2.9 ATP Production I: Glycolysis

Learning Objectives

- Be able to draw a diagram showing the relationship among glycolysis, tricarboxylic acid cycle, and electron transport chain
- Explain what is meant in describing ATP as the "energy currency" of the cell
- Write the empirical formula for glucose
- List three sources of glucose in the body
- Define glycogenolysis
- Define gluconeogenesis
- Describe how glycogenolysis is regulated in the liver by epinephrine
- Explain why muscle tissue does not contribute to plasma glucose directly
- Describe how glucose gets into cells
- Describe what is meant by substrate-level phosphorylation
- Explain the function of lactate dehydrogenase during rapid glycolysis
- Describe how the rate of gluconeogenesis can be increased

TAKE A GLOBAL VIEW OF METABOLISM

Intermediary metabolism comprises all of the transformations of biological chemicals that allow the cell to produce energy and synthesize materials that make it up. It is a bewildering array of chemicals and their interconnected pathways. Within this, there are processes that are the composite of many of the individual processes. **Glycolysis**, for example, occupies a special place in the metabolic scheme. We ought to have some appreciation of its place without having to recall all of the transformations that occur within it. The same is true of the **citric acid cycle**, also known as the **Kreb's cycle** or the **tricarboxylic acid cycle**. This set of metabolic transformations is central to energy production in cells. We ought to understand the role of the metabolic pathways without necessarily knowing all of the individual transformations that occur within them.

ENERGY PRODUCTION OCCURS IN THREE STAGES: BREAKDOWN INTO UNITS, FORMATION OF ACETYL COA, AND COMPLETE OXIDATION OF ACETYL COA

Figure 2.9.1 shows the overall plan of energy-producing reactions in cells. These occur in three stages. In Stage 1, foodstuffs consisting of proteins, lipids, and carbohydrates are broken down into their constituent subunits. These are the amino acids, simple sugars like glucose, and fatty acids and glycerol.

In the second stage, these simple subunits are broken down to form **acetyl coenzyme A**. Coenzyme A is a chemical that acts as a carrier for the two-carbon acetyl group, but it is not a carrier in the sense of being transported across a membrane. It is being carried forward in a biochemical reaction. The formation of acetyl CoA is accompanied by the incorporation of some of the energy of the food into ATP, and some limited formation of another compound, **NADH**. NADH is **nicotinamide adenine dinucleotide**. It acts as a carrier for **reducing equivalents**. We will learn more about NADH later on in this chapter. These reducing equivalents are later used to produce ATP in the mitochondria.

The third stage of energy production takes place in the mitochondria and involves the complete **oxidation** of acetyl CoA to water and CO_2 and produces the major proportion of reducing equivalents. The energy stored in NADH produced in this stage is converted to energy stored in ATP via the **electron transport chain** which is coupled indirectly to the **ATP synthetase** in the inner mitochondrial membrane.

ATP IS THE ENERGY CURRENCY OF THE CELL

Electricity is a very versatile form of energy that has come to dominate modern society. We use it to operate heavy machinery, melt metal for casting or extrusion, power drills, pumps, and saws; run television, toasters, ovens, and computers—we use it for almost everything. We generate this electric power by burning coal, natural gas, and even public refuse, but we can also "burn" nuclear material. These methods

DOI: http://dx.doi.org/10.1016/B978-0-12-800883-6.00020-3

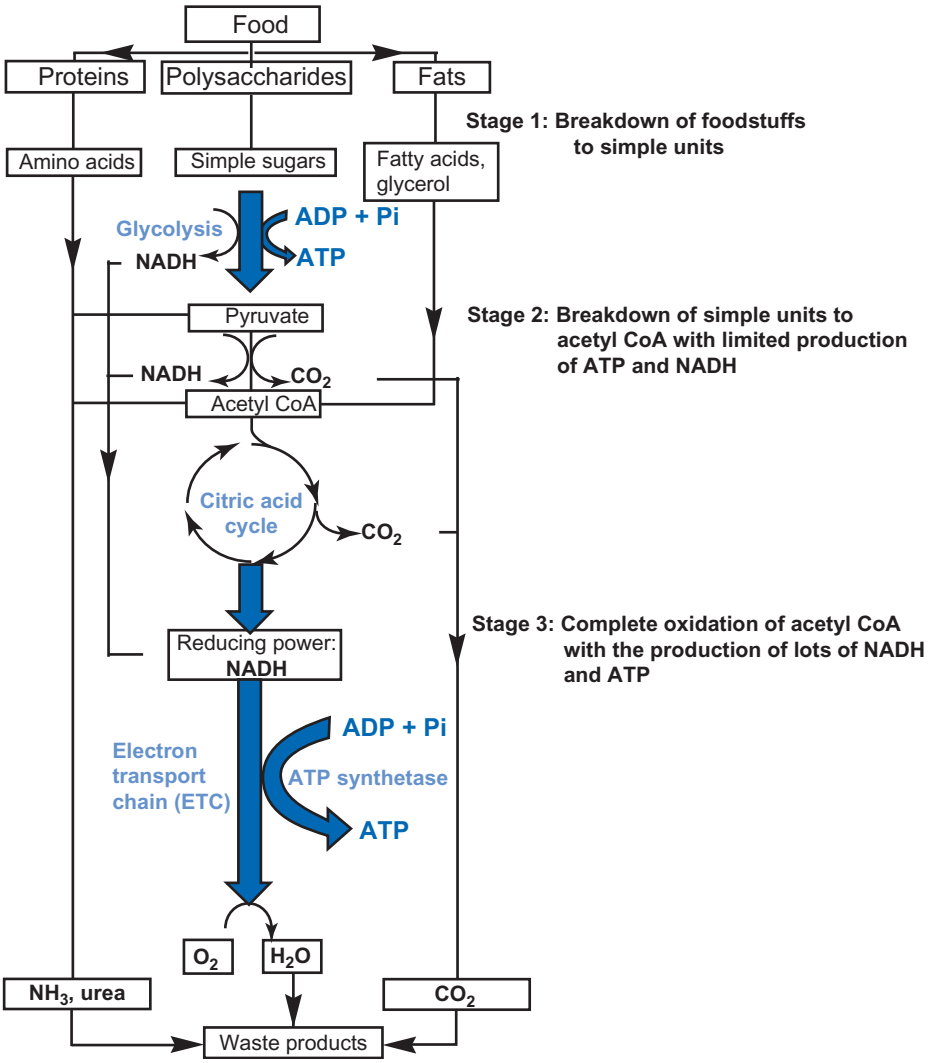


FIGURE 2.9.1 Overall scheme of intermediary metabolism. In the first stage, macronutrients found in food are broken down into their constituent subunits. In the second stage, these are converted to acetyl CoA in a process that produces only a little ATP and NADH. In the third stage, the acetyl CoA is completely oxidized, accompanied by the production of lots of ATP and NADH.

generate power by boiling water to turn a turbine connected to a dynamo. We can also generate electric power by turning a dynamo by moving water or wind. We can also use solar radiation to generate useful electrical power.

Our cells have an analogue of the power plant: the mitochondrion. It does not make electric power, but it does generate chemical energy in the form of ATP. Just like electric power, ATP can be generated from multiple kinds of fuel. Carbohydrates, fats, or amino acids can all be "burned" to produce energy that is stored in the terminal phosphate bond of ATP. Just like societal production of electrical energy, ATP formation has a final common pathway in the mitochondria, the "power house of the cell." Analogous to electricity, ATP can also be produced outside of the mitochondria. Like electricity, this form of chemical energy is very versatile. ATP fuels chemical work such as the synthesis of materials. It fuels mechanical work such as muscle contraction and movement of the cytoskeleton. It fuels electrical work in moving ions across membranes. The extra energy not directly captured by these processes is used to heat the body. All of these activities require ATP to be split into ADP and inorganic phosphate, Pi. The human body continuously splits ATP, and the steady state requires that this continuous splitting is matched to a continuous reformation of ATP from ADP and Pi. This idea is shown in Figure 2.9.2.

FUEL RESERVES ARE STORED IN THE BODY PRIMARILY IN FAT DEPOTS AND GLYCOGEN

Energy that the body uses for movement, biochemical synthesis, and transport all ultimately derives from chemical energy stored in food. However, the body stores some of this energy in its own materials. These include the **fat deposits** in **adipose tissue** and **glycogen** granules stored in the muscles and liver. Energy is not stored as protein deposits, but body proteins can be

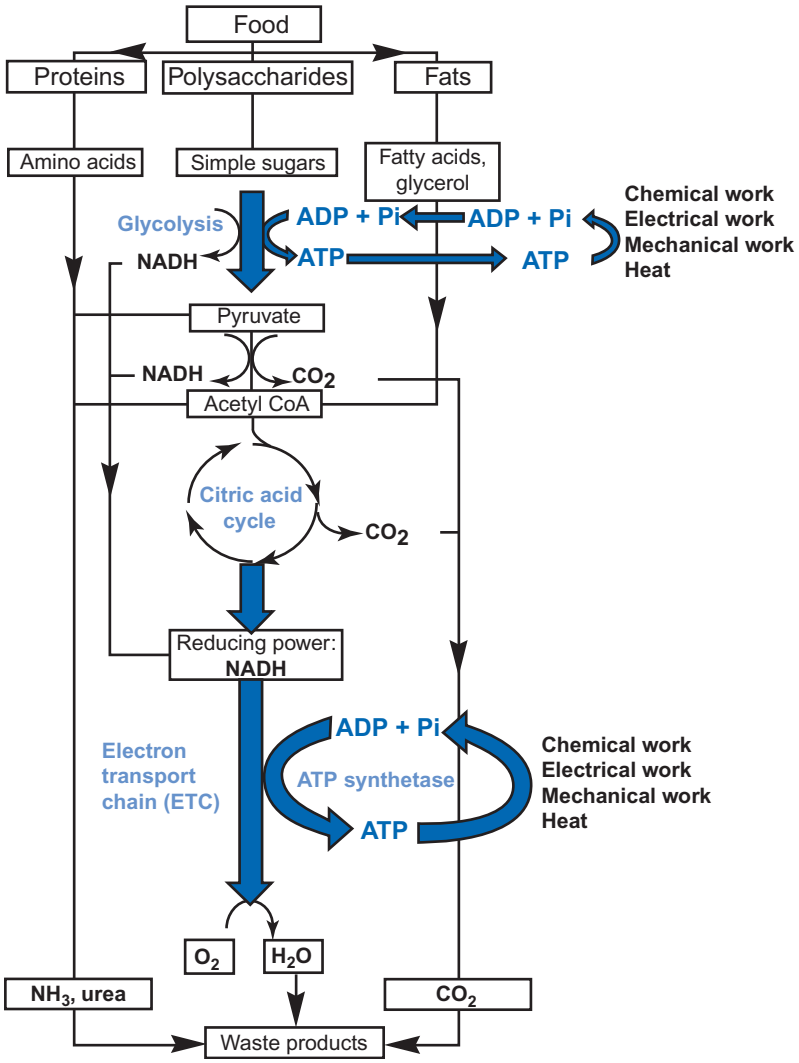
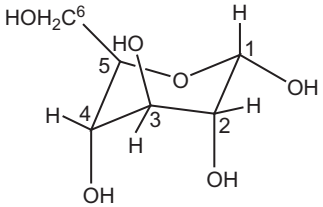


FIGURE 2.9.2 ATP as the energy currency of the cell. ATP is continuously being used for a variety of purposes that include chemical synthesis, production of mechanical force and transport of materials, and movement of ions that constitutes an electrical current. ATP hydrolysis also generates heat. This continuous use of ATP varies with the state of activation. ATP hydrolysis is coupled to resynthesis of ATP in order to maintain a constant supply of energy so that activation that is coupled to increased rates of ATP hydrolysis is simultaneously linked to increased rates of ATP synthesis.

and are continuously used as energy sources. We begin our discussion of energy metabolism with glucose.

GLUCOSE IS A READILY AVAILABLE SOURCE OF ENERGY

Many cells of the body, particularly those in the central nervous system, depend crucially on glucose as an energy source. Glucose is a six-carbon compound with the empirical formula of $C_6H_{12}O_6$. It is called a **carbohydrate** because its chemical formula is close to $C_n(H_2O)_n$, indicating a 1:1 ratio between carbon and water. Thus its empirical formula is equivalent to a hydrated carbon atom. The chemical structure of glucose is shown in Figure 2.9.3. The blood plasma



α-D glucopyranose

FIGURE 2.9.3 Chemical structure of α-D-glucopyranose. Glucose can exist in several configurations, one of which is shown here. The glucose atoms within the molecule are numbered 1 through 6 as shown in the figure. The pyranose ring forms a six-membered structure that approximates a plane. The hydroxyl side groups project from the plane either up or down. At C-1, C-2, and C-4 it is down and at C-3 it is up. When the hydroxyl group is down it is designated as α; when it is up it is designated as β.

typically contains glucose at levels between 80 and 120 mg%. Recall that mg% is mg of glucose per 100 mL of plasma (=1 dL). Glucose enters the circulation from several sources. The first source is directly from foodstuffs. Plant starches in the food we eat are broken down to glucose which is absorbed from the intestine into the portal blood (blood that flows from intestine to liver) and then into the general circulation.

Another source of glucose is from **glycogen** stored in the liver and in muscle. Glycogen is a polymer of glucose in which the glucose subunits are stuck together end to end. There are two ways of doing this, called an α-1,4 glucosidic bond and an α-1,6 glucosidic bond. This nomenclature merely names the numbers of the carbon atoms that are attached to one another and the α signifies the stereochemistry of how the bond is formed. The chemical structure of glycogen is shown in Figure 2.9.4. Glucose is stored as glycogen in many cells, but in large quantities in the muscles and liver. The glucose in glycogen cannot release its chemical energy while it is bound in the glycogen. It must first be broken down to the constituent subunits, the glucose molecules, by a process called **glycogenolysis**. The root word "lysis" means "break down," so glycogenolysis means "glycogen break down." Liver glycogen can contribute to blood glucose, whereas muscle glycogen is converted to glucose in the muscle fiber and used only for muscle activities.

A third source of glucose is from amino acids. Some amino acids can be used to produce glucose through a process called **gluconeogenesis.** Literally, this means "new glucose formation."

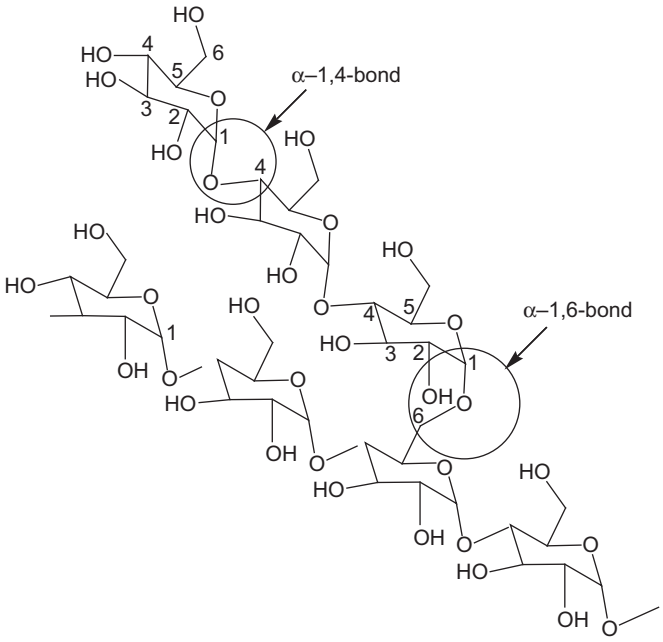


FIGURE 2.9.4 Structure of glycogen. Note that glycogen is a **branched** polymer of glucose. The α-1,4 glucosidic bond connects linear chains of glucose molecules. The α-1,6 glucosidic bond causes the chain to branch.

GLUCOSE RELEASE BY THE LIVER IS CONTROLLED BY HORMONES THROUGH A SECOND MESSENGER SYSTEM

Glycogenolysis in the liver is controlled partly by hormones. A hormone is a material which is released from **secretory cells** in the body that travels through the body via the blood, and has an effect on **target cells** located some distance away (see Chapter 2.8). One of the important hormones regulating glycogenolysis in the liver is **epinephrine.** Epinephrine does not enter the liver cell. It binds to a receptor on the **hepatocyte** (liver cell) surface and a "second messenger" is produced within the cell. The receptor for epinephrine is a G-protein-coupled receptor (**GPCR**), as discussed in Chapter 2.8. The receptor is coupled to a heterotrimeric **G-protein**, a class of protein that binds GTP, guanosine triphosphate. In the case of the epinephrine receptor, the G protein is a Gαs, meaning that the α subunit of the heterotrimeric G-protein stimulates **adenylyl cyclase** to increase the cytosolic concentration of **cyclic AMP** (3′,5′cyclic adenosine monophosphate). The cAMP is the "second messenger" within the hepatocyte.

The cAMP then activates an enzyme, **protein kinase A** (**PKA**), in the liver cell. PKA begins a cascade of phosphorylation reactions that shuts down glycogen synthesis and activates glycogen breakdown according to the scheme shown in Figure 2.9.5.

After activation by cAMP, the system returns to its inactivated state in two ways. First, the cAMP produced by adenylyl cyclase is degraded to AMP (adenosine monophosphate) by another enzyme, **cAMP phosphodiesterase**. This turns off the second messenger signal. Second, **protein phosphatases** dephosphorylate the proteins that were phosphorylated during activation of the cascade. There are four classes of serine/threonine phosphoprotein phosphatases: **PP1**, **PP2a**, **PP2b**, and **PP2c**. PP1 dephosphorylates many of the proteins phosphorylated by PKA. The balance between phosphorylated and dephosphorylated proteins is set by the competing activity of the kinases and phosphatases.

THE LIVER EXPORTS GLUCOSE INTO THE BLOOD BECAUSE IT CAN DEPHOSPHORYLATE GLUCOSE-6-P

In the liver, glycogenolysis ends at glucose-1-P. This is converted to glucose-6-P by another enzyme, **phosphoglucomutase**. The glucose-6-P is then converted to glucose by **glucose-6-phosphatase.** This enzyme is extremely important because only the liver, kidney, and intestine have it, allowing them to release glucose into the blood from glucose-6-P; neither glucose-1-P nor glucose-6-P can exit the cell. Muscle cells have glycogen stores that can be broken down to provide energy, but only for the muscle cell because they lack

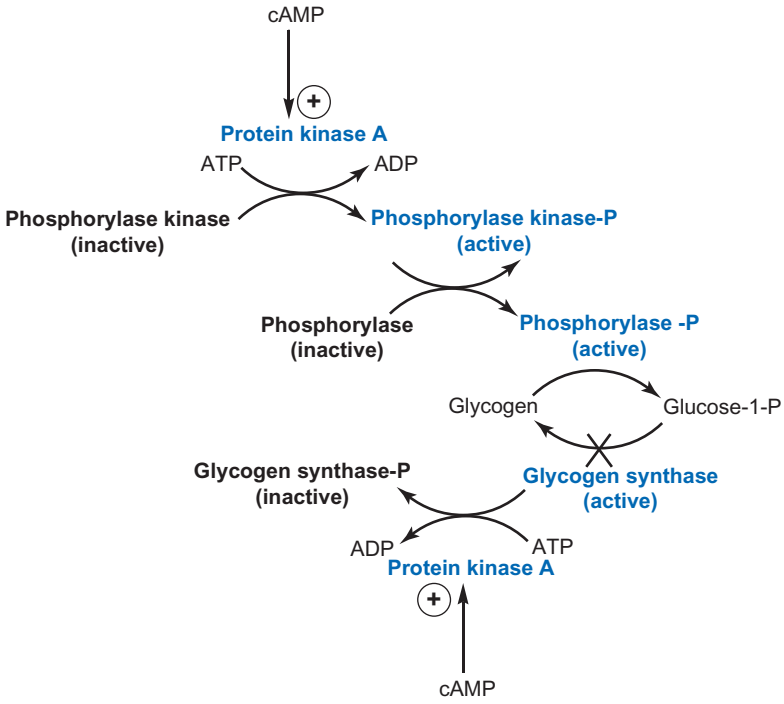


FIGURE 2.9.5 Cascade of activation events to shut down glycogen synthesis and activate glycogenolysis upon stimulation of the liver with epinephrine. Epinephrine binds to a G-Protein-Coupled Receptor on the surface of the hepatocytes which stimulates adenylyl cyclase to increase formation of 3′,5′ cyclic adenosine monophosphate (cAMP). The increased cAMP stimulates protein kinase A, which then phosphorylates the enzyme phosphorylase kinase, so-named because it phosphorylates another enzyme, phosphorylase. Phosphorylase has its name because it phosphorylates glycogen during glycogenolysis to produce glucose-1-phosphate. PKA also phosphorylates glycogen synthase, converting it from its active form to an inactive form.

glucose-6-phosphatase. Muscles cannot contribute glucose to the blood.

A SPECIFIC GLUCOSE CARRIER TAKES GLUCOSE UP INTO CELLS

In muscle cells, glucose can be taken up from the blood by a glucose transporter, GLUT, of which there are multiple isoforms. The one in muscle and fat is GLUT4. The number of these receptors is regulated hormonally, and they exist in a latent form in vesicles stored within the cell. The GLUT4 transporters are particularly sensitive to the hormone **insulin**. Brain, liver, and red blood cells have GLUT transporters that are not regulated by insulin, and therefore these tissues are insensitive to insulin. Muscle cells can also derive glucose from glycogenolysis within the cell. The fate of glucose, whether derived from blood or glycogen, is conversion to pyruvate through the process of **glycolysis**.

GLYCOLYSIS IS A SERIES OF BIOCHEMICAL TRANSFORMATIONS LEADING FROM GLUCOSE TO PYRUVATE

Figures 2.9.6 and 2.9.7 show the reactions of glycolysis that produce pyruvate from glucose. These reactions occur in the cytoplasm. The pyruvate then enters the mitochondria where it is completely oxidized and produces a number of ATP molecules per molecule of pyruvate.

GLYCOLYSIS GENERATES ATP QUICKLY IN THE ABSENCE OF OXYGEN

Glycolysis can generate ATP in the absence of oxygen. This is described as **anaerobic** metabolism. It results from **substrate-level phosphorylation**. This is distinct from **oxidative phosphorylation** that occurs in the mitochondria. Substrate-level phosphorylation refers to the formation of ATP from ADP and a phosphorylated intermediate, rather than from ADP and inorganic phosphate, Pi, as is done in oxidative phosphorylation.

The amount of ATP that is generated by glycolysis is relatively low. Two ATP molecules are required to start glycolysis (from glucose), and four are generated by substrate-level phosphorylation. An additional two NADH molecules are generated, which can be used to generate another three to five ATP molecules through the electron transport chain in the mitochondria. So a net gain of 5–7 moles of ATP can be generated from the conversion of 1 mole of glucose to 2 moles of pyruvate. The total energy in the oxidation of glucose is 2867 kJ mol^{-1}. The energy in 7 moles of ATP is about 7×57.1 kJ $mol^{-1} = 399.7$ kJ mol^{-1}. This represents capture of only some 14% of the total energy available from glucose oxidation.

GLYCOLYSIS REQUIRES NAD^+

Glycolysis occurs in the cytoplasm and it generates some NADH from NAD^+. The NAD^+ is an obligatory substrate for the reaction of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate. If NAD^+ is not

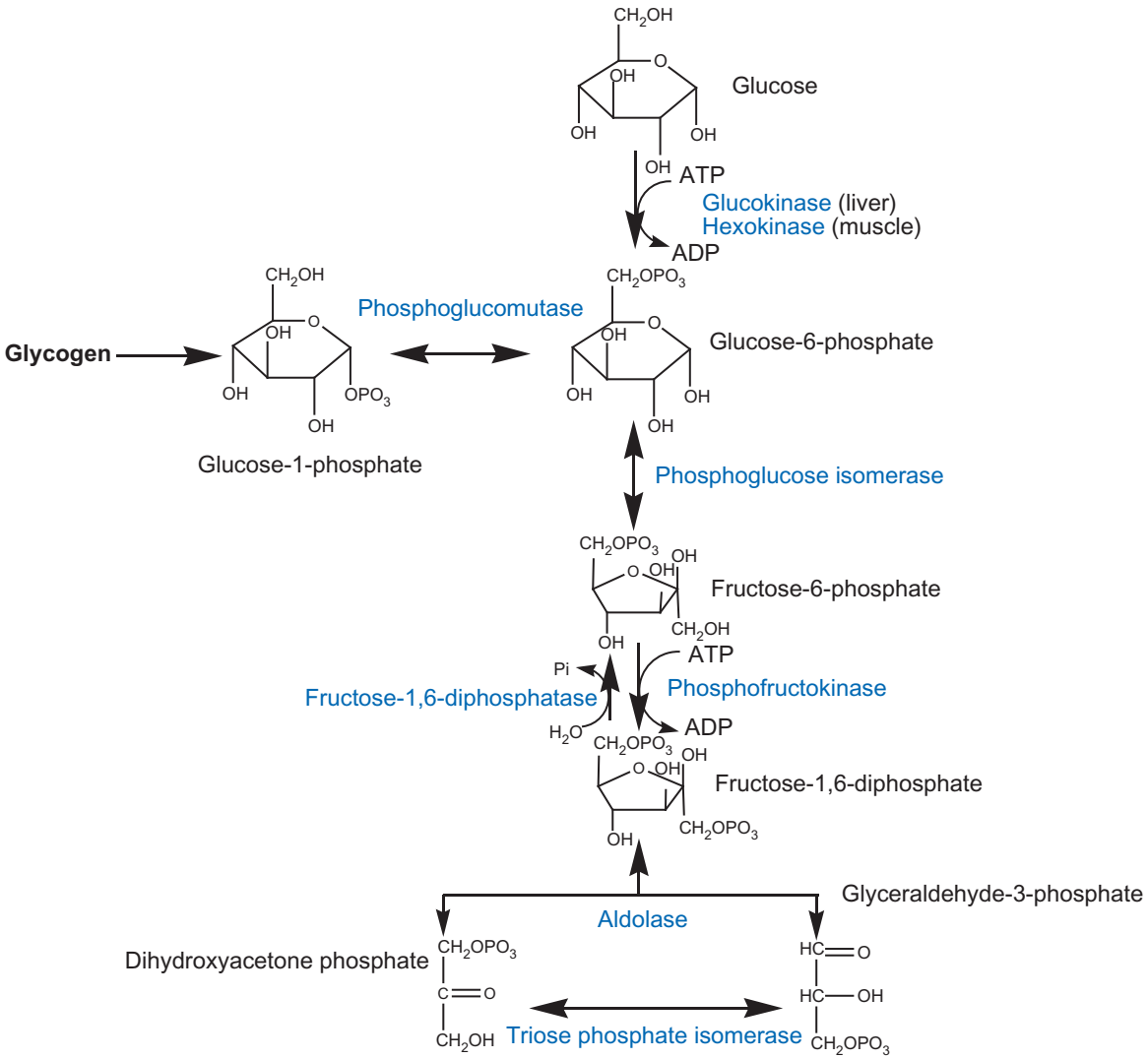


FIGURE 2.9.6 First part of glycolysis, leading from glucose to two three-carbon intermediates that are readily interconvertible. Chemical structures and names of the intermediates are shown in black. The enzymes that participate in the interconversions are shown in blue. Glycolysis begins by phosphorylation of glucose in two successive steps, forming glucose-6-phosphate and then forming fructose-1,6-diphosphate. These steps in glycolysis require ATP to "prime" the process.

regenerated, glycolysis will halt. In the presence of oxygen, NADH is oxidized in the mitochondria to regenerate NAD^+, but NADH itself cannot cross the mitochondrial membrane. Two shuttles transfer the "reducing equivalents" across the mitochondrial membrane. These are the **glycerolphosphate shuttle** and the **malate/aspartate shuttle** (see Chapter 2.10). Figure 2.9.8 illustrates the requirement of glycolysis for NAD^+.

If the glycolytic generation of NADH exceeds the mitochondrial oxidation of cytoplasmic NADH, then cytoplasmic NAD^+ will become depleted and its absence will limit the metabolic flux through glycolysis. Under these conditions, the cell must regenerate NAD^+ from NADH in order to allow glycolysis to continue. This is achieved by making lactic acid from pyruvate through the enzyme **lactate dehydrogenase, LDH**. Lactic acid production occurs all the time, but increases when glycolysis is going faster than the mitochondria can accommodate the metabolic flux of cytoplasmic NADH, regardless of the state of oxygenation of the tissue.

A good example of this occurring physiologically is in muscle during brief strenuous exercise such as a 200-m sprint. In this case, nearly all of the energy will be supplied by glycolysis. In order for glycolysis to continue, the muscle will produce lactic acid, which will leave the muscle and travel to the liver. The oxygen necessary to oxidize the accumulated lactic acid constitutes part of the "oxygen debt" that must be repaid when oxygen is available.

Provided the liver is adequately oxygenated, the liver will reoxidize the lactic acid to pyruvate, which can then

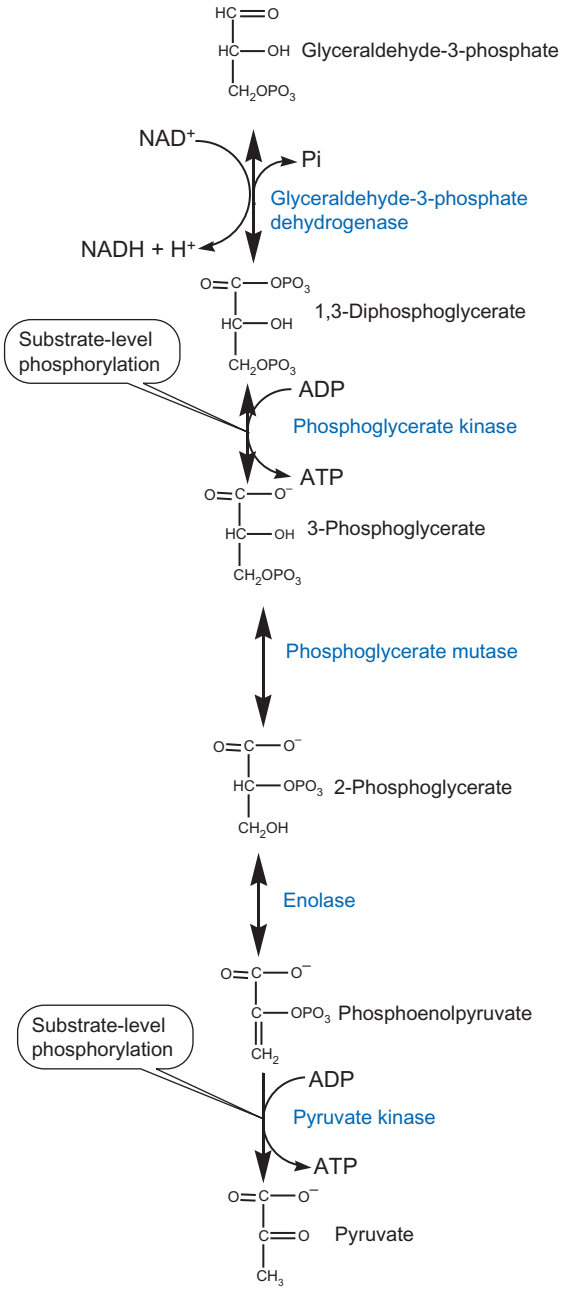


FIGURE 2.9.7 Second part of glycolysis leading from glyceraldehyde-3-P to pyruvate. Chemical structures and names of the intermediates are shown in black. The enzymes that participate in the interconversions are shown in blue. ATP is formed twice in this sequence, once in the conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate and for a second time in the conversion of phosphoenolpyruvate to pyruvate. The formation of ATP directly from phosphorylated intermediary metabolites is called substrate-level phosphorylation. Two molecules of glyceraldehyde-3-phosphate are formed from every molecule of glucose. Thus glycolysis produces 4 ATP per molecule of glucose.

be converted to glucose by gluconeogenesis. The glucose so formed can be released by the liver into the blood for use again by the muscle. The overall process by which muscle glucose becomes blood lactic acid which is converted to muscle glucose is called the **Cori cycle** (see Chapter 3.7).

GLUCONEOGENESIS REQUIRES REVERSAL OF GLYCOLYSIS

Energy transduction in cells involves glycolysis, as we have reviewed it, plus the complete oxidation of pyruvate in the mitochondria, plus the oxidation of other fuels such as fats and proteins. Some tissues (liver, intestine, kidney) export glucose into the blood for the muscles to use during exercise. As mentioned earlier, the liver can mobilize glycogen stores for this purpose, but it can also make new glucose from the amino acids derived from proteins. The process of making new glucose from proteins is called **gluconeogenesis**. It involves chemically transforming the hydrocarbon parts of amino acids into intermediates of carbohydrate metabolism, and then running glycolysis backwards to form glucose. How this is accomplished is illustrated in Figure 2.9.9 for the effect of glucagon on liver cells. Briefly, glucagon activates glycogenolysis through means similar to what we have described earlier for epinephrine. This produces glucose-1-phosphate. In the liver, phosphoglucomutase converts glucose-1-phosphate to glucose-6-phosphate. Glucose-6-phosphatase removes the phosphate from glucose-6-phosphate to produce glucose, which is then released into the blood stream. Activated PKA also phosphorylates **CREB**, the **cyclic AMP responsive element binding protein**. This activates its binding to the **CRE**, **cAMP responsive element**. Activation of CRE increases the transcription of another transcriptional activator that then turns on the synthesis of **PEPCK, phosphoenolpyruvate carboxy kinase**. This enzyme converts **oxaloacetate** to **phosphoenolpyruvate**. The oxaloacetate is a common carbohydrate intermediate formed from the glucogenic amino acids. These are amino acids that form glucose (see Chapter 2.11). PKA also indirectly regulates a key controlling enzyme in glycolysis: **phosphofructokinase 1/fructose biphosphatase 1(PFK1/FBPase1)**. PFK converts fructose-6-phosphate to fructose-1,6-biphosphate; FBPase converts fructose-1,6-biphosphate to fructose-6-phosphate. The FBPase1 activity and PFK1 activities are regulated by cytosolic levels of **fructose-2,6-biphosphate (FBP)**. Fructose-2,6-biphosphate stimulates PFK activity and it inhibits FBPase activity. Fructose-2,6-biphosphate levels are determined by the activity of **phosphofructose kinase 2** and **fructose-2,6-biphosphatase (FBPase2)** which convert fructose-6-phosphate to fructose-2,6-biphosphate. The activities of PFK2/FBPase 2 reside on a single polypeptide chain. PKA phosphorylates PFK2/FBPase2, stimulating the FBPase2 activity and inhibiting the PFK2 activity. This reduces the level of fructose-2,6-biphosphate, which subsequently removes activation of PFK1 and removes inhibition of FBPase1. The net result is an inhibition of PFK1, which thereby slows glycolysis, and activation of FBPase1, which increases gluconeogenesis.

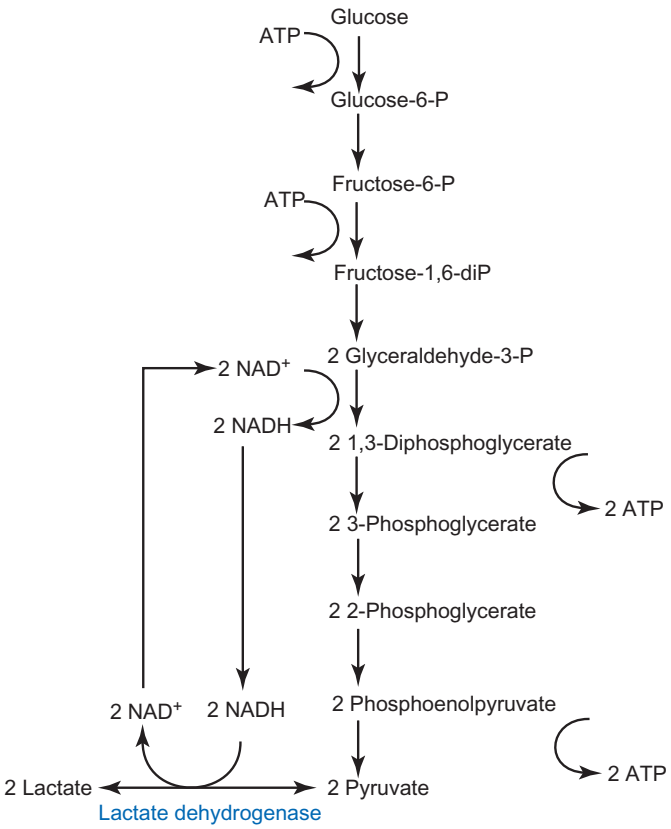


FIGURE 2.9.8 Necessity for regenerating NAD^+ during rapid glycolysis. When NADH oxidation by the mitochondria cannot keep pace with glycolysis, [NAD^+] falls and [NADH] rises. The oxidation of NADH by lactate dehydrogenase, converting pyruvate to lactate, occurs to regenerate NAD^+ so that glycolysis can continue to generate some ATP.

SUMMARY

Cells use chemical energy to power their synthetic, mechanical, and transport work. The chemical energy stored in the terminal phosphate bond of ATP is used as a common energy source for all of these processes. Cells produce ATP by linking the energy of oxidation of foodstuffs to the chemical synthesis of ATP. Oxidation of carbohydrates, fats, and proteins all give rise to ATP as a common energy currency for the cell.

The overall process of energy production occurs in three stages: (1) breakdown of foodstuffs into component units (amino acids for the proteins; fatty acids and glycerol for fats; glucose and fructose for carbohydrates); (2) formation of acetyl CoA with limited formation of ATP and NADH; (3) complete oxidation of acetyl CoA with the production of lots of NADH and ATP through the electron transport chain in the mitochondria.

Carbohydrates provide the most rapid source of ATP. Glucose in the blood can be taken up by tissues through specific glucose transporters in their cell membranes (GLUT1, GLUT4) to provide a ready source of energy. Liver and muscle cells store carbohydrates in a readily usable form called glycogen. Liver can convert glycogen stores to blood glucose but muscle uses its glycogen stores inside the muscle by converting it to glucose-1-phosphate. Glycogen utilization begins with its breakdown into component glucose molecules, a process called glycogenolysis. In liver cells this is regulated by hormones. One important hormone, epinephrine, helps raise blood glucose by mobilizing liver glycogen. It achieves this task by binding to a receptor on the outside surface of hepatocytes. This receptor is coupled to a G-protein, so-named because it binds and then hydrolyzes guanosine triphosphate, GTP. This G-protein consists of three subunits: α, β, and γ. Upon binding of hormone, the α subunit dissociates and activates adenylyl cyclase, which converts intracellular ATP to 3′,5′-cyclic AMP (cAMP). The cAMP then activates protein kinase A (PKA), which phosphorylates a number of target proteins involved in glycogen metabolism. PKA phosphorylates phosphorylase kinase, which then phosphorylates phosphorylase, the enzyme that breaks down glycogen. It also phosphorylates glycogen synthase, inactivating it. In this way, increasing cAMP turns on glycogenolysis and inhibits glycogen synthesis.

The end product of glycogenolysis is glucose-1-phosphate. This is converted to glucose-6-phosphate by phosphoglucomutase. Glucose-6-phosphate can then enter glycolysis, the conversion of glucose to pyruvic acid that occurs in the cytoplasm. Liver cells can convert

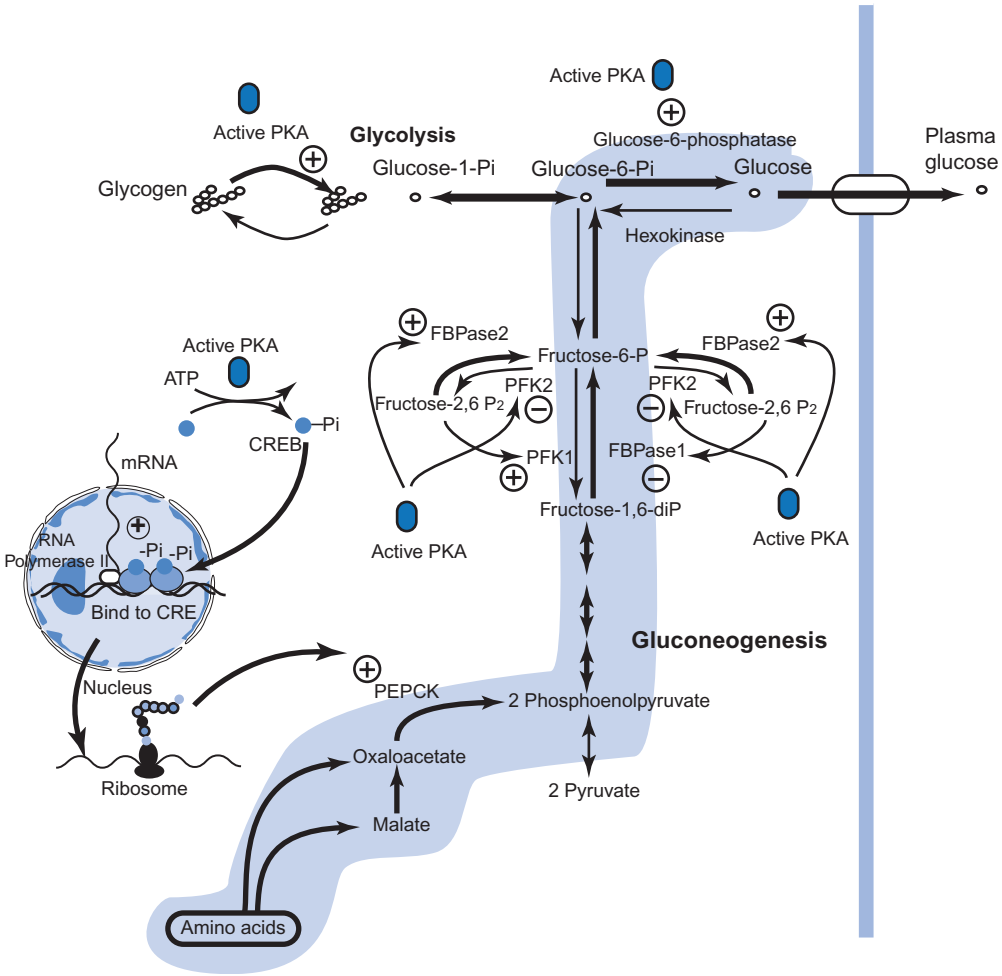


FIGURE 2.9.9 Mechanism of action of glucagon on liver cells to put glucose into the blood. Glucagon increases key processes, indicated by the circled + signs, through increasing cAMP in the hepatocytes. Gluconeogenesis is the synthesis of new glucose from amino acids, indicated by the pathway highlighted in blue. Gluconeogenesis requires the conversion of fructose-1,6 diphosphate to fructose-6-phosphate, the reverse of the reaction that occurs during glycolysis. This is accomplished by inhibiting PFK1 and activating FBPase. Inhibition of PFK1 and activation of FBPase are brought about by decreasing fructose-2,6 diphosphate levels by stimulating FBPase2 and inhibiting PFK2 through phosphorylation mediated by PKA.

glucose-6-phosphate to glucose, which it then exports into the blood. Muscle cells lack glucose-6-phosphatase and so cannot export glucose into the blood.

The first stage in glucose oxidation is glycolysis, in which one molecule of glucose is converted to two molecules of pyruvic acid. Glycolysis generates some ATP by substrate-level phosphorylation (occurring at the level of the phosphorylated intermediates of glycolysis as opposed to synthesis from ADP and Pi). It also requires another compound, nicotinamide adenine dinucleotide (NAD^+). This compound is converted to NADH during glycolysis, and under aerobic conditions is regenerated from NADH by the mitochondria. When glycolysis outstrips the ability of mitochondria to regenerate NAD^+, NADH can be converted to NAD^+ by linking this conversion to the production of lactic acid from pyruvate through the enzyme lactic dehydrogenase. Thus in strenuous activity lactic acid is produced to regenerate NAD^+ so that glycolysis can continue.

REVIEW QUESTIONS

1. What is glucose? Name three processes that supply body cells with glucose.
2. What is glycogen? What is glycolysis? Why can liver convert glycogen to blood glucose but muscle cannot?
3. Why is ATP first consumed in glycolysis instead of being produced? What is substrate-level phosphorylation?
4. Why do muscle cells produce lactic acid during bursts of activity?
5. What is lactate dehydrogenase?
6. What is the Cori cycle?
7. What is gluconeogenesis?