the mitochondrion determines whether pyruvate is converted to lactate or to acetyl coenzyme A (acetyl-CoA). The regulation shown for pyruvate kinase occurs only
for the liver (L) isoenzyme.

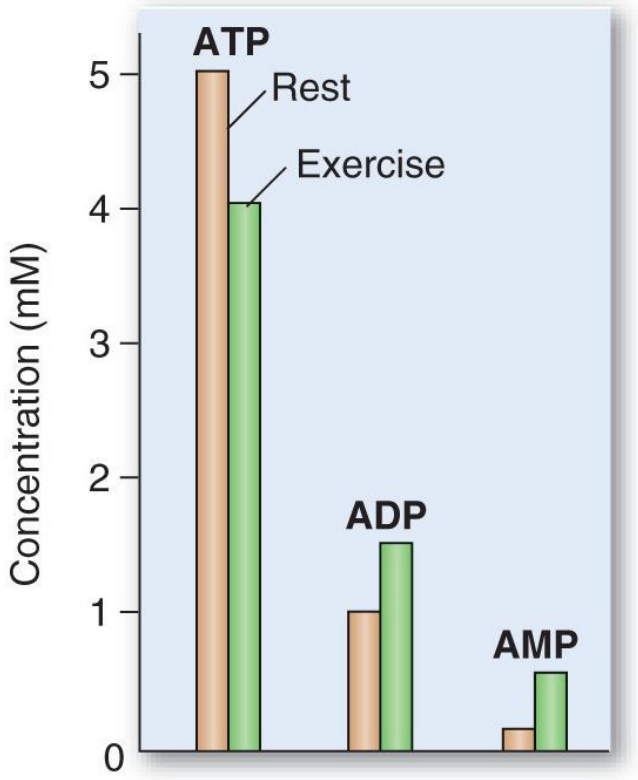
- One of the major functions of glycolysis is the **generation of ATP**, so the pathway is regulated to maintain ATP homeostasis in all cells.

- PFK-1 and PDH, which links glycolysis and the TCA cycle, are both major **regulatory sites** that respond to feedback indicators of the rate of ATP use. The supply of glucose 6-P for glycolysis is tissue-dependent and can be regulated at the steps of glucose transport into cells, glycogenolysis (the degradation of glycogen to form glucose), or the rate of glucose phosphorylation by hexokinase isoenzymes. Other regulatory mechanisms integrate the ATP-generating role of glycolysis with its anabolic roles.

- All of the regulatory enzymes of glycolysis exist as **tissue-specific isoenzymes**, which alter the regulation of the pathway to match variations in conditions and needs in different tissues. For example, in the liver, an isoenzyme of pyruvate kinase introduces an additional regulatory site in glycolysis that contributes to the inhibition of glycolysis when the reverse pathway, gluconeogenesis, is activated.

- **Hexokinases** exist as tissue-specific isoenzymes whose regulatory properties reflect the role of glycolysis in different tissues. In most tissues, hexokinase is a **low-K_m enzyme** with a high affinity for glucose. It is inhibited by physiologic concentrations of its product, glucose 6-P. If glucose 6-P does not enter glycolysis or another pathway, it accumulates and decreases the activity of hexokinase.

- **PFK-1** is the rate-limiting enzyme of glycolysis and controls the rate of glucose 6-P entry into glycolysis in most tissues. PFK-1 is an **allosteric enzyme** that has a total of six binding sites: Two are for substrates (**Mg-ATP** and **fructose 6-P**) and four are allosteric regulatory sites. The allosteric regulatory sites occupy a physically different domain on the enzyme than the catalytic site. When an allosteric effector binds, it changes the conformation at the active site and may activate or inhibit the enzyme. The allosteric sites for PFK-1 include an inhibitory site for Mg-ATP, an inhibitory site for citrate and other anions, an allosteric activation site for AMP, and an allosteric activation site for fructose 2,6-bisphosphate (**fructose 2,6-bisP**) and other bisphosphates. Several different **tissue-specific isoforms** of PFK-1 are affected in different ways by the concentration of these substrates and allosteric effectors but all contain these four allosteric sites.



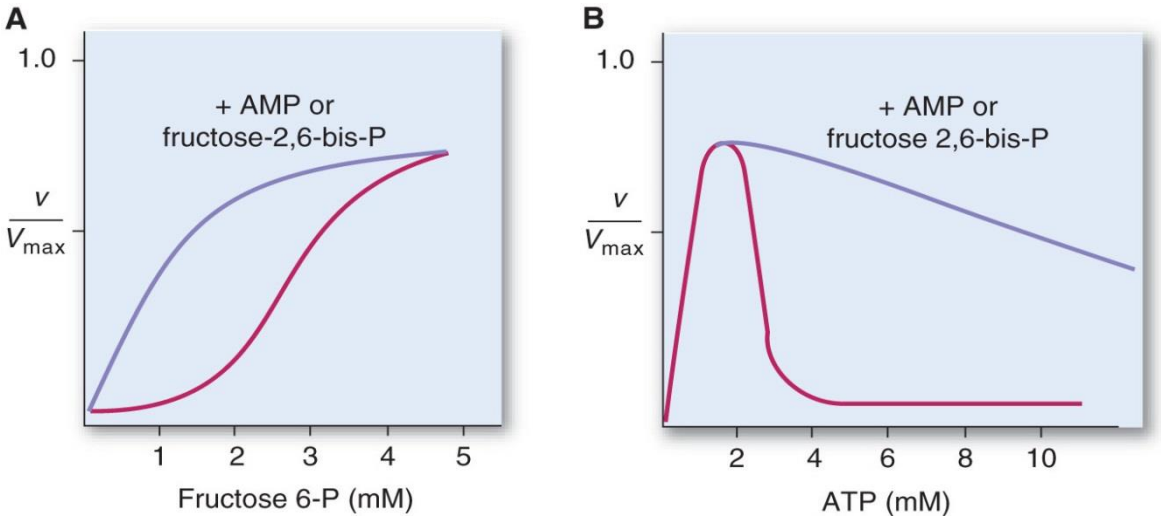
Changes in ATP, ADP, and AMP concentrations in skeletal muscles during exercise.
The concentration of ATP decreases by only approximately 20% during exercise, and the concentration of ADP rises. The concentration of AMP, produced by the adenylate kinase reaction, increases many fold and serves as a sensitive indicator of decreasing ATP levels.

- The **AMP levels** within the cytosol provide a better indicator of the rate of ATP utilization than the ATP concentration itself. The concentration of AMP in the cytosol is determined by the equilibrium position of the adenylate kinase reaction, which catalyzes the following reaction:

$$2\ ADP \leftrightarrow AMP + ATP$$

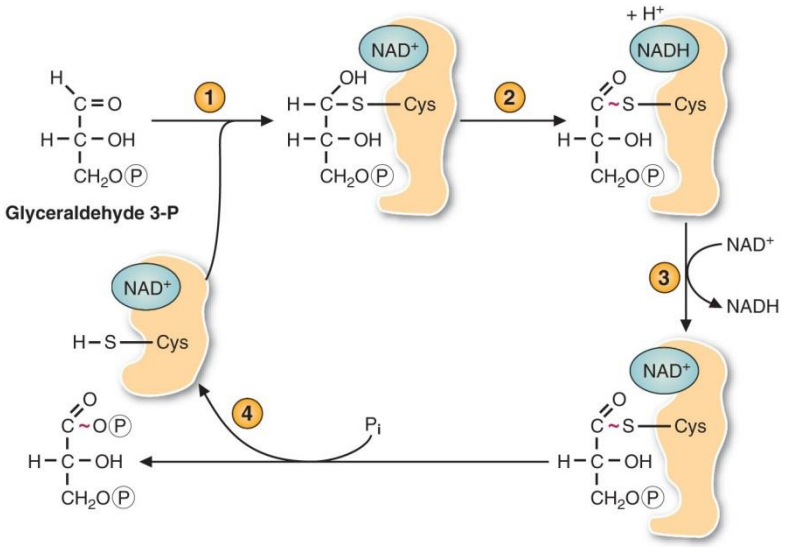
- The equilibrium is such that hydrolysis of ATP to ADP in **energy-requiring reactions** increases both the ADP and AMP contents of the cytosol. However, ATP is present in much higher quantities than AMP or ADP, so a small decrease of ATP concentration in the cytosol causes a much larger percentage increase in the small AMP pool.

- In skeletal muscles, for instance, ATP levels are approximately 5 mM and decrease by no more than 20% during strenuous exercise. At the same time, ADP levels may increase by 50%, and AMP levels, which are in the micromolar range, may increase by 300%. AMP activates several metabolic pathways, including glycolysis, glycogenolysis, and fatty acid oxidation (particularly in muscle tissues), to ensure that **ATP homeostasis** is maintained.



Regulation of PFK-1 by AMP, ATP, and fructose 2,6-bisP.
A. AMP and fructose 2,6-bisP activate PFK-1. **B.** ATP, as a substrate, increases the rate of the reaction at low concentrations but allosterically inhibits the enzyme at high concentrations.

- **ATP** binds to two different sites on the enzyme: the **substrate-binding site** and an **allosteric inhibitory site**. Under physiologic conditions in the cell, the ATP concentration is usually high enough to saturate the substrate-binding site and inhibit the enzyme by binding to the ATP allosteric site. This effect of **ATP** is opposed by **AMP**, which binds to a separate allosteric activator site. For most of the **PFK-1 isoenzymes**, the binding of **AMP** increases the affinity of the enzyme for **fructose 6-P** (e.g., it shifts the kinetic curve to the left). Thus, increases in **AMP concentration** can greatly increase the rate of the enzyme, particularly when **fructose 6-P concentrations** are low.



Mechanism of the glyceraldehyde 3-phosphate dehydrogenase reaction.
(1) The enzyme forms a covalent linkage with the substrate, using a cysteine group at the active site. The enzyme also contains bound NAD^+ close to the active site. *(2)* The substrate is oxidized, forming a high-energy thioester linkage (in *red*) and NADH. *(3)* NADH has a low affinity for the enzyme and is replaced by a new molecule of NAD. *(4)* Inorganic phosphate attacks the thioester linkage, releasing the product 1,3-bisphosphoglycerate and regenerating the active enzyme in a form ready to initiate another reaction.

- This is the work of the **glyceraldehyde 3-P dehydrogenase** reaction, which converts **glyceraldehyde 3-P** to **1,3-BPG**. This reaction can be considered to be two separate half-reactions:
- The first being the oxidation of glyceraldehyde 3-P to 3-phosphoglycerate, and the second being the addition of inorganic phosphate (P_i) to 3-phosphoglycerate to produce 1,3-BPG. The $\Delta G^{0'}$ for the first reaction is approximately -12 kcal/mol; for the second reaction, it is approximately +12 kcal/mol.
- Thus, although the first half-reaction is extremely **favorable**, the second half-reaction is **unfavorable** and does not proceed under cellular conditions. So the enzyme help this reaction through the enzyme forming a covalent bond with the substrate, using an essential cysteine residue at the active site to form a high-energy thioester linkage during the course of the reaction. Thus, the energy that would be released as heat in the oxidation of glyceraldehyde 3-P to 3- phosphoglycerate is conserved in the thioester linkage that is formed (such that the $\Delta G^{0'}$ of the formation of the thioester intermediate from glyceraldehyde 3-P is close to zero).
- Then, replacement of the sulfur with inorganic phosphate to form the final product, 1,3-BPG, is relatively straightforward, as the $\Delta G^{0'}$ for that conversion is also close to zero, and the acylphosphate bond retains the energy from the oxidation of the aldehyde. This is one example of how covalent catalysis by an enzyme can result in the conservation of energy between different bond types.