Fuel Oxidation and The Generation of ATP

All physiologic processes in living cells require energy transformation. Cells convert the chemical bond energy in foods into other forms, such as an electrochemical gradient across the plasma membrane, or the movement of muscle fibers in an arm, or assembly of complex molecules such as DNA (Fig. 1). These energy transformations can be divided into three principal phases: (1) oxidation of fuels (fat, carbohydrate, and protein), (2) conversion of energy from fuel oxidation into the high-energy phosphate bonds of ATP, and (3) utilization of ATP phosphate bond energy to drive energy-requiring processes.

The first two phases of energy transformation are part of cellular respiration, the overall process of using O_2 and energy derived from oxidizing fuels to generate ATP. We need to breathe principally because our cells require O_2 to generate adequate amounts of ATP from the oxidation of fuels to CO_2. Cellular respiration uses over 90% of the O_2 we inhale.

In phase 1 of respiration, energy is conserved from fuel oxidation by enzymes that transfer electrons from the fuels to the electron-accepting coenzymes NAD^+ and FAD, which are reduced to NADH and FAD(2H), respectively (Fig. 2). The pathways for the oxidation of most fuels (glucose, fatty acids, ketone bodies, and many amino acids) converge in the generation of the activated 2-carbon acetyl group in acetyl CoA. The complete oxidation of the acetyl group to CO_2 occurs in the tricarboxylic acid (TCA) cycle, which collects the energy mostly as NADH and FAD(2H).

In phase 2 of cellular respiration, the energy derived from fuel oxidation is converted to the high-energy phosphate bonds of ATP by the process of oxidative phosphorylation (see Fig. 2). Electrons are transferred from NADH and FAD(2H) to O_2 by the electron transport chain, a series of electron transfer proteins that are located in the inner mitochondrial membrane. Oxidation of NADH and FAD(2H) by O_2 generates an electrochemical potential across the inner mitochondrial membrane in the form of a transmembrane proton gradient (Δp). This electrochemical potential drives the synthesis of ATP form ADP and Pi by a transmembrane enzyme called ATP synthase (or F_0F_1ATPase).

In phase 3 of cellular respiration, the high-energy phosphate bonds of ATP are used for processes such as muscle contraction (mechanical work), maintaining low intracellular Na^+ concentrations (transport work), synthesis of larger molecules such as DNA in anabolic pathways (biosynthetic work), or detoxification (biochemical work). As a consequence of these processes, ATP is either directly or indirectly hydrolyzed to ADP and inorganic phosphate (Pi), or to AMP and pyrophosphate (PPi).

Cellular respiration occurs in mitochondria (Fig. 3). The mitochondrial matrix, which is the compartment enclosed by the inner mitochondrial membrance, contains almost all of the enzymes for the TCA cycle and oxidation of fatty acids,

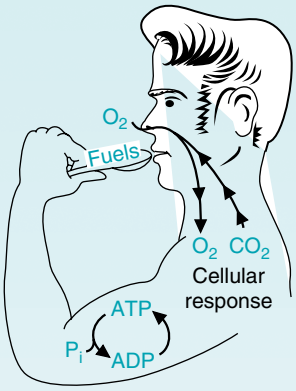


Fig. 1. Energy transformations in fuel metabolism. When ATP energy is transformed into cellular responses, such as muscle contraction, ATP is cleaved to ADP and Pi (inorganic phosphate). In cellular respiration, O_2 is used for regenerating ATP from oxidation of fuels to CO_2.

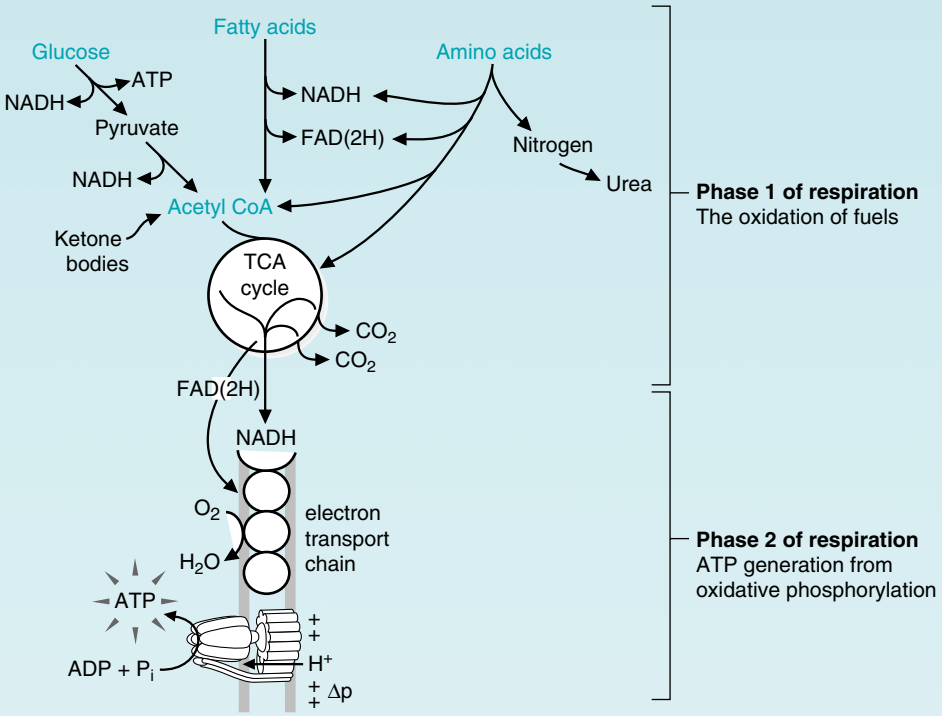


Fig. 2. Cellular respiration. Δp = the proton gradient.

Definitions of prefixes and suffixes used in describing clinical conditions:

an-:	Without
-emia:	Blood
hyper-:	Excessive, above normal
hypo-:	Deficient, below normal
-osis:	Abnormal or diseased state
-uria:	Urine

ketone bodies, and most amino acids. The inner mitochondrial membrane contains the protein complexes of the electron transport chain and ATP synthase, the enzyme complex that generates ATP from ADP and Pi. Some of the subunits of these complexes are encoded by mitochondrial DNA, which resides in the matrix. ATP is generated in the matrix, but most of the energy-using processes in the cell occur outside of the mitochondrion. As a consequence, newly generated ATP must be continuously transported to the cytosol by protein transporters in the impermeable inner mitochondrial membrane and by diffusion through pores in the more permeable outer mitochondrial membrane.

The rates of fuel oxidation and ATP utilization are tightly coordinated through feedback regulation of the electron transport chain and the pathways of fuel oxidation. Thus, if less energy is required for work, more fuel is stored as glycogen or fat in adipose tissue. The basal metabolic rate (BMR), caloric balance, and ΔG (the change in Gibbs free energy, which is the amount of energy available to do useful work) are quantitative ways of describing energy requirements and the energy that can be derived from fuel oxidation. The various types of enzyme regulation described in Chapter 9 are all used to regulate the rate of oxidation of different fuels to meet energy requirements.

Fatty acids are a major fuel in the body. After eating, we store excess fatty acids and carbohydrates that are not oxidized as fat (triacylglycerols) in adipose tissue. Between meals, these fatty acids are released and circulate in blood bound to albumin. In muscle, liver, and other tissues, fatty acids are oxidized to acetyl CoA in the pathway of β-oxidation. NADH and FAD(2H) generated from β-oxidation are reoxidized by O_2 in the electron transport chain, thereby generating ATP (see Fig. 2). Small amounts of certain fatty acids are oxidized through other pathways that convert them to either oxidizable fuels or urinary excretion products (e.g., peroxisomal β-oxidation).

Not all acetyl CoA generated from β-oxidation enters the TCA cycle. In the liver, acetyl CoA generated from β-oxidation of fatty acids can also be converted to the

ketone bodies acetoacetate and β-hydroxybutyrate. Ketone bodies are taken up by muscle and other tissues, which convert them back to acetyl CoA for oxidation in the TCA cycle. They become a major fuel for the brain during prolonged fasting.

Amino acids derived from dietary or body proteins are also potential fuels that can be oxidized to acetyl CoA, or converted to glucose and then oxidized (see Fig. 2). These oxidation pathways, like those of fatty acids, generate NADH or FAD(2H). Ammonia, which can be formed during amino acid oxidation, is toxic. It is therefore converted to urea in the liver and excreted in the urine. There are more than 20 different amino acids, each with a somewhat different pathway for oxidation of the carbon skeleton and conversion of its nitrogen to urea. Because of the complexity of amino acid metabolism, use of amino acids as fuels is considered separately in Section Seven, Nitrogen Metabolism.

Glucose is a universal fuel used to generate ATP in every cell type in the body (Fig. 4). In glycolysis, 1 mole of glucose is converted to 2 moles of pyruvate and 2 moles of NADH by cytosolic enzymes. Small amounts of ATP are generated when high-energy pathway intermediates transfer phosphate to ADP in a process termed substrate level phosphorylation. In aerobic glycolysis, the NADH produced from glycolysis is reoxidized by O_2 via the electron transport chain, and pyruvate enters the TCA cycle. In anaerobic glycolysis, the NADH is reoxidized by conversion of pyruvate to lactate, which enters the blood. Although anaerobic glycolysis has a low ATP yield, it is important for tissues with a low oxygen supply and few mitochondria (e.g., the kidney medulla), or tissues experiencing diminished blood flow (ischemia).

All cells continuously use ATP and require a constant supply of fuels to provide energy for the generation of ATP. Chapters 1 through 3 of this text outline the basic patterns of fuel utilization in the human and provide information about dietary components.

The pathologic consequences of metabolic problems in fuel oxidation can be grouped into 2 categories: (1) lack of a required product, or (2) excess of a substrate or pathway intermediate. The product of fuel oxidation is ATP, and an inadequate rate of ATP production occurs under a wide variety of medical conditions. Extreme conditions that interfere with ATP generation from oxidative phosphorylation, such as complete oxygen deprivation (anoxia), or cyanide poisoning, are fatal. A myocardial infarction is caused by a lack of adequate blood flow to regions of the heart (ischemia), thereby depriving cardiomyocytes of oxygen and fuel. Hyperthyroidism is associated with excessive heat generation from fuel oxidation, and in hypothyroidism, ATP generation can decrease to a fatal level. Conditions such as malnutrition, anorexia nervosa, or excessive alcohol consumption may decrease availability of thiamine, Fe^{2+}, and other vitamins and minerals required by the enzymes of fuel oxidation. Mutations in mitochondrial DNA or nuclear DNA result in deficient ATP generation from oxidative metabolism.

In contrast, problems arising from an excess of substrate or fuel are seen in diabetes mellitus, which may result in a potentially fatal ketoacidosis. Lactic acidosis occurs with problems of oxidative metabolism.

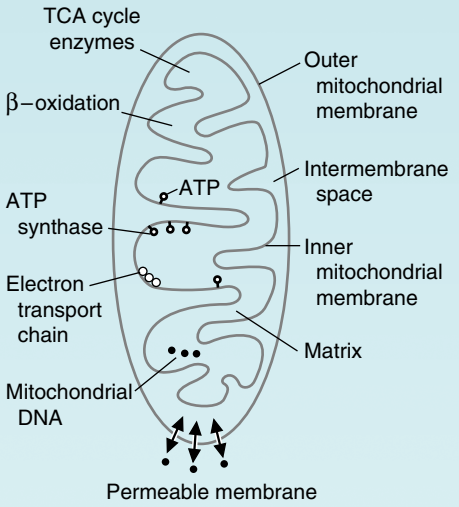


Fig. 3. Oxidative metabolism in mitochondria. The inner mitochondrial membrane forms infoldings, called cristae, which enclose the mitochondrial matrix. Most of the enzymes for the TCA cycle, the β-oxidation of fatty acids, and for mitochondrial DNA synthesis are found in the matrix. ATP synthase and the protein complexes of the electron transport chain are embedded in the inner mitochondrial membrane. The outer mitochondrial membrane is permeable to small ions, but the inner mitochondrial membrane is impermeable.

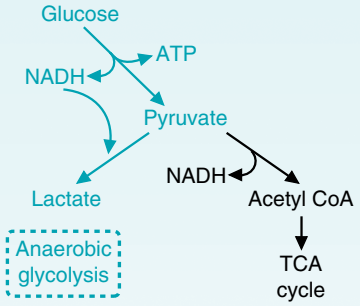


Fig. 4. Glycolysis. In glycolysis, glucose is converted to pyruvate. If the pyruvate is reduced to lactate, the pathway does not require O_2 and is called anaerobic glycolysis. If this pyruvate is converted instead to acetyl CoA and oxidized in the TCA cycle, glycolysis requires O_2 and is aerobic.

19 Cellular Bioenergetics: ATP And O_2

Bioenergetics *refers to* ***cellular energy transformations.***

The ATP-ADP cycle. *In cells, the* ***chemical bond energy*** *of fuels is transformed into the physiologic responses necessary for life. The central role of the* ***high-energy phosphate bonds of ATP*** *in these processes is summarized in the* ***ATP-ADP cycle*** *(Fig. 19.1). To generate ATP through cellular respiration, fuels are degraded by oxidative reactions that transfer most of their* ***chemical bond energy*** *to NAD^+ and FAD to generate the reduced form of these coenzymes,* ***NADH*** *and* ***FAD(2H).*** *When NADH and FAD(2H) are oxidized by O_2 in the electron transport chain, the energy is used to regenerate ATP in the process of* ***oxidative phosphorylation.*** *Energy available from cleavage of the high-energy phosphate bonds of ATP can be used directly for* ***mechanical work*** *(e.g., muscle contraction) or for* ***transport work*** *(e.g., a Na^+ gradient generated by* ***Na^+, K^+-ATPase****). It can also be used for* ***biochemical work*** *(energy-requiring chemical reactions), such as* ***anabolic pathways*** *(biosynthesis of large molecules like proteins) or detoxification reactions.* ***Phosphoryl transfer*** *reactions,* ***protein conformational changes,*** *and the formation of* ***activated intermediates*** *containing* ***high energy bonds*** *(e.g., UDP-sugars) facilitate these energy transformations. Energy released from foods that is not used for work against the environment is transformed into* ***heat.***

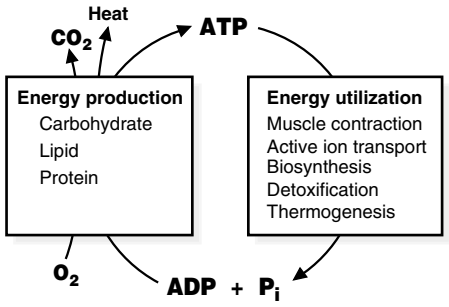


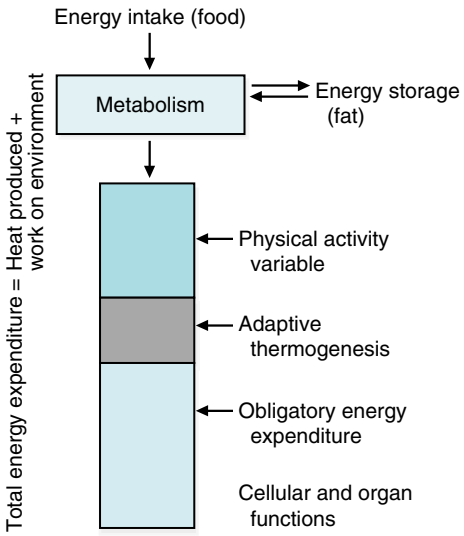
Fig. 19.1. ATP-ADP cycle.

ATP homeostasis. *Fuel oxidation is regulated to maintain* ***ATP homeostasis*** *(homeo, same; stasis, state). Regardless of whether the level of cellular fuel utilization is high (with increased ATP consumption), or low (with decreased ATP consumption), the available ATP within the cell is maintained at a constant level by appropriate increases or decreases in the rate of fuel oxidation. Problems in ATP homeostasis and energy balance occur in obesity, hyperthyroidism, and myocardial infarction.*

Energy from Fuel Oxidation. *Fuel oxidation is* ***exergonic;*** *it releases energy. The maximum quantity of energy released that is available for useful work (e.g., ATP synthesis) is called $\Delta G^{0\prime}$, the* ***change in Gibbs free energy*** *at pH 7.0 under standard conditions. Fuel oxidation has a* ***negative*** *$\Delta G^{0\prime}$, that is, the products have a lower chemical bond energy than the reactants and their formation is energetically favored. ATP synthesis from ADP and inorganic phosphate is* ***endergonic;*** *it requires energy and has a positive $\Delta G^{0\prime}$. To proceed in our cells, all pathways must have a negative $\Delta G^{0\prime}$. How is this accomplished for anabolic pathways such as glycogen synthesis? These metabolic pathways incorporate reactions that expend high-energy bonds to compensate for the energy-requiring steps. Because the $\Delta G^{0\prime}$s for a sequence of reactions* ***are additive,*** *the overall pathway becomes energetically favorable.*

Fuels are oxidized principally by donating electrons to NAD^+ and FAD, which then donate electrons to O_2 in the electron transport chain. The ***caloric value*** *of a fuel is related to its $\Delta G^{0\prime}$ for transfer of electrons to O_2, and its* ***reduction potential,*** **E°′** *(a measure of its willingness to donate, or accept,*

In the thermodynamic perspective of energy expenditure, where energy intake to the body exceeds energy expended, the difference is effectively stored as fat.



The portion of food that is metabolized is regulated to match the total energy expenditure of the body. A certain amount of the energy is obligatory (the amount of energy expended to do the work of the cells, the BMR). Some energy is also expended for adaptive thermogenesis, heat generated in response to cold or diet. An additional amount of energy is used for physical exercise (work against the environment). To voluntarily store less energy as fat, we can vary our caloric intake through dietary changes or our energy expenditure through changes in our physical exercise.

Cora Nari suffered a heart attack 8 months ago and had a significant loss of functional heart muscle. The pain she is experiencing is called angina pectoris, which is a crushing or constricting pain located in the center of the chest, often radiating to the neck or arms (see Ann Jeina, Chapters 6 and 7). The most common cause of angina pectoris is partial blockage of coronary arteries from atherosclerosis. The heart muscle cells beyond the block receive an inadequate blood flow and oxygen, and die when ATP production falls too low.

electrons). Because fatty acids are more reduced than carbohydrates, they have a higher caloric value. The high affinity of oxygen for electrons (a high positive reduction potential) drives fuel oxidation forward, with release of energy that can be used for ATP synthesis in oxidative phosphorylation. However, smaller amounts of ATP can be generated without the use of O_2 in ***anaerobic glycolysis.***

Fuel oxidation can also generate ***NADPH,*** *which usually donates electrons to biosynthetic pathways and detoxification reactions. For example, in some reactions catalyzed by* ***oxygenases,*** *NADPH is the electron donor and O_2 the electron acceptor.*

THE WAITING ROOM

Otto Shape is a 26-year old medical student who has completed his first year of medical school. He is 70 inches tall and began medical school weighing 154 lb, within his ideal weight range (see Chapter 1). By the time he finished his last examination in his first year, he weighed 187 lb. He had calculated his BMR at approximately 1,680 kcal, and his energy expenditure for physical exercise equal to 30% of his BMR. He planned on returning to his premedical school weight in 6 weeks over the summer by eating 576 kcal less each day and playing 7 hours of tennis every day. However, he did a summer internship instead of playing tennis. When Otto started his second year of medical school, he weighed 210 lb.

X.S. Teefore (excess T_4) is a 26-year-old man who noted heat intolerance with heavy sweating, heart palpitations, and tremulousness. Over the past 4 months, he has lost weight in spite of a good appetite. He is sleeping poorly and describes himself as feeling "jittery inside."

On physical examination, his heart rate is rapid (116 beats/min) and he appears restless and fidgety. His skin feels warm, and he is perspiring profusely. A fine hand tremor is observed as he extends his arms in front of his chest. His thyroid gland appears to be diffusely enlarged and, on palpation, is approximately 3 times normal size. Thyroid function tests confirm that Mr. Teefore's thyroid gland is secreting excessive amounts of the thyroid hormones T_4 (tetraiodothyronine) and T_3 (triiodothyronine), the major thyroid hormones present in the blood.

Cora Nari is a 64-year-old woman who had a myocardial infarction 8 months ago. Although she managed to lose 6 lb since that time, she remains overweight and has not reduced the fat content of her diet adequately. The graded aerobic exercise program she started 5 weeks after her infarction is now followed irregularly, falling far short of the cardiac conditioning intensity prescribed by her cardiologist. She is readmitted to the hospital cardiac care unit (CCU) after experiencing a severe "viselike pressure" in the mid-chest area while cleaning ice from the windshield of her car. The electrocardiogram (ECG) shows evidence of a new posterior wall myocardial infarction. Signs and symptoms of left ventricular failure are present.

I. ENERGY AVAILABLE TO DO WORK

The basic principle of the ATP-ADP cycle is that fuel oxidation generates ATP, and hydrolysis of ATP to ADP provides the energy to perform most of the work required in the cell. ATP has therefore been called the energy currency of our cells. Like the

one dollar bill, it has a defined value, is required to obtain goods and services, and disappears before we know it. To keep up with the demand, we must constantly replenish our ATP supply through the use of O_2 for fuel oxidation.

The amount of energy from ATP cleavage available to do useful work is related to the difference in energy levels between the products and substrates of the reaction and is called the change in Gibbs free energy, ΔG (Δ, difference; G, Gibbs free energy). In cells, the Δ G for energy production from fuel oxidation must be greater than the ΔG of energy-requiring processes, such as protein synthesis and muscle contraction, for life to continue.

The heart is a specialist in the transformation of ATP chemical bond energy into mechanical work. Each single heartbeat uses approximately 2% of the ATP in the heart. If the heart were not able to regenerate ATP, all its ATP would be hydrolyzed in less than 1 minute. Because the amount of ATP required by the heart is so high, it must rely on the pathway of oxidative phosphorylation for generation of this ATP. In **Cora Nari's** heart, hypoxia is affecting her ability to generate ATP.

A. The High-Energy Phosphate Bonds of ATP

The amount of energy released or required by bond cleavage or formation is determined by the chemical properties of the substrates and products. The bonds between the phosphate groups in ATP are called phosphoanhydride bonds (Fig. 19.2). When these bonds are hydrolyzed, energy is released because the products of the reaction (ADP and phosphate) are more stable, with lower bond energies, than the reactants (ATP and H_2O). The instability of the phosphoanhydride bonds arises from their negatively charged phosphate groups, which repel each other and strain the bonds between them. It takes energy to make the phosphate groups stay together. In contrast, there are fewer negative charges in ADP to repel each other. The phosphate group as a free anion is more stable than it is in ATP because of an increase in resonance structures (i.e., the electrons of the oxygen double bond are shared by all the oxygen atoms). As a consequence, ATP hydrolysis is energetically favorable and proceeds with release of energy as heat.

In the cell, ATP is not directly hydrolyzed. Energy released as heat from ATP hydrolysis cannot be transferred efficiently into energy-requiring processes, such as biosynthetic reactions or maintaining an ion gradient. Instead, cellular enzymes directly transfer the phosphate group to a metabolic intermediate or protein that is part of the energy-requiring process (a phosphoryl transfer reaction).

B. Change in Free Energy (ΔG) During a Reaction

How much energy can be obtained from ATP hydrolysis to do the work required in the cell? The maximum amount of useful energy that can be obtained from a

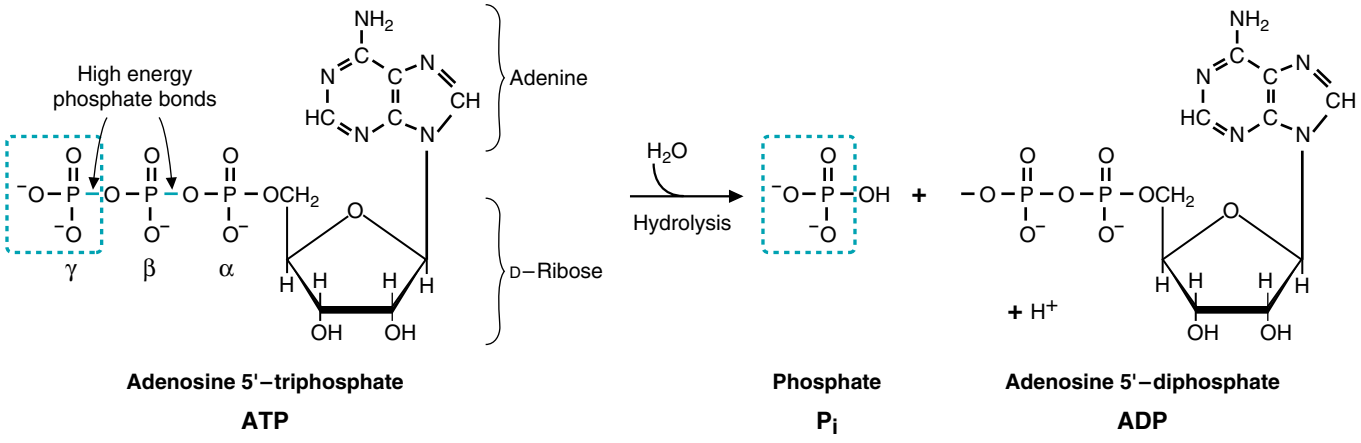


Fig. 19.2. Hydrolysis of ATP to ADP and inorganic phosphate (Pi). Cleavage of the phosphoanhydride bonds between either the β and γ phosphates or the α and β phosphates releases the same amount of energy, approximately −7.3 kcal/mole. However, hydrolysis of the phosphate-adenosine bond (a phosphoester bond) releases less energy (−3.4 kcal/mole), and consequently, this bond is not considered a high-energy phosphate bond. During ATP hydrolysis, the change in disorder during the reaction is small and so ΔG values at physiologic temperature (37°C) are similar to those at standard temperature (25°C). ΔG is affected by pH, which alters the ionization state of the phosphate groups of ATP and by the intracellular concentration of Mg^{2+} ions, which bind to the β and γ phosphate groups of ATP.

reaction is called ΔG, the change in Gibbs free energy. The value of ΔG for a reaction can be influenced by the initial concentration of substrates and products, by temperature, pH, and pressure. The ΔG^0 for a reaction refers to the energy change for a reaction starting at 1 M substrate and product concentrations and proceeding to equilibrium (equilibrium, by definition, occurs when there is no change in substrate and product concentrations with time). $\Delta G^{0\prime}$ is the value for ΔG^0 under standard conditions (pH = 7.0, $[H_2O]$ = 55 M, and 25ºC), as well as standard concentrations (Table 19.1).

$\Delta G^{0\prime}$ is equivalent to the chemical bond energy of the products minus that of the reactants, corrected for energy that has gone into entropy (an increase in amount of molecular disorder). This correction for change in entropy is very small for most reactions occurring in cells, and, thus, the $\Delta G^{0\prime}$ for hydrolysis of various chemical bonds reflects the amount of energy available from that bond.

The value of −7.3 kcal/mole (-30.5 kJ/mole) that is generally used for the $\Delta G^{0\prime}$ of ATP hydrolysis is, thus, the amount of energy available from hydrolysis of ATP under standard conditions that can be spent on energy-requiring processes; it defines the "monetary value" of our "ATP currency." Although the difference between cellular conditions (pH 7.3, 37°C) and standard conditions is very small, the difference between cellular concentrations of ATP, ADP, and Pi and the standard 1-M concentrations is huge and greatly affects the availability of energy in the cell.

C. Exothermic and Endothermic Reactions

The value of $\Delta G^{0\prime}$tells you whether the reaction requires or releases energy, the amount of energy involved, and the ratio of products to substrates at equilibrium. The negative value for the $\Delta G^{0\prime}$ of ATP hydrolysis indicates that, if you begin with equimolar (1 M) concentrations of substrates and products, the reaction proceeds in

Q: The reaction catalyzed by phosphoglucomutase (PGM) is reversible and functions in the synthesis of glycogen from glucose as well as the degradation of glycogen back to glucose. If the $\Delta G^{0\prime}$ for conversion of glucose 6-P to glucose 1-P is +1.65 kcal/mole, what is the $\Delta G^{0\prime}$ of the reverse reaction?

Table 19.1. Thermodynamic Expressions, Laws, and Constants

Definitions	
ΔG	Change in free energy, or Gibbs free energy
ΔG^0	Standard free energy change, ΔG at 1 M concentrations of substrates and products
$\Delta G^{0\prime}$	Standard free energy change at 25ºC, pH 7.0
ΔH	Change in enthalpy, or heat content
ΔS	Change in entropy, or increase in disorder
K'_{eq}	Equilibrium constant at 25ºC, pH 7.0, incorporating $[H_2O]$ = 55.5 M and $[H+] = 10^{-7}$ M in the constant
$\Delta E^{0\prime}$	Change in reduction potential
~P	Biochemical symbol for a high-energy phosphate bond, i.e., a bond which is hydrolyzed with the release of more than about 7 kcal/mole of heat
Laws of thermodynamics	
First law of thermodynamics, the conservation of energy: In any physical or chemical change, the total energy of a system, including its surroundings, remains constant.	
Second law of thermodynamics: The universe tends toward disorder. In all natural processes, the total entropy of a system always increases.	
Constants	
Units of ΔG and ΔH = cal/mole or J/mole: 1 cal = 4.18 J	
T, Absolute temperature: K, Kelvin = 273 + ºC (25ºC = 298º K)	
R, Universal gas constant: 1.99 cal/mole·K or 8.31 J/mole·K	
F, Faraday constant: F = 23 kcal/mole-volt or 96,500 J/V·mole	
Units of E_0', volts	
Formulas	
$\Delta G = \Delta H - T\Delta S$	
$\Delta G^{0\prime} = -RT\ln K'_{eq}$	
$\Delta G^{0\prime} = -nF\Delta E^{\circ\prime}$	
$\ln = 2.303 \log_{10}$	

Table 19.2. A General Expression for ΔG

To generalize the expression for ΔG, consider a reaction in which

$$aA + bB \rightleftarrows cC + dD$$

The small letters denote a moles of A will combine with b moles of B to produce c moles of C and d moles of D.

$$\Delta G^{0'} = -RT \ln K'_{eq} = -RT \ln \frac{[C]^c_{eq}\,[D]^d_{eq}}{[A]^a_{eq}\,[B]^b_{eq}}$$

and

$$\Delta G = \Delta G^{0'} + RT \ln \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b}$$

the forward direction with the release of energy. From initial concentrations of 1 M, the ATP concentration will decrease, and ADP and Pi will increase until equilibrium is reached.

For a reaction in which a substrate S is converted to a product P, the ratio of the product concentration to the substrate concentration at equilibrium is given by:

Equation 1:

$$\Delta G^{0\prime} = -RT \ln [P]/[S]$$

(see Table 19.2. for a more general form of this equation; R is equal to the gas constant (1.98 calories/mole-degree Kelvin), and T is equal to the temperature in degrees Kelvin).

Thus, the difference in chemical bond energies of the substrate and product ($\Delta G^{0\prime}$) determines the concentration of each at equilibrium.

Reactions such as ATP hydrolysis are exergonic (release energy) or exothermic (release heat). They have a negative $\Delta G^{0\prime}$ and release energy while proceeding in the forward direction to equilibrium. Endergonic, or endothermic, reactions have a positive $\Delta G^{0\prime}$ for the forward direction (the direction shown), and the backward direction is favored. For example, in the pathway of glycogen synthesis, phosphoglucomutase converts glucose-6-P to glucose-1-P. Glucose-1-P has a higher phosphate bond energy than glucose-6-P because the phosphate is on the aldehyde carbon (Fig 19.3). The $\Delta G^{0\prime}$ for the forward direction (glucose-1-P → glucose-6-P) is, therefore, positive. Beginning at equimolar concentrations of both compounds, there is a net conversion of glucose-1-P back to glucose-6-P and, at equilibrium, the concentration of glucose-6-P is higher than glucose-1-P. The exact ratio is determined by $\Delta G^{0\prime}$ for the reaction.

It is often said that a reaction with a negative $\Delta G'$ proceeds spontaneously in the forward direction, meaning that products accumulate at the expense of reactants. However, $\Delta G'$ is not an indicator of the velocity of the reaction, or the rate at which equilibrium can be reached. In the cell, the velocity of the reaction depends on the efficiency and amount of enzyme available to catalyze the reaction (see Chapter 9), and, therefore, "spontaneously" in this context can be misleading.

II. ENERGY TRANSFORMATIONS TO DO MECHANICAL AND TRANSPORT WORK

To do work in the cell, a mechanism must be available for converting the chemical bond energy of ATP into another form, such as an Na^+ gradient across a membrane. These energy transformations usually involve intermediate steps in which ATP is bound to a protein, and cleavage of the bound ATP results in a conformational change of the protein.

A: The $\Delta G^{0\prime}$ for the reverse reaction is −1.65 kcal. The change in free energy is the same for the forward and reverse directions, but has an opposite sign. Because negative $\Delta G^{0\prime}$ values indicate favorable reactions, this reaction under standard conditions favors the conversion of glucose 1-P to glucose 6-P.

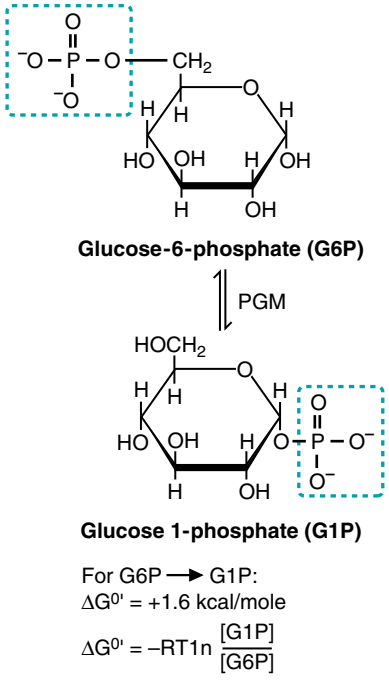


Fig. 19.3. The phosphoglucomutase reaction. The forward direction is involved in converting glucose to glycogen, and the reverse direction in converting glycogen to glucose 6-P.

Q: The $\Delta G^{0\prime}$ for the conversion of glucose 6-P to glucose 1-P is +1.65 kcal/mole. What is the ratio of [glucose-1-P] to [glucose-6-P] at equilibrium?

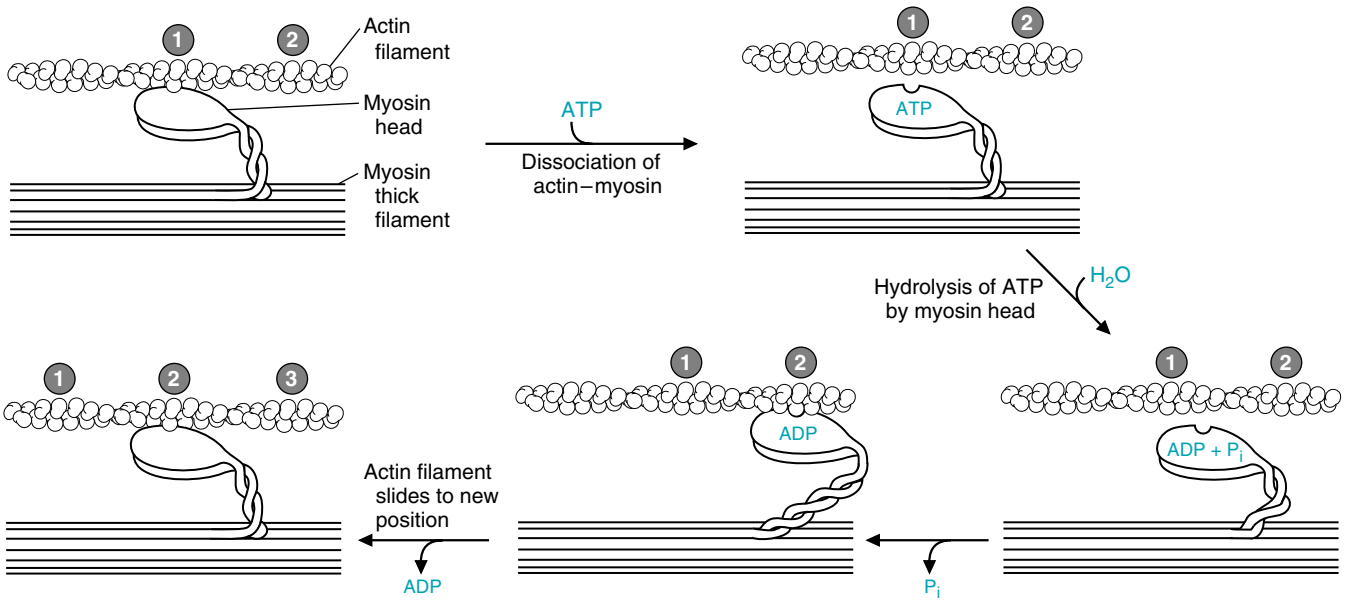


Fig. 19.4. A simplified diagram of myosin ATPase. Muscle fiber is made of thick filaments composed of bundles of the protein myosin, and thin filaments composed of the protein actin (which is activated by Ca^{2+} binding). At many positions along the actin filament, a terminal domain of a myosin molecule, referred to as the "head," binds to a specific site on the actin. The myosin head has an ATP binding site and is an ATPase; it can hydrolyze ATP to ADP and Pi. (1) As ATP binds to myosin, the conformation of myosin changes, and it dissociates from the actin. (2) Myosin hydrolyzes the ATP, again changing conformation. (3) When Pi dissociates, the myosin head reassociates with activated actin at a new position. (4) As ADP dissociates, the myosin again changes conformation, or tightens. This change of conformation at multiple association points between actin and myosin slides the actin filament forward (5).

A: In equation 1 of Table 19.2, $\Delta G^{0\prime} = RT \ln K_{eq}$. For this reaction, K_{eq} = [glucose-1-phosphate]/[glucose-6-phosphate]. The constant R is 1.99×10^{-3} kcal/mole- °K, and T is (273 + 25) °K, so RT equals 0.593 kcal/mole. Substituting in equation 1 then gives $1.65 = -0.593 \ln$ [glucose-1-P]/[glucose-6-P]. Thus, ln[glucose 1-P]/[glucose 6-P] $= -2.78$, and [glucose-1-P]/[glucose-6-phosphate] $= e^{-2.78}$, or 0.062. So the ratio of [glucose-1-P] to [glucose-6-P] at equilibrium is 0.062.

Otto Shape has not followed his proposed diet and exercise regimen and has been gaining weight. He has a positive caloric balance, because his daily energy expenditure is less than his daily energy intake (see Chapter 2). Although the energy expenditure for physical exercise is only approximately 30% of the BMR (basal metabolic rate) in a sedentary individual, it can be 100% or more of the BMR in a person who exercises strenuously for several hours or more. The large increase in ATP utilization for muscle contraction during exercise accounts for its contribution to the daily energy expenditure.

A. Mechanical Work

In mechanical work, the high-energy phosphate bond of ATP is converted into movement by changing the conformation of a protein (Fig.19.4.). For example, in contracting muscle fibers, the hydrolysis of ATP while it is bound to myosin ATPase changes the conformation of myosin so that it is in a "cocked" position ready to associate with the sliding actin filament. Thus, exercising muscle fibers have almost a hundred-fold higher rate of ATP utilization and caloric requirements than resting muscle fibers. Motor proteins, such as kinesins that transport chemicals along fibers, provide another example of mechanical work in a cell.

B. Transport Work

In transport work, called active transport, the high-energy phosphate bond of ATP is used to transport compounds against a concentration gradient (see Chapter10,

The equations for calculating ΔG are based on the first law of thermodynamics (see Table 19.1). The change in chemical bond energy that occurs during a reaction is ΔH, the change in enthalpy of the reaction. At constant temperature and pressure, ΔH is equivalent to the chemical bond energy of the products minus that of the reactants. ΔG, the maximum amount of useful work available from a reaction, is equal to ΔH minus TΔS. TΔS is a correction for the amount of energy that has gone into an increase in the entropy (disorder in arrangement of molecules) of the system.

$$\Delta G = \Delta H - T\Delta S$$

where ΔH = the change in enthalpy, T is the temperature of the system in Kelvin, and ΔS is the change in entropy, or increased disorder of the system. ΔS is often negligible in reactions such as ATP hydrolysis in which the number of substrates (H_2O, ATP) and products (ADP, Pi) are equal and no gas is formed. Under these conditions, the values for ΔG at physiologic temperature (37°C) are similar to those at standard temperature (25°C)

Fig.10.12). In P-ATPases (plasma membrane ATPases) and V-ATPases (vesicular ATPases), the chemical bond energy of ATP is used to reversibly phosphorylate the transport protein and change its conformation. For example, as Na^+, K^+-ATPase binds and cleaves ATP, it becomes phosphorylated and changes its conformation to release 3 Na^+ ions to the outside of the cell, thereby building up a higher extracellular than intracellular concentration of Na^+. Na^+ re-enters the cell on cotransport proteins that drive the uptake of amino acids and many other compounds into the cell. Thus, Na^+ must be continuously transported back out. The expenditure of ATP for Na^+ transport occurs even while we sleep and is estimated to account for 10 to 30% of our BMR.

A large number of other active transporters also convert ATP chemical bond energy into an ion gradient (membrane potential). Vesicular ATPases pump protons into lysosomes. Ca^{2+}ATPases in the plasma membrane move Ca^{2+} out of the cell against a concentration gradient. Similar Ca^{2+}ATPases pump Ca^{2+} into the lumen of the endoplasmic reticulum and the sarcoplasmic reticulum (in muscle). Thus, a considerable amount of energy is expended in maintaining a low cytoplasmic Ca^{2+} level.

III. BIOCHEMICAL WORK

The high-energy phosphate bonds of ATP are also used for biochemical work. Biochemical work occurs in anabolic pathways, which are pathways that synthesize large molecules (e.g., DNA, glycogen, triacylglycerols, and proteins) from smaller compounds. Biochemical work also occurs when toxic compounds are converted to nontoxic compounds that can be excreted (e.g., the liver converts NH_4^+ ions to urea in the urea cycle). In general, formation of chemical bonds between two organic molecules (e.g., C-C bonds in fatty acid synthesis or C-N bonds in protein synthesis) requires energy and is therefore biochemical work. How do our cells get these necessary energy-requiring reactions to occur?

To answer this question, the next sections consider how energy is used to synthesize glycogen from glucose (Fig 19.5). Glycogen is a storage polysaccharide consisting of glucosyl units linked together through glycosidic bonds. If an anabolic pathway, such as glycogen synthesis, were to have an overall positive $\Delta G^{0\prime}$, the cell would be full of glucose and intermediates of the pathway, but very little glycogen would be formed. To avoid this, cells do biochemical work and spend enough of their ATP currency to give anabolic pathways an overall negative $\Delta G^{0\prime}$.

Approximately 70% of our resting daily energy requirement arises from work carried out by our largest organs: the heart, brain, kidneys, and liver. Using their rate of oxygen consumption and a P/O ratio of 2.5, it can be estimated that each of these organs is using and producing several times its own weight in ATP each day. The heart, which rhythmically contracts, is using this ATP for mechanical work. In contrast, skeletal muscles in a resting individual use far less ATP per gram of tissue. The kidney has an ATP consumption per gram of tissue similar to that of the heart and is using this ATP largely for transport work to recover usable nutrients and maintain pH and electrolyte balance. The brain, likewise, uses most of its ATP for transport work, maintaining the ion gradients necessary for conduction of the nerve impulse. The liver, in contrast, has a high rate of ATP consumption and utilization to carry out metabolic work (biosynthesis and detoxification).

Estimated Daily Use of ATP (g ATP/g tissue)	
Heart	16
Brain	6
Kidneys	24
Liver	6
Skeletal Muscle (rest)	0.3
Skeletal Muscle (running)	23.6

X.S. Teefore has increased blood levels of thyroid hormones, which accelerate basal metabolic processes that use ATP in our organs (e.g., Na^+,K^+-ATPase), thereby increasing the BMR. An increased BMR was used for a presumptive diagnosis of hyperthyroidism before development of the tests to measure T3 and T4. Because **X.S. Teefore** did not fully compensate for his increased ATP requirements with an increased caloric intake, he was in negative caloric balance and lost weight.

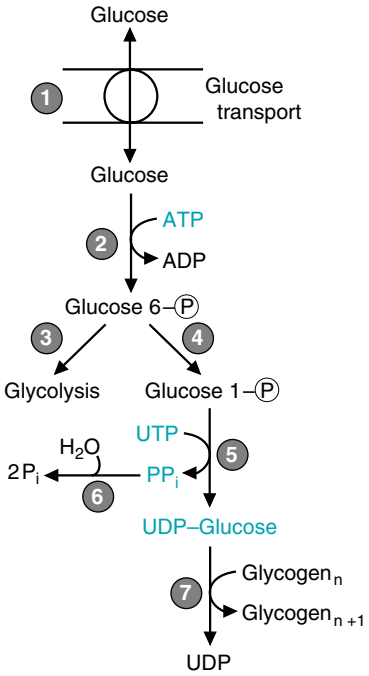


Fig. 19.5. Energetics of glycogen synthesis. Compounds containing high-energy bonds are shown in blue. (1) Glucose is transported into the cell. (2) Glucose phosphorylation uses the high-energy phosphate bond (~P) of ATP in a phosphoryl transfer step. (4) Conversion of glucose 6-phosphate to glucose 1-phosphate by phosphoglucomutase. (5) UDP-Glucose pyrophosphorylase cleaves a ~P bond in UTP, releasing pyrophosphate and forming UDP-glucose, an activated intermediate. (6) The pyrophosphate is hydrolyzed, releasing additional energy. (7) The phosphoester bond of UDP-glucose is cleaved during the addition of a glucosyl unit to the end of a glycogen polysaccharide chain. The UDP-glucose acts as the leaving group in this reaction. Glucose-6-phosphate also can be metabolized via glycolysis (3) when energy is required.

A. ΔG^0 Values Are Additive

Reactions in which chemical bonds are formed between two organic molecules are usually catalyzed by enzymes that transfer energy from cleavage of ATP in a phosphoryl transfer reaction or by enzymes that cleave a high-energy bond in an activated intermediate of the pathway. Because the $\Delta G^{0\prime}$ values in a reaction sequence are additive, the pathway acquires an overall negative $\Delta G^{0\prime}$, and the reactions in the pathway will occur to move toward an equilibrium state where the concentration of final products is greater than that of the initial reactants.

1. PHOSPHORYL TRANSFER REACTIONS

One of the characteristics of Gibbs free energy is that ΔG^0 values for consecutive steps or reactions in a sequence can be added together to obtain a single value for the overall process. Thus, the high-energy phosphate bonds of ATP can be used to drive a reaction forward that would otherwise be highly unfavorable energetically. Consider, for example, synthesis of glucose 6-P from glucose, the first step in glycolysis and glycogen synthesis (see Fig.19.5, circle 2). If the reaction were to proceed by addition of inorganic phosphate to glucose, glucose-6-P synthesis would have a positive $\Delta G^{0\prime}$ value of 3.3 kcal/mole (Table 19.3). However, when this reaction is coupled to cleavage of the high-energy ATP bond through a phosphoryl transfer reaction, the $\Delta G^{0\prime}$ for glucose-6-P synthesis acquires a net negative value of minus 4.0 kcal/mole, which can be calculated from the sum of the two reactions. Glucose 6-P cannot be transported back out of the cell, and therefore the net negative $\Delta G^{0\prime}$ for glucose 6-P synthesis helps the cell to trap glucose for its own metabolic needs.

Q: Given a $\Delta G^{0\prime}$ of +1.65 kcal/mole for the conversion of glucose-6-P to glucose-1-P, and a $\Delta G^{0\prime}$ of −4.0 kcal/mole for the conversion of glucose + ATP to glucose-6-P + ADP, what is the value of $\Delta G^{0\prime}$ for the conversion of glucose to glucose-1-P?

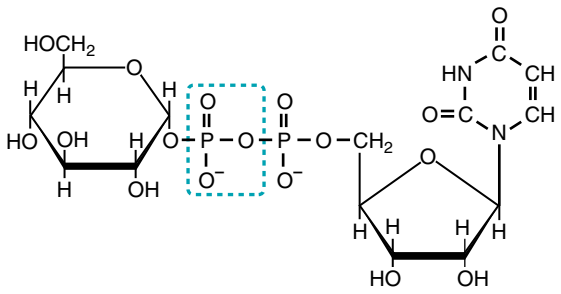
The net value for synthesis of glucose 6-P from glucose and ATP would be the same whether or not the two reactions were catalyzed by the same enzyme, were catalyzed by two separate enzymes, or were not catalyzed by an enzyme at all, because it is dictated by the amount of energy in the chemical bonds being broken and formed.

2. ACTIVATED INTERMEDIATES IN GLYCOGEN SYNTHESIS

To synthesize glycogen from glucose, energy is provided by the cleavage of 3 high-energy phosphate bonds in ATP, UTP, and pyrophosphate (PPi)(see Fig. 19.5, Steps 2, 5, and 6). Energy transfer is facilitated by phosphoryl group transfer and by formation of an activated intermediate (UDP-glucose). Step 4, the conversion of glucose 6-phosphate to glucose 1-P, has a positive $\Delta G^{0\prime}$. This step is pulled and pushed in the desired direction by the accumulation of substrate and removal of product in reactions that have a negative $\Delta G^{0\prime}$ from cleavage of high-energy bonds. In Step 5, the UTP high-energy phosphate bond is cleaved to form the activated sugar, UDP-glucose (Fig 19.6). This reaction is further facilitated by cleavage of the high-energy bond in the pyrophosphate (Step 6) that is released in Step 5 (approximately −7.7 kcal). In Step 7, cleavage of the bond between UDP and glucose in the activated intermediate provides the energy for attaching the glucose moiety to the end of the glycogen molecule (approximately −3.3 kcal). In general, the amount of ATP phosphate bond energy used in an anabolic pathway, or detoxification pathway, must provide the pathway with an overall negative $\Delta G^{0\prime}$, so that the concentration of products is favored over that of reactants.

Table 19.3. $\Delta G^{0\prime}$ for the Transfer of a Phosphate from ATP to Glucose

Glucose + Pi → glucose-6-P + H_2O	$\Delta G^{0\prime}$ = +3.3 kcal/mole
ATP + H_2O → ADP + Pi	$\Delta G^{0\prime}$ = −7.3 kcal/mole
Sum: glucose + ATP → glucose-6-P + ADP	$\Delta G^{0\prime}$ = −4.0 kcal/mole



Uridine diphosphate glucose (UDP–glucose)

Fig. 19.6. UDP-glucose contains a high-energy pyrophosphate bond, shown in blue.

B. ΔG Depends on Substrate and Product Concentration

$\Delta G^{0\prime}$ reflects the energy difference between reactants and products at specific concentrations (each at 1 M) and standard conditions (pH 7.0, 25°C). However, these are not the conditions prevailing in cells, where variations from "standard conditions" are relevant to determining actual free energy changes and hence the direction in which reactions are likely to occur. One aspect of free energy changes contributing to the forward direction of anabolic pathways is the dependence of ΔG, the free energy change of a reaction, on the initial substrate and product concentrations. Reactions in the cell with a positive $\Delta G^{0\prime}$ can proceed in the forward direction if the concentration of substrate is raised to high enough levels, or if the concentration of product is decreased to very low levels. Product concentrations can be very low if, for example, the product is rapidly used in a subsequent energetically favorable reaction, or if the product diffuses or is transported away.

1. THE DIFFERENCE BETWEEN ΔG AND $\Delta G^{0\prime}$

The driving force toward equilibrium starting at any concentration of substrate and product is expressed by ΔG, and not by $\Delta G^{0\prime}$, which is the free energy change to reach equilibrium starting with 1 M concentrations of substrate and product. For a reaction in which the substrate S is converted to the product P:

Equation 2

$$\Delta G = \Delta G^{0\prime} + RT \ln [P]/[S]$$

(see Table 19.2, for the general form of this equation).

The expression for ΔG has two terms: $\Delta G^{0\prime}$, the energy change to reach equilibrium starting at equal and 1 M concentrations of substrates and products, and the second term, the energy change to reach equal concentrations of substrate and product starting from any initial concentration. (When [P] = [S] and [P]/[S] = 1, the ln of [P]/[S] is 0, and $\Delta G = \Delta G^{0\prime}$). The second term will be negative for all concentrations of substrate greater than product, and the greater the substrate concentration, the more negative this term will be. Thus, if the substrate concentration is suddenly raised high enough or the product concentration decreased low enough, ΔG (the sum of the first and second terms) will also be negative, and conversion of substrate to product becomes thermodynamically favorable.

2. THE REVERSIBILITY OF THE PHOSPHOGLUCOMUTASE REACTION IN THE CELL

The effect of substrate and product concentration on ΔG and the direction of a reaction in the cell can be illustrated with conversion of glucose-6-P to glucose-1-P, the

A: $\Delta G^{0\prime}$ for the overall reaction is the sum of the individual reactions, and is −2.35 kcal. The individual reactions are:

Glucose + ATP → glucose 6-P + ADP

$\Delta G^{0\prime}$ = −4.0 kcal/mole

Glucose 6-P → glucose 1-P

$\Delta G^{0\prime}$ = +1.65 kcal/mole

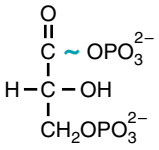
Sum: Glucose + ATP → glucose 1-P + ADP

$\Delta G^{0\prime}$ = −2.35 kcal/mole

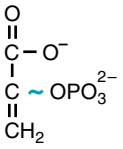
Thus, the cleavage of ATP has made the synthesis of glucose-1-P from glucose energetically favorable.

reaction catalyzed by phosphoglucomutase in the pathway of glycogen synthesis (see Fig. 19.3). The reaction has a small positive $\Delta G^{0\prime}$ for glucose 1-P synthesis (+1.65.kcal/mole) and at equilibrium, the ratio of [glucose 1-P]/[glucose 6-P] is approximately 6 to 94 (which you calculated in Question 2). However, if another reaction uses glucose 1-P such that this ratio suddenly becomes 3 to 94, there is now a driving force for converting more glucose 6-P to glucose 1-P and restoring the equilibrium ratio. Substitution in equation 2 gives ΔG, the driving force to equilibrium, as $+1.65 + RT \ln [G1P]/[G6P] = 1.65 + (-2.06) = -0.41$, which is a negative value. Thus, a decrease in the ratio of product to substrate has converted the synthesis of glucose 1-P from a thermodynamically unfavorable to a thermodynamically favorable reaction that will proceed in the forward direction until equilibrium is reached.

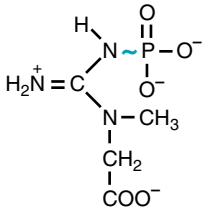
The ΔG of ATP hydrolysis in cells can be very different than $\Delta G^{0\prime}$. The synthesis of ATP rather than its hydrolysis becomes the energetically favorable direction if the [ATP] drops just a little or the [ADP] or [Pi] increase just a little over equilibrium values. Thus, as ATP is hydrolyzed in energy-requiring reactions, the change in the sign of ΔG promotes ATP synthesis.



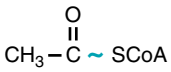
1,3–Bisphosphoglycerate



Phosphoenolpyruvate



Creatine phosphate



Acetyl CoA

Fig. 19.7. Some compounds with high-energy bonds. 1,3-bisphosphoglycerate and phosphoenolpyruvate are intermediates of glycolysis. Creatine phosphate is a high-energy phosphate reservoir and shuttle in brain, muscle, and spermatozoa. Acetyl CoA is a precursor of the TCA cycle. The high-energy bonds are shown in blue.

C. Activated Intermediates with High Energy Bonds

Many biochemical pathways form activated intermediates containing high-energy bonds to facilitate biochemical work. The term "high-energy bond" is a biologic term defined by the $\Delta G^{0\prime}$ for ATP hydrolysis; any bond that can be hydrolyzed with the release of approximately as much, or more, energy than ATP is called a high-energy bond. The high-energy bond in activated intermediates, such as UDP-glucose in glycogen synthesis, facilitate energy transfer.

1. ATP, UTP, GTP, AND CTP

Cells use GTP and CTP, as well as UTP and ATP, to form activated intermediates. Different anabolic pathways generally use different nucleotides as their direct source of high phosphate bond energy: UTP is used for combining sugars, CTP in lipid synthesis, and GTP in protein synthesis.

The high-energy phosphate bonds of UTP, GTP, and CTP are energetically equivalent to ATP and are synthesized from ATP by nucleoside diphosphokinases and nucleoside monophosphokinases. For example, UTP is formed from UDP by a nucleoside diphosphokinase in the reaction:

$$ATP + UDP \leftrightarrow UTP + ADP.$$

ADP is converted back to ATP by the process of oxidative phosphorylation, using energy supplied by fuel oxidation.

Energy-requiring reactions often generate the nucleoside diphosphate ADP. Adenylate kinase, an important enzyme in cellular energy balance, is a nucleoside monophosphate kinase that transfers a phosphate from one ADP to another ADP to form ATP and AMP:

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

This enzyme, thus, can regenerate ATP under conditions in which ATP utilization is required.

2. OTHER COMPOUNDS WITH HIGH-ENERGY BONDS

In addition to the nucleoside triphosphates, other compounds containing high-energy bonds are formed to facilitate energy transfer in anabolic and catabolic pathways (e.g., 1,3- bisphosphoglycerate in glycolysis and acetyl CoA in the TCA cycle) (Fig.19.7). Creatine phosphate contains a high-energy phosphate bond that allows it to serve as an energy reservoir for ATP synthesis and transport in muscle cells, neurons, and spermatozoa. All of these high-energy bonds are "unstable," and their hydrolysis yields substantial free energy because the products are much more stable, as a result of electron resonance within their structures.

IV. THERMOGENESIS

According to the first law of thermodynamics, energy cannot be destroyed. Thus, energy from oxidation of a fuel (its caloric content) must be equal to the amount of heat released, the work performed against the environment, and the increase in order of molecules in our bodies. Some of the energy from fuel oxidation is converted into heat as the fuel is oxidized and some heat is generated as ATP is used to do work. If we become less efficient in converting energy from fuel oxidation into ATP, or if we use an additional amount of ATP for muscular contraction, we will oxidize an additional amount of fuel to maintain ATP homeostasis (constant cellular ATP levels). With the oxidation of additional fuel, we release additional heat. Thus, heat production is a natural consequence of "burning fuel."

Thermogenesis refers to energy expended for the purpose of generating heat in addition to that expended for ATP production. To maintain our body at 37°C, despite changes in environmental temperature, it is necessary to regulate fuel oxidation and its efficiency (as well as heat dissipation). In shivering thermogenesis, we respond to sudden cold with asynchronous muscle contractions (shivers) that increase ATP utilization and, therefore, fuel oxidation and the release of energy as heat. In nonshivering thermogenesis (adaptive thermogenesis), the efficiency of converting energy from fuel oxidation into ATP is decreased. More fuel needs to be oxidized to maintain constant ATP levels and, thus, more heat is generated.

X.S. Teefore has increased thyroid hormone levels that increase his rate of ATP utilization and fuel oxidation. An excess of thyroid hormones also may affect the efficiency of ATP production, resulting in fewer ATP produced for a given O_2 consumption. The increased rate of ATP utilization and diminished efficiency stimulates oxidative metabolism, resulting in a much greater rate of heat production. The hyperthyroid patient, therefore, complains of constantly feeling hot (heat intolerance) and sweaty. (Perspiration allows dissipation of excess heat through evaporation from the skin surface.)

V. ENERGY FROM FUEL OXIDATION

Fuel oxidation provides energy for bodily processes principally through generation of the reduced coenzymes, NADH and FAD(2H). They are used principally to generate ATP in oxidative phosphorylation. However, fuel oxidation also generates NADPH, which is most often used directly in energy-requiring processes. Carbohydrates also may be used to generate ATP through a nonoxidative pathway, called anaerobic glycolysis.

A. Energy Transfer from Fuels through Oxidative Phosphorylation

Fuel oxidation is our major source of ATP and our major means of transferring energy from the chemical bonds of the fuels to cellular energy-requiring processes. The amount of energy available from a fuel is equivalent to the amount of heat that is generated when a fuel is burned. To conserve this energy for the generation of ATP, the process of cellular respiration transforms the energy from the chemical bonds of fuels into the reduction state of electron-accepting coenzymes, NAD^+ and FAD (circle 1, Fig. 19.8). As these compounds transfer electrons to O_2 in the electron transport chain, most of this energy is transformed into an electrochemical gradient across the inner mitochondrial membrane (circle 2, Fig. 19.8). Much of the energy in the electrochemical gradient is used to regenerate ATP from ADP in oxidative phosphorylation (phosphorylation that requires O_2).

1. OXIDATION-REDUCTION REACTIONS

Oxidation-reduction reactions always involve a pair of chemicals: an electron donor, which is oxidized in the reactions, and an electron acceptor, which is reduced in the reaction. In fuel metabolism, the fuel donates electrons, and is oxidized, and NAD^+ and FAD accept electrons, and are reduced.

When is NAD^+, rather than FAD, used in a particular oxidation-reduction reaction? It depends on the chemical properties of the electron donor and the enzyme catalyzing the reaction. In oxidation reactions, NAD^+ accepts two electrons as a hydride ion to form NADH, and a proton (H^+) is released into the medium (Fig 19.9). It is generally used for metabolic reactions involving oxidation of alcohols and aldehydes. In contrast,

Oxidation is the loss of electrons, and reduction is the gain of electrons. Remember LEO GER:
Loss of Electrons = Oxidation;
Gain of Electrons = Reduction.
Compounds are oxidized in the body in essentially three ways: (1) the transfer of electrons from the compound as a hydrogen atom or a hydride ion, (2) the direct addition of oxygen from O_2, and (3) the direct donation of electrons (e.g., $Fe^{2+} \rightarrow Fe^{3+}$) (see Chapter 5). Fuel oxidation involves the transfer of electrons as a hydrogen atom or a hydride ion and, thus, reduced compounds have more hydrogen relative to oxygen than the oxidized compounds. Consequently, aldehydes are more reduced than acids, and alcohols are more reduced than aldehydes.

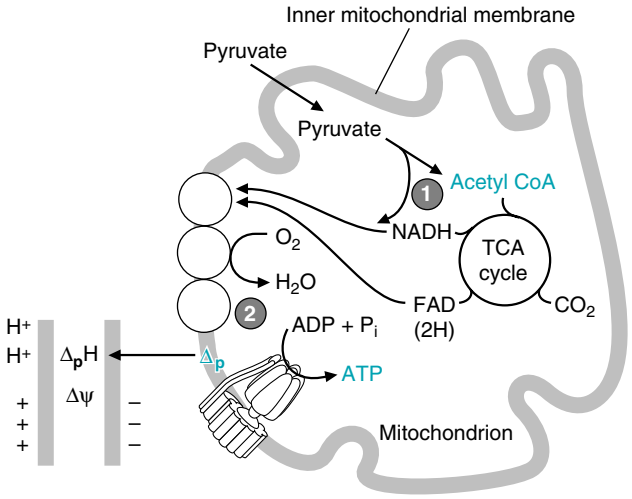


Fig. 19.8. Overview of energy transformations in oxidative phosphorylation. The electrochemical potential gradient across the mitochondrial membrane is represented by ΔpH, the proton gradient, and ΔΨ, the membrane potential. The role of the electrochemical potential in oxidative phosphorylation is discussed in more depth in Chapter 21.

FAD accepts two electrons as hydrogen atoms, which are donated singly from separate atoms (e.g., formation of a double bond or a disulfide)(Fig. 19.10).

As the reduced coenzymes donate these electrons to O_2 through the electron transport chain, they are reoxidized. The energy derived from reoxidation of NADH and FAD(2H) is available for the generation of ATP by oxidative phosphorylation. In our analogy of ATP as currency, the reduced coenzymes are our "paychecks" for oxidizing fuels. Because our cells spend ATP so fast, we must immediately convert our paychecks into ATP cash.

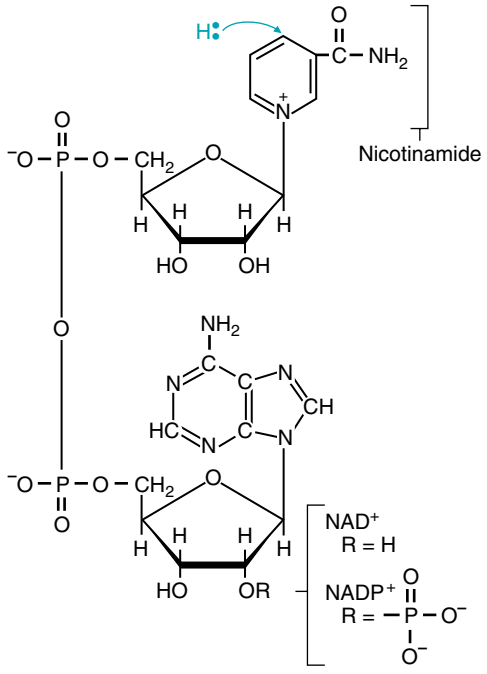


Fig. 19.9. Reduction of NAD^+ and $NADP^+$. These structurally related coenzymes are reduced by accepting two electrons as $H:^-$, the hydride ion.

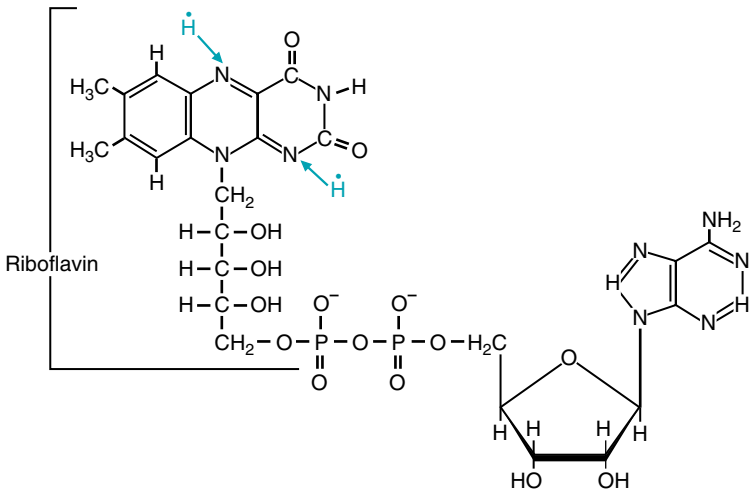


Fig. 19.10. Reduction of FAD. FAD accepts two electrons as two hydrogen atoms and is reduced. The reduced coenzyme is denoted in this text as FAD(2H) because it often accepts a total of two electrons one at a time, never going to the fully reduced form, $FADH_2$. FMN (flavin mononucleotide) consists of riboflavin with one phosphate group attached.

2. REDUCTION POTENTIAL

Each oxidation/reduction reaction makes or takes a fixed amount of energy, ($\Delta G^{0\prime}$), which is directly proportional to the $\Delta E^{\circ\prime}$ (the difference in reduction potentials of the oxidation-reduction pair). The reduction potential of a compound, $E^{\circ\prime}$, is a measure in volts of the energy change when that compound accepts electrons (becomes reduced); minus $E^{\circ\prime}$ is the energy change when the compound donates electrons (becomes oxidized). $E^{\circ\prime}$ can be considered an expression of the willingness of the compound to accept electrons. Some examples of reduction potentials are shown in Table 19.4. Oxygen, which is the best electron acceptor, has the largest positive reduction potential (i.e., is the most willing to accept electrons and be reduced). As a consequence, the transfer of electrons from all compounds to O_2 is energetically favorable and occurs with energy release.

The more negative the reduction potential of a compound, the greater is the energy available for ATP generation when that compound passes its electrons to oxygen. The $\Delta G^{0\prime}$ for transfer of electrons from NADH to O_2 is greater than the transfer from FAD(2H) to O_2 (see the reduction potential values for NADH and FAD(2H) in Table 19.4). Thus, the energy available for ATP synthesis from NADH is approximately −53 kcal, and approximately −41 kcal from the FAD-containing flavoproteins in the electron transport chain.

Table 19.4. Reduction Potentials of Some Oxidation-Reduction Half-Reactions

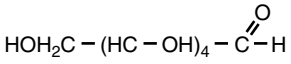
Reduction Half-Reactions	$E^{0\prime}$ at pH 7.0
$1/2\ O_2 + 2H^+ + 2\ e^- \rightarrow H_2O$	0.816
Cytochrome a-Fe^{3+} + 1 $e^- \rightarrow$ cytochrome a-Fe^{2+}	0.290
$CoQ + 2H^+ + 2\ e^- \rightarrow CoQH_2$	0.060
Fumarate + $2H^+$ + 2 e^- → succinate	0.030
Oxalacetate + $2H^+$ + 2 e^- → malate	−0.102
Acetaldehyde + $2H^+$ + 2 e^- → ethanol	−0.163
Pyruvate + $2H^+$ + 2 e^- → lactate	−0.190
Riboflavin + $2H^+$ + 2 e^- → riboflavin-H_2	−0.200
$NAD^+ + 2H^+ + 2\ e^- \rightarrow NADH + H^+$	−0.320
Acetate + $2H^+$ + 2 e^- → acetaldehyde	−0.468

To calculate the free energy change of an oxidation-reduction reaction, the reduction potential of the electron donor (NADH) is added to that of the acceptor (O_2). The $\Delta E^{0\prime}$ for the net reaction is calculated from the sum of the half reactions. For NADH donation of electrons, it is = +0.320 volts, opposite of that shown in Table 4 (remember, Table 4 shows the $E^{0\prime}$ for accepting electrons), and for O_2 acceptance, it is +0.816. The number of electrons being transferred is 2 (so, n = 2). The direct relationship between the energy changes in oxidation-reduction reactions and $\Delta G^{0\prime}$ is expressed by the equation

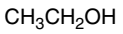
$$\Delta G^{0\prime} = -n\ F\ \Delta E^{0\prime}$$

where n is the number of electrons transferred and F is Faraday's constant (23 kcal/mole - volt). Thus, a value of approximately -53 kcal/mole is obtained for the energy available for ATP synthesis by transferring two electrons from NADH to oxygen.

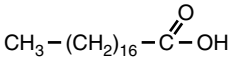
Otto Shape decided to lose weight by decreasing his intake of fat and alcohol (ethanol), and increasing his content of carbohydrates. Compare the structure of ethanol with that of glucose and fatty acids (below). On the basis of their oxidation state, which compound provides the most energy (calories) per gram?



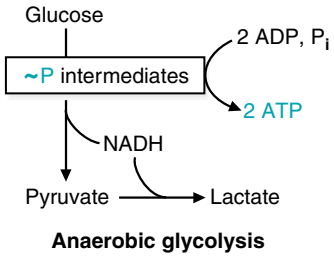
Glucose



Ethanol



A fatty acid



Anaerobic glycolysis

Fig. 19.11. Anaerobic glycolysis. Phosphate is transferred from high-energy intermediates of the pathway to ADP. Because NADH from the pathway is reoxidized by reduction of pyruvate to lactate, no oxygen is required.

Oxidases

$O_2 + 4e^-, 4H^+ \longrightarrow 2H_2O$

$O_2 + SH_2 \longrightarrow S + H_2O_2$

Monooxygenases

$O_2 + S + \text{Electron donor}-XH_2 \longrightarrow H_2O + \text{Electron donor}-X + S-OH$

Dioxygenases

$S + O_2 \longrightarrow SO_2$

Fig. 19.12. Oxidases and oxygenases. The fate of O_2 is shown in blue. S represents an organic substrate.

3. CALORIC VALUES OF FUELS

The caloric value of a food is directly related to its oxidation state, which is a measure of $\Delta G^{0\prime}$ for transfer of electrons from that fuel to O_2. The electrons donated by the fuel are from its C-H and C-C bonds. Fatty acids such as palmitate ($CH_3(CH_2)_{14}COOH$) have a caloric value of roughly 9 kcal/g. Glucose is already partially oxidized and has a caloric value of only about 4 kcal/g. The carbons, on an average, contain fewer C-H bonds from which to donate electrons.

The caloric value of a food is applicable in humans only if our cells have enzymes that can oxidize that fuel by transferring electrons from the fuel to NAD^+, $NADP^+$, or FAD. When we burn wood in a fireplace, electrons are transferred from cellulose and other carbohydrates to O_2, releasing energy as heat. However, wood has no caloric content for humans; we cannot digest it and convert cellulose to a form that can be oxidized by our enzymes. Cholesterol, although a lipid, also has no caloric value for us because we cannot oxidize the carbons in its complex ring structure in reactions that generate NADH, FAD(2H), or NADPH.

B. NADPH in Oxidation-Reduction Reactions

$NADP^+$ is similar to NAD^+ and has the same reduction potential. However, $NADP^+$ has an extra phosphate group on the ribose, which affects its enzyme binding (see Fig. 19.9). Consequently, most enzymes use either NAD^+ or $NADP^+$, but seldom both. In certain reactions, fuels are oxidized by transfer of electrons to $NADP^+$ to form NADPH. For example, glucose 6-P dehydrogenase, in the pentose phosphate pathway, transfers electrons from glucose 6-P to $NADP^+$ instead of NAD^+. NADPH usually donates electrons to biosynthetic reactions such as fatty acid synthesis, and to detoxification reactions that use oxygen directly. Consequently, the energy in its reduction potential is usually used in energy-requiring reactions without first being converted to ATP currency.

C. Anaerobic Glycolysis

Not all ATP is generated by fuel oxidation. In anaerobic glycolysis, glucose is degraded in reactions that form high-energy phosphorylated intermediates of the pathway (Fig.19.11). These activated high-energy intermediates provide the energy for the generation of ATP from ADP without involving electron transfer to O_2. Therefore, this pathway is called anaerobic glycolysis, and ATP is generated from substrate level phosphorylation rather than oxidative phosphorylation (see Chapter 22). Anaerobic glycolysis is a critical source of ATP for cells that have a decreased O_2 supply, either because they are physiologically designed that way (e.g., cells in the kidney medulla), or because their supply of O_2 has been pathologically decreased (e.g., coronary artery disease).

VI. OXYGENASES AND OXIDASES NOT INVOLVED IN ATP GENERATION

Approximately 90 to 95% of the oxygen we consume is used by the terminal oxidase in the electron transport chain for ATP generation via oxidative phosphorylation. The remainder of the O_2 is used directly by oxygenases and other oxidases, enzymes that oxidize a compound in the body by transferring electrons directly to O_2 (Fig. 19.12). The large positive reduction potential of O_2 makes all of these reactions extremely favorable thermodynamically, but the electronic structure of O_2 slows the speed of electron transfer. These enzymes, therefore, contain a metal ion that facilitates reduction of O_2.

A. Oxidases

Oxidases transfer electrons from the substrate to O_2, which is reduced to water (H_2O) or to hydrogen peroxide (H_2O_2). The terminal protein complex in the electron transport chain, called cytochrome oxidase, is an oxidase because it accepts electrons donated to the chain by NADH and FAD(2H) and uses these to reduce O_2 to water. Most of the other oxidases in the cell form hydrogen peroxide (H_2O_2), instead of H_2O, and are called peroxidases. Peroxidases are generally confined to peroxisomes to protect DNA and other cellular components from toxic free radicals (compounds containing single electrons in an outer orbital) generated by hydrogen peroxide.

B. Oxygenases

Oxygenases, in contrast to oxidases, incorporate one or both of the atoms of oxygen into the organic substrate (see Fig 19.12). Monooxygenases, enzymes that incorporate one atom of oxygen into the substrate and the other into H_2O, are often named hydroxylases (e.g., phenylalanine hydroxylase, which adds a hydroxyl group to phenylalanine to form tyrosine) or mixed function oxidases. Monooxygenases require an electron donor-substrate, such as NADPH, a coenzyme such as FAD, which can transfer single electrons, and a metal or similar compound that can form a reactive oxygen complex (Fig.19.13). They are usually found in the endoplasmic reticulum, and occasionally in mitochondria. Dioxygenases, enzymes that incorporate both atoms of oxygen into the substrate, are used in the pathways for converting arachidonate into prostaglandins, thromboxanes, and leukotrienes.

VII. ENERGY BALANCE

Our total energy expenditure is equivalent to our oxygen consumption (Fig. 19.14). The resting metabolic rate (energy expenditure of a person at rest, at 25°C, after an overnight fast) accounts for approximately 60 to 70% of our total energy expenditure and O_2 consumption, and physical exercise accounts for the

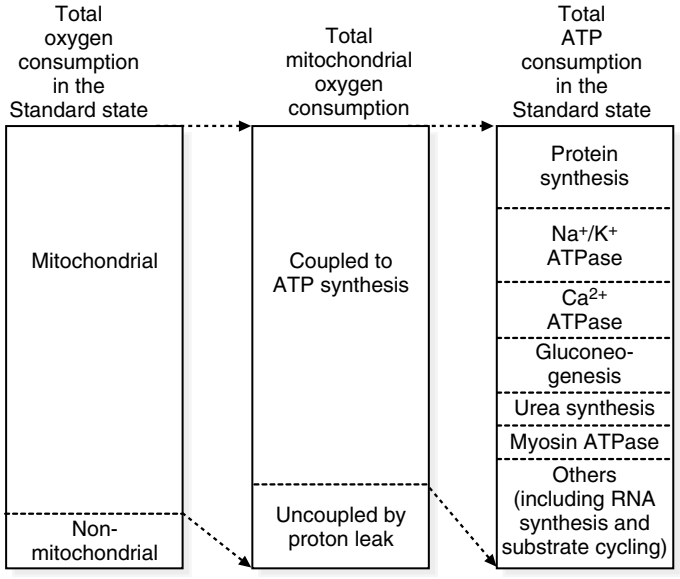


Fig. 19.14. Estimated contribution of processes to energy utilization in standard state. Copied, with permission, from Rolfe DFS, Brown GC. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol Rev 1997;77:731-758.

A: In palmitate and other fatty acids, most carbons are more reduced than those in glucose or ethanol (more of the carbons have electrons in carbon–hydrogen bonds). Therefore, fatty acids have the greatest caloric content/gram, 9 kcal. In glucose, the carbons have already formed bonds with oxygen, and fewer electrons in C-H bonds are available to generate energy. Thus, the complete oxidation of glucose gives roughly 4 kcal/g. In ethanol, one carbon is a methyl group with C–H bonds, and one has an OH group. Therefore the oxidation state is intermediate between glucose and fatty acids, and ethanol thus has 7 kcal/g.

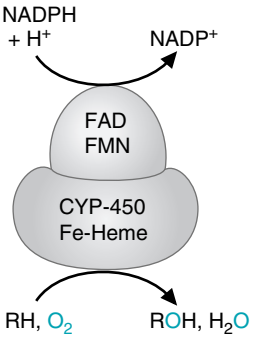


Fig. 19.13. Cytochrome P450 mono-oxygenases. Electrons are donated by NADPH to O_2 and the substrate. The flavin coenzymes FAD and FMN in one subunit transfer single electrons to cytochrome P450, which is an Fe-heme containing protein that absorbs light at a wavelength of 450 nm. The enzyme is embedded in a membrane, usually the endoplasmic reticulum.

remainder. Of the resting metabolic rate, approximately 90 to 95% of O_2 consumption is used by the mitochondrial electron transport chain, and only 5 to 10% is required for nonmitochondrial oxidases and oxygenases and is not related to ATP synthesis. Approximately 20 to 30% of the energy from this mitochondrial O_2 consumption is lost by proton leak back across the mitochondrial membrane, which dissipates the electrochemical gradient without ATP synthesis. The remainder of our O_2 consumption is used for ATPases that maintain ion gradients and for biosynthetic pathways.

ATP homeostasis refers to the ability of our cells to maintain constant levels of ATP despite fluctuations in the rate of utilization. Thus, increased utilization of ATP for exercise or biosynthetic reactions increases the rate of fuel oxidation. The major mechanism employed is feedback regulation; all of the pathways of fuel oxidation leading to generation of ATP are feedback-regulated by ATP levels, or by compounds related to the concentration of ATP. In general, the less ATP used, the less fuel will be oxidized to generate ATP.

According to the first law of thermodynamics, the energy (cal) in our consumed fuel can never be lost. Consumed fuel is either oxidized to meet the energy demands of the basal metabolic rate + exercise, or it is stored as fat. Thus, an intake of calories in excess of those expended results in weight gain. The simple statement, "If you eat too much and don't exercise, you will get fat," is really a summary of the bioenergetics of the ATP-ADP cycle.

CLINICAL COMMENTS

Otto Shape. Otto Shape visited his physician, who noted the increased weight. He recommended several diet modifications to Otto that would decrease the caloric content of his diet and pointed out the importance of exercise for weight reduction. He reminded Otto that the American Heart Association and the American Cancer Society recommended 45 to 60 minutes of moderate-to-vigorous exercise 5 to 7 days per week. He also reminded Otto that he would want to be a role model for his patients. Otto decided to begin an exercise regimen that includes an hour of running each day.

X.S. Teefore. Mr. Teefore exhibited the classical signs and symptoms of hyperthyroidism (increased secretion of the thyroid hormones, T_3 and T_4) including a goiter (enlarged thyroid gland). Thyroid function tests confirmed this diagnosis.

Thyroid hormones (principally T_3) modulate cellular energy production and utilization through their ability to increase the gene transcription of many proteins involved in intermediary metabolism, including enzymes in the TCA cycle and oxidative phosphorylation. They increase the rate of ATP utilization by Na^+, K^+-ATPase, and other enzymes. They also affect the efficiency of energy transformations, so that either more fuel must be oxidized to maintain a given level of ATP, or more ATP must be expended to achieve the desired physiological response. The loss of weight experienced by **X.S. Teefore,** in spite of a very good appetite, reflects his increased caloric requirements and a less efficient utilization of fuels. The result is an enhanced oxidation of adipose tissue stores as well as a catabolic effect on muscle and other protein-containing tissues. Through mechanisms that are not well understood, increased levels of thyroid hormone in the blood also increase the activity or "tone" of the sympathetic (adrenergic) nervous system. An activated sympathetic nervous system leads to a more rapid and forceful heartbeat (tachycardia and palpitations), increased nervousness (anxiety and insomnia), tremulousness (a sense of shakiness or jitteriness), and other symptoms.

The thyroid gland secretes the thyroid hormones tetraiodothyronine (T_4) and triiodothyronine (T_3) (see Fig. 11.8 for the structure of T_3). T_3 is the most active form of the hormone. T_4 is synthesized and secreted in approximately 10 times greater amounts than T_3. Hepatocytes (liver cells) and other cells contain a deiodinase that removes one of the iodines from T_4, converting it to T_3. T_3 exerts its effects on tissues by regulating the transcription of specific genes involved in energy metabolism (see Chapter 16, section III.C.2., Fig. 16.14).

Cora Nari. Cora Nari was in left ventricular heart failure (LVF) when she presented to the hospital with her second heart attack in 8 months. The diagnosis of LVF was based, in part, on her rapid heart rate (104 beats/min) and respiratory rate. On examining her lungs, her physician heard respiratory rales, caused by inspired air bubbling in fluid that had filled her lung air spaces secondary to LVF. This condition is referred to as congestive heart failure.

Cora Nari's rapid heart rate (tachycardia) resulted from a reduced capacity of her ischemic, failing left ventricular muscle to eject a normal amount of blood into the arteries leading away from the heart with each contraction. The resultant drop in intraarterial pressure signaled a reflex response in the central nervous system that, in turn, caused an increase in heart rate in an attempt to bring the total amount of blood leaving the left ventricle each minute (the cardiac output) back toward a more appropriate level to maintain systemic blood pressure.

Treatment of Cora's congestive heart failure will include efforts to reduce the workload of the heart with diuretics and other "load reducers," attempts to improve the force of left ventricular contraction with digitalis and other "inotropes," and the administration of oxygen by nasal cannula to reduce the injury caused by lack of blood flow (ischemia) to the viable heart tissue in the vicinity of the infarction.

BIOCHEMICAL COMMENTS

Active Transport and Cell Death. Most of us cannot remember when we first learned that we would die if we stopped breathing. But exactly how cells die from a lack of oxygen is an intriguing question. Pathologists generally describe two histologically distinct types of cell death: necrosis and apoptosis (programmed cell death). Cell death from a lack of O_2, such as occurs during a myocardial infarction, can be very rapid, and is considered necrosis. The lack of ATP for the active transport of Na^+ and Ca^{2+} triggers some of the death cascades leading to necrosis (Fig. 19.15).

The influx of Na^+ and loss of the Na^+ gradient across the plasma membrane is an early event accompanying ATP depletion during interruption of the O_2 supply. One consequence of the increased intracellular Na^+ concentration is that other transport processes driven by the Na^+ gradient are impaired. For example, the Na^+ / H^+ exchanger, which normally pumps out H^+ generated from metabolism in exchange for extracellular Na^+, can no longer function, and intracellular pH may drop. The increased intracellular H^+ may impair ATP generation from anaerobic glycolysis. As a consequence of increased intracellular ion concentrations, water enters the cells and hydropic swelling occurs. Swelling is accompanied by the release of creatine kinase MB subunits, troponin I, and troponin C into the blood. These enzymes are measured in the blood as indicators of a myocardial infarction (see Chapters 6 and 7). Swelling is an early event and is considered a reversible stage of cell injury.

Normally, intracellular Ca^{2+} concentration is carefully regulated to fluctuate at low levels (intracellular Ca^{2+} concentration is less than 10^{-7} M, compared with approximately 10^{-3} M in extracellular fluid). Fluctuations of Ca^{2+} concentration at these low levels regulate myofibrillar contraction, energy metabolism, and other cellular processes. However, when Ca^{2+} concentration is increased above this normal range, it triggers cell death (necrosis). High Ca^{2+} concentrations activate a phospholipase that increases membrane permeability, resulting in further loss of ion gradients across the cell membrane. They also trigger opening of the mitochondrial permeability transition pore, which results in loss of mitochondrial function and further impairs oxidative phosphorylation.

Intracellular Ca^{2+} levels may increase as a result of cell swelling, the lack of ATP for ATP-dependent Ca^{2+} pumps, or the loss of the Na^+ gradient. Normally,

Congestive heart failure occurs when the weakened pumping action of the ischemic left ventricular heart muscle causes back pressure to increase in the vessels which bring oxygenated blood from the lungs to the left side of the heart. The pressure inside these pulmonary vessels eventually reaches a critical level above which water from the blood moves down a "pressure gradient" from the capillary lumen into alveolar air spaces of the lung (transudation). The patient experiences shortness of breath as the fluid in the air spaces interferes with oxygen exchange from the inspired air into arterial blood, causing hypoxia. The hypoxia then stimulates the respiratory center in the central nervous system, leading to a more rapid respiratory rate in an effort to increase the oxygen content of the blood. As the patient inhales deeply, the physician hears gurgling sounds (known as inspiratory rales) with a stethoscope placed over the posterior lung bases. These sounds represent the bubbling of inspired air as it enters the fluid-filled pulmonary alveolar air spaces.

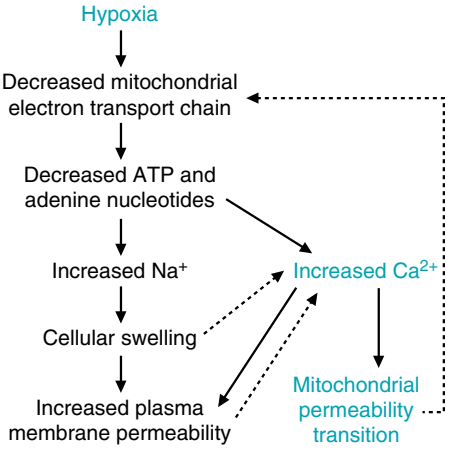


Fig. 19.15. Hypoxia, Ca^{2+}, Na^+, and cell death. Without an adequate O_2 supply, decreased ATP synthesis from oxidative phosphorylation results in an increase of cytoplasmic Na^+ and Ca^{2+} ions. Increased ions levels can trigger death cascades that involve increased permeability of the plasma membrane, loss of ion gradients, decreased cytosolic pH, mitochondrial Ca^{2+} overload, and a change in mitochondrial permeability called the mitochondrial permeability transition. The solid lines show the first sequence of events; the dashed lines show how these events feedback to accelerate the mitochondrial deterioration, making recovery of oxidative phosphorylation impossible.

Ca^{2+}-ATPases located in the plasma membrane pump Ca^{2+}- out of the cell. Ca^{2+}-ATPases in the endoplasmic reticulum, and in the sarcoplasmic reticulum of heart and other muscles, sequester Ca^{2+} within the membranes, where it is bound by a low-affinity binding protein. Ca^{2+} is released from the sarcoplasmic reticulum in response to a nerve impulse, which signals contraction, and the increase of Ca^{2+} stimulates both muscle contraction and the oxidation of fuels. Within the heart, another Ca^{2+} transporter protein, the Na^+/Ca^{2+} exchange transporter, coordinates the efflux of Ca^{2+} in exchange for Na^+, so that Ca^{2+} is extruded with each contraction.

Suggested References

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REVIEW QUESTIONS—CHAPTER 19

1. The highest-energy phosphate bond in ATP is located between which of the following groups?

   (A) Adenosine and phosphate
   (B) Ribose and phosphate
   (C) Ribose and adenine
   (D) Two hydroxyl groups in the ribose ring
   (E) Two phosphate groups

2. Which of the following bioenergetic terms or phrases is correctly defined?

   (A) The first law of thermodynamics states that the universe tends towards a state of increased order.
   (B) The second law of thermodynamics states that the total energy of a system remains constant.
   (C) The change in enthalpy of a reaction is a measure of the total amount of heat that can be released from changes in the chemical bonds.
   (D) $\Delta G^{\circ\prime}$ of a reaction is the standard free energy change measured at 37°C and a pH of 7.4.
   (E) A high-energy bond is a bond that releases more than 3 kcal/mole of heat when it is hydrolyzed.

3. Which statement best describes the direction a chemical reaction will follow?

   (A) A reaction with a positive free energy will proceed in the forward direction if the substrate concentration is raised high enough.
   (B) Under standard conditions, a reaction will proceed in the forward direction if the free energy $\Delta G^{\circ\prime}$ is positive.
   (C) The direction of a reaction is independent of the initial substrate and product concentrations because the direction is determined by the change in free energy.
   (D) The concentration of all of the substrates must be higher than all of the products to proceed in the forward direction.
   (E) The enzyme for the reaction must be working at better than 50% of its maximum efficiency for the reaction to proceed in the forward direction.

4. A patient, Mr. Perkins, has just suffered a heart attack. As a consequence, his heart would display which of the following changes?

   (A) An increased intracellular O_2 concentration
   (B) An increased intracellular ATP concentration
   (C) An increased intracellular H^+ concentration
   (D) A decreased intracellular Ca^{2+} concentration
   (E) A decreased intracellular Na^+ concentration

5. Which of the following statements correctly describes reduction of one of the electron carriers, NAD^+ or FAD?

   (A) NAD^+ accepts two electrons as hydrogen atoms to form $NADH_2$.
   (B) NAD^+ accepts two electrons that are each donated from a separate atom of the substrate.
   (C) NAD^+ accepts two electrons as a hydride ion to form NADH.
   (D) FAD releases a proton as it accepts two electrons.
   (E) FAD must accept two electrons at a time.