ATP Production II: The TCA Cycle and Oxidative Phosphorylation 2.10

Learning Objectives

- Describe the reaction catalyzed by pyruvate dehydrogenase
- Describe in general terms the function of the water-soluble vitamins
- Describe what it means to say that NADH is a carrier for reducing equivalents
- Define reduction potential and describe how it can be measured
- Be able to calculate the energy released in a reduction–oxidation reaction
- List the number of NADH molecules generated per turn of the TCA cycle
- List the number of $FADH_2$ molecules generated per turn of the TCA cycle
- List the number of ATP molecules (or equivalent) produced by substrate-level phosphorylation per turn of the TCA cycle
- Indicate where CO_2 is released during glucose oxidation
- Describe what is meant by the "electron transport chain"
- Tell the approximate magnitude and sign of the membrane potential across the inner mitochondrial membrane
- Give the stoichiometry of ATP formation from NADH; from $FADH_2$
- Be able to calculate the electrochemical potential difference for H^+ ions across the inner mitochondrial membrane
- Describe in words how the ATP synthase makes ATP
- Describe the chemiosmotic hypothesis for oxidative phosphorylation
- Describe in general terms how cytoplasmic NADH enters the mitochondria
- Describe how ADP and Pi get into the mitochondrion and how ATP leaves it

OXIDATION OF PYRUVATE OCCURS IN THE MITOCHONDRIA VIA THE TCA CYCLE

Pyruvate is the end product of glycolysis. Its metabolism continues in the mitochondria via the "**TCA cycle**," the **tricarboxylic acid cycle**, so named because many of the intermediates have three carboxyl groups. It is also referred to as the **Krebs cycle** in honor of Sir Hans Krebs, who did much of the pioneering work in describing it, and it is also referred to as the **citric acid cycle** because citric acid is formed in it. This series of metabolic transformations occurs in the inner mitochondria of cells. Its fuel source is pyruvic acid derived from glycolysis in the cytosol. The TCA cycle can also be initiated within the mitochondria by the oxidation of fatty acids to form acetyl CoA (see Chapter 2.11).

PYRUVATE ENTERS THE MITOCHONDRIA AND IS CONVERTED TO ACETYL CoA

The mitochondria have two membranes, an outer membrane and an inner membrane. The outer membrane is relatively permeable, whereas the inner membrane is highly impermeable to most materials. Pyruvate produced in the cytosol by glycolysis crosses the inner mitochondrial membrane by facilitated diffusion on its own **pyruvate carrier**. Inside the **matrix** of the mitochondria, pyruvate is converted to **acetyl coenzyme A**. This conversion of pyruvate to **acetyl CoA** requires three different enzymes and five different coenzymes, which are organized into a multienzyme complex called **pyruvate dehydrogenase**. Three of the coenzymes required here are vitamins: thiamine, riboflavin, and niacin. **The water-soluble vitamins all find their use in mammals as part of enzymatic reactions, and most of the B vitamins are involved in carbohydrate metabolism**. The overall reaction is shown in Figure 2.10.1 along with the structure of coenzyme A.

PYRUVATE DEHYDROGENASE RELEASES CO_2 AND MAKES NADH

The production of acetyl CoA from pyruvate is noteworthy because here is the first production of CO_2 from glucose. This gas forms a major waste product that must be eliminated, largely through the lungs. Second, the reaction produces NADH from NAD^+. NADH is a carrier for **reducing equivalents** in the cell. The structures of NAD^+ and NADH are shown in Figure 2.10.2.

The conversion of NAD^+ to NADH is a **reduction reaction**. Oxidation and reduction are two halves of the same process, dealing with the exchange of electrons

DOI: http://dx.doi.org/10.1016/B978-0-12-800883-6.00021-5

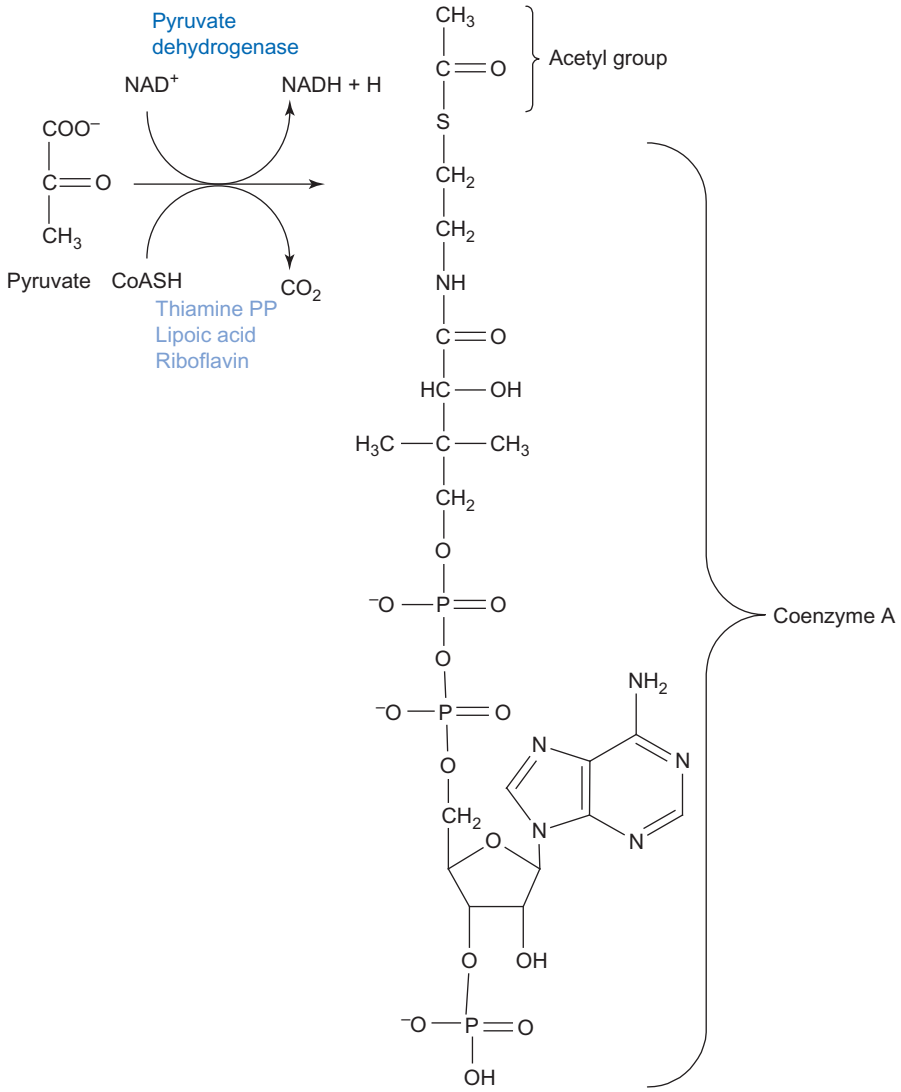

FIGURE 2.10.1 Conversion of pyruvate to acetyl CoA by pyruvate dehydrogenase, indicated in blue. Note that the reaction releases CO_2. Coenzyme A is a complex of ATP with pantothenic acid. It carries the acetyl group on through biochemical reactions in the cell. The reaction requires five different coenzymes (coenzyme A, NAD^+, thiamine pyrophosphate, lipoic acid, and riboflavin). Three of these coenzymes are vitamins (niacin, thiamine, and riboflavin).

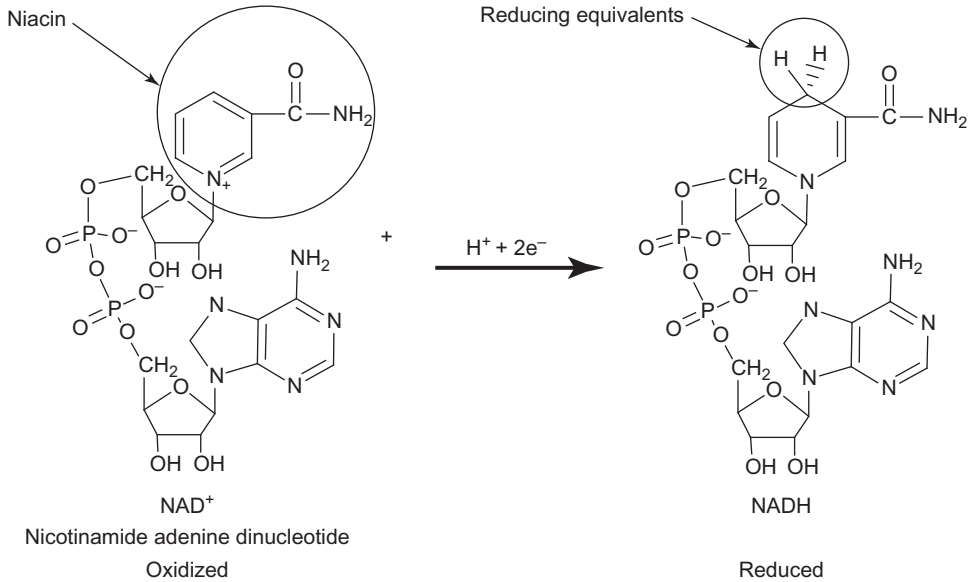

FIGURE 2.10.2 Formation of NADH from NAD^+. NAD^+ is the oxidized form; NADH is the reduced form.

between chemicals. A mnemonic device for oxidation/reduction reactions is

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which stands for "Loss of Electrons, Oxidation; Gain of Electrons, Reduction." When NAD^+ gains two electrons in the form of H^-, it is reduced. At the same time, the chemical from which the H^- is extracted is oxidized. In oxidation/reduction reactions, one chemical is reduced while the other is oxidized. Now the electrons in the reduction reaction had to come from someplace, and that someplace is another chemical. So in a reduction–oxidation reaction there are always two redox pairs, one being reduced and the other being oxidized in the process.

THE AFFINITY OF A CHEMICAL FOR ELECTRONS IS MEASURED BY ITS STANDARD REDUCTION POTENTIAL

If compound A binds electrons more tightly than compound B, we expect that A will take electrons from B in the reaction $A + B \rightarrow A^- + B^+$. In this reaction, A is reduced and B is oxidized. This can be written as the sum of two **half-reactions**:

$$A + e^- \rightarrow A^- \text{ and } B \rightarrow B^+ + e^-$$

The relative tendency for a compound to be reduced is called its **reduction potential**. The **standard redox potential** is measured against a standard half-reaction, arbitrarily assigned the reduction potential of zero. This is the reduction/oxidation of hydrogen:

$$2H^+(aq) + 2e^- \rightarrow H_2(g)\ E_0 = 0.00$$

Here $\mathbf{E_0}$ is referred to as the **standard reduction potential** of hydrogen. Because oxidation is the reverse reaction of reduction, the standard oxidation potential is the negative of the standard reduction potential.

Standard reduction potentials are measured as shown in Figure 2.10.3 against a **Standard Hydrogen Electrode**. It is measured under standard conditions of 1 M concentration of all reactants and 1 atm pressure of H_2 gas. The standard reduction potential is given the symbol E_0.

Recall from Chapter 1.3 that the potential is the work done in bringing a **positive** unit charge from infinite separation to the point at which the potential is to be defined. The work done in moving a charge through a potential is just the charge times the potential. This was expressed in Eqn [1.3.13]:

$$[2.10.1] \qquad \Delta\text{Energy} = q\Delta U$$

where U is the potential and q is the total charge. In electrochemistry we generally use E for the potential, as we have done above, in units of volts, and q is in coulombs. This gives the energy change in volt-coulombs or joules. This energy change is the change in free energy, and so Eqn [2.10.1] can be rewritten as

$$[2.10.2] \qquad \Delta G = z\Im\ \Delta E_0'$$

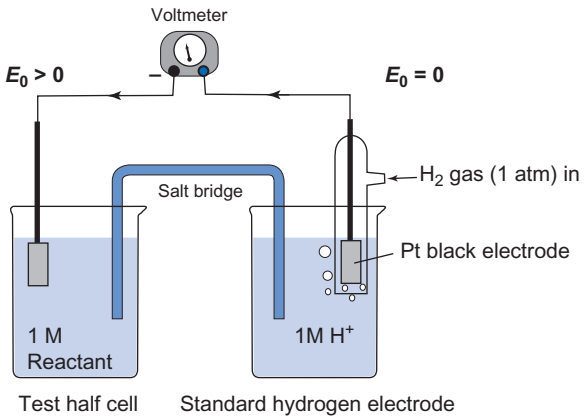

FIGURE 2.10.3 Measurement of the standard electrode potential. One half-cell containing standard concentrations of reactant (1 M) is connected to the standard hydrogen electrode (SHE) with a finely divided Pt black electrode bubbled with 1 atm of H_2 gas and 1 M H^+ in solution. The voltage between the two half-cells is the reduction potential. If electrons flow to the reactant, then the reactant is being reduced and has a positive standard reduction potential—it has a higher affinity for electrons than hydrogen. Note that current is defined as positive charge flow, which is opposite to electron flow. Voltages are typically measured with a potentiometer that finds the voltage necessary to stop current flow, so that the measurement occurs at equilibrium when no current flows.

where $\Delta E_0'$ is the difference in reduction potential between the two half-cells, z is the valence of the carrier, and $\Im$ is the Faraday = 98,500 coulombs mol^{-1}. The Faraday converts the charges to coulombs and normalizes the free energy change to the free energy change per mole. Since the charge carrier is the electron, $z = -1$, this equation becomes

$$[2.10.3] \qquad \Delta G = -\Im\Delta E_0'$$

This is the free energy change *per mole of electrons*. If there are n electrons involved per reaction, the free energy change *per mole of reaction* is

$$[2.10.4] \qquad \Delta G = -n\Im\Delta E_0'$$

THE REDUCTION POTENTIAL DEPENDS ON THE CONCENTRATION OF OXIDIZED AND REDUCED FORMS, AND THE TEMPERATURE

The standard reduction potential is defined at unit concentrations of all reactants. When the concentrations are not 1 M, the measured reduction potential changes. Consider the reduction of A in contact with a Standard Hydrogen Electrode, as described earlier. We can write the two half-cell reactions as

$$A + n\ H^+_{test} + n\ e^- \rightarrow AH_n$$

$$n/2\ H_2 \rightarrow n\ H_{SHE} + n\ e^-$$

$$A + n/2\ H_2 + n\ H^+_{test} \rightarrow AH_n + n\ H^+_{SHE}$$

where SHE denotes Standard Hydrogen Electrode. The free energy per mole for the overall reaction is calculated as

$$\Delta\mu = \mu^0_{AH_n} + RT\ln[AH_n] + n\,\mu^0_{H_{SHE}} + n\,RT\ln[H^+_{SHE}]$$

$$-\mu^0_A - RT\ln[A] - \frac{n}{2}\mu^0_{H_2} - \frac{n}{2}RT\ln f_{H_2}$$

$$-n\,\mu^0_{H_{test}} - n\,RT\ln[H^+_{test}]$$

[2.10.5]

where f_{H2} is the fugacity of hydrogen, analogous to the activity in aqueous solutions. Since the pressure of H_2 gas at the Standard Hydrogen Electrode is 1 atm, f_{H2} is 1.0. Collecting terms in Eqn [2.10.5], we get

$$\Delta\mu = \mu^0_{AH_n} + n\,\mu^0_{H_{SHE}} - \mu^0_A - \frac{n}{2}\mu^0_{H_2} - n\,\mu^0_{H_{test}} + RT\ln[AH_n]$$

$$-RT\ln[A] + n\,RT\ln[H^+_{SHE}] - n\,RT\ln[H^+{}_{test}]$$

[2.10.6]

The top line of Eqn [2.10.6] is the free energy per mole for the standard reduction potential for A. This is given by Eqn [2.10.4]. Remembering that $[H^+]$ for the Standard Hydrogen Electrode = 1 M, its term drops out, because ln $[H^+]$ = ln 1 = 0, and we write

$$\Delta\mu = -n\Im\Delta E_0 + RT\ln\frac{[AH_n]}{[A]} - n\,RT\ln\left[H^+{}_{test}\right]$$

[2.10.7]

The observed potential is related to the free energy change of the reaction through Eqn [2.10.4] and so we have

$$-n\Im\Delta E = -n\Im\Delta E_0 + n\,RT\ln\left[H^+{}_{test}\right] + RT\ln\frac{[AH_n]}{[A]}$$

[2.10.8]

In most situations, the $[H^+]$ in the test half-cell can be kept nearly constant by the use of chemical buffers. In this case, its contribution to the free energy will also be constant, and we can define a practical reduction potential ($\Delta E_0'$) that incorporates the standard reduction potential and the pH term. Doing this, plus dividing both sides by $-n\Im$, we come to

$$\Delta E = \Delta E_0' + \frac{RT}{n\Im}\ln\frac{[A]}{[AH_n]} \quad [2.10.9]$$

The argument of the logarithm is inverted from Eqn [2.10.8] because of multiplying through by −1 to convert the minus sign in $-n\Im$ to positive values. What this equation means is that the actual reduction potential depends on the relative concentrations of the oxidized form ([A]) and reduced form ($[AH_n]$). The reduction potential as a function of the oxidation state of a redox reaction is shown in Figure 2.10.4 for NADH, Ubiquinone, and Cytochrome C (more on these later). Note that when [A] = [AH], which occurs when the reactant is 50% oxidized, the measured ΔE is equal to $\Delta E_0'$.

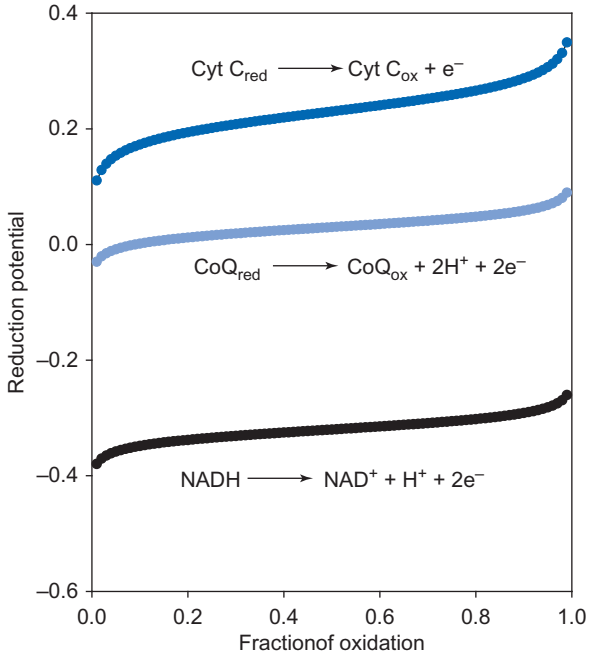

FIGURE 2.10.4 Reduction potential for various redox reactions found in the cell as a function of their oxidation state. The reduced form is AH_n in Eqn [2.10.9] and the oxidized form is A. As AH_n is oxidized by addition of a strong oxidant, it is converted to A and the reduction potential changes according to Eqn [2.10.9]. When the reaction is 50% complete, [A] = $[AH_n]$ and the argument of the logarithm becomes 1.0, and ln 1.0 = 0. At this point, the measured reduction potential is the practical standard reduction potential: $\Delta E = \Delta E_0'$. For NADH this is −0.32 volts; for Coenzyme Q (CoQ) this is +0.030 volts; for cytochrome C this is +0.23 volts. The higher reduction potential means that oxidized CoQ will oxidize NADH by taking electrons from it, and oxidized Cyt C will oxidize CoQ by taking electrons from it.

THE TCA CYCLE IS A CATALYTIC CYCLE

The biochemical transformations that constitute the TCA cycle are shown in Figure 2.10.5. This is a catalytic cycle in that the intermediates themselves are not altered by the cycle. It starts with oxaloacetate, a 4-carbon dicarboxylic acid, condensing with acetyl CoA to produce citrate. As the cycle continues, NADH is generated in each of three separate reactions (at isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase) and $FADH_2$ is generated at succinate dehydrogenase. $FADH_2$ is the oxidized form of FAD, flavin adenine dinucleotide. It is another chemical carrier of reducing equivalents whose structure is shown in Figure 2.10.6. GTP is generated at succinyl CoA synthetase, and CO_2 is generated twice, at isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. The GTP generated in the cycle by substrate-level phosphorylation is formally equivalent to ATP as the two high-energy compounds are readily interconverted. The overall TCA cycle is

$$\text{Acetyl CoA} + 2H_2O + GDP + Pi + FAD + 3NAD^+ \rightarrow$$
$$CoASH + 2\,CO_2 + GTP + 3\,NADH + 3\,H^+ + FADH_2$$

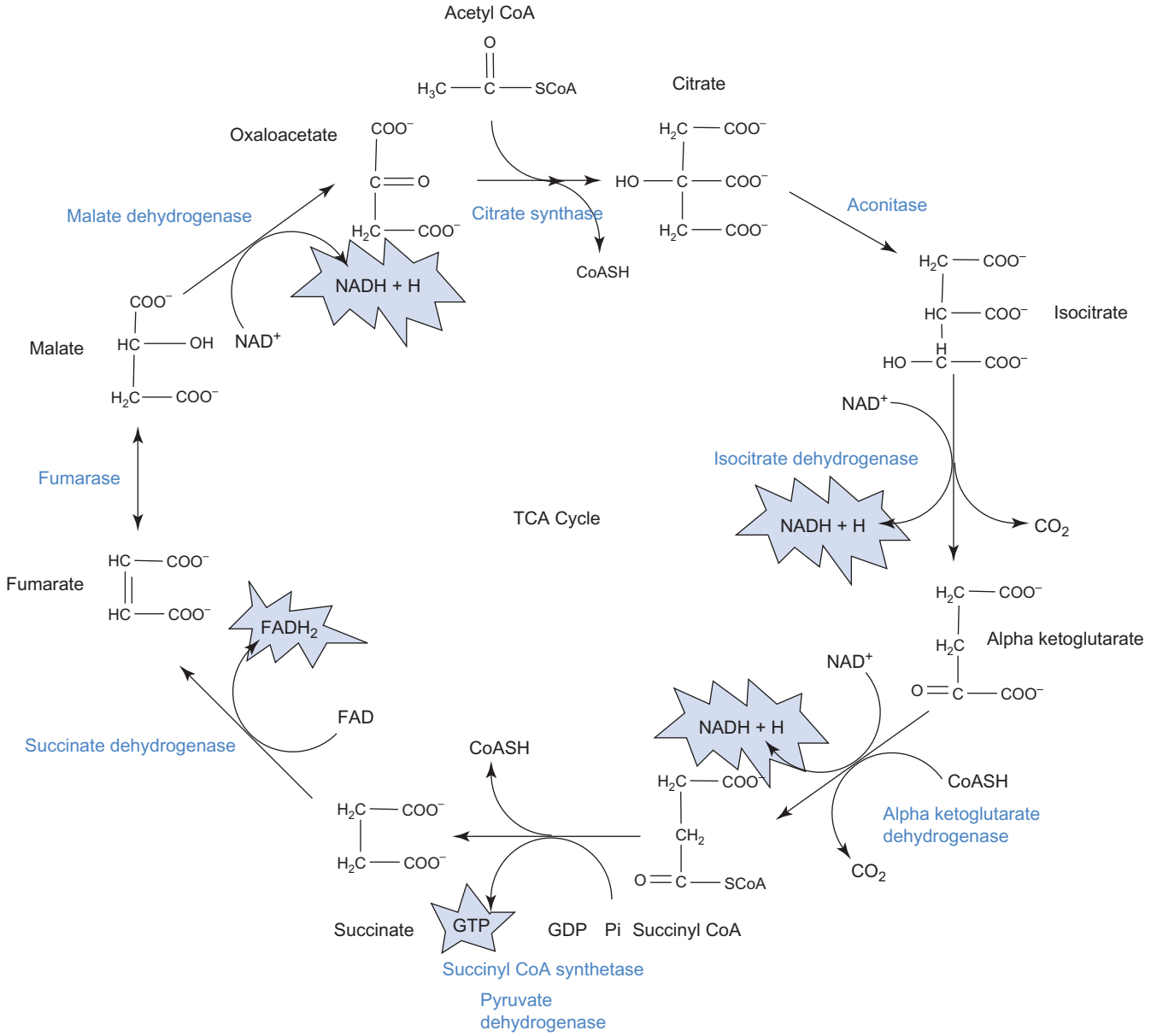

FIGURE 2.10.5 Metabolic interconversions in the TCA cycle. Note that the two-carbon fragment, acetic acid, is carried by CoA to combine with oxaloacetate to form citrate. The oxaloacetate is regenerated by the cycle. Thus the cycle's intermediates are catalysts in that they are not consumed. The two carbons in acetate are converted to CO_2 by the cycle.

The result is that the two carbons in acetate are converted to CO_2 and a bunch of reducing equivalents (8 per 2-carbon acetate). Note that O_2 is not explicitly required for this process, but it is required for the continued operation of this cycle. If we add in the formation of acetyl CoA from pyruvate, the overall reaction is

$$\text{Pyruvate} + 3\ H_2O + GDP + Pi + FAD + 4\ NAD^+ \rightarrow$$

$$3\ CO_2 + GTP + 4\ NADH + 4\ H^+ + FADH_2$$

The NADH and $FADH_2$ produced during this overall series of reactions must be returned to NAD^+ and FAD, or the process will stop. The reduced NADH and $FADH_2$ are oxidized by a special system called the **electron transport chain (ETC)**. In the process of being oxidized, the energy stored in these compounds enables the synthesis of ATP through a process called **oxidative phosphorylation**.

THE ETC LINKS CHEMICAL ENERGY TO H^+ PUMPING OUT OF THE MITOCHONDRIA

The ETC consists of an array of proteins inserted in the inner mitochondrial membrane. The overall plan is this: NADH delivers two electrons to a series of chemicals that differ in their chemical affinity for these electrons (see Figure 2.10.7). This is expressed in their reduction potential (see above) which is related to

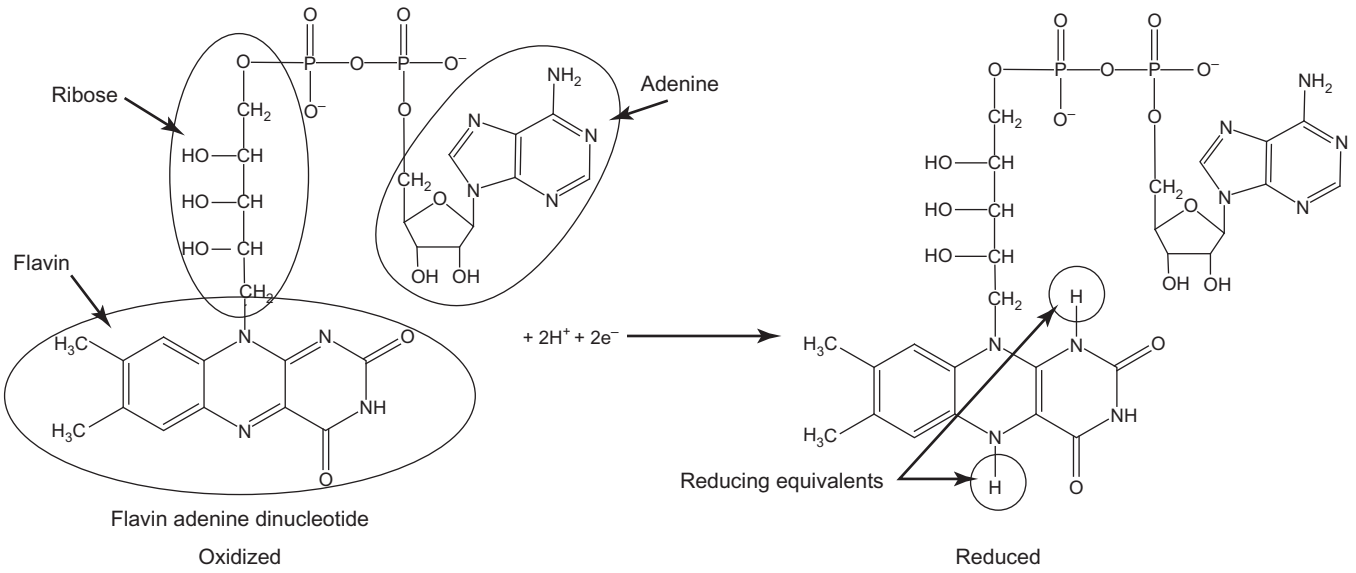

FIGURE 2.10.6 Structure of oxidized and reduced FAD. FAD stands for "flavin adenine dinucleotide." It consists of a flavin part, a ribose part, and an adenine part. The ribose + flavin is better known as riboflavin or vitamin B_2. The binding of reducing equivalents is associated with gain of energy, which can be released on oxidation.

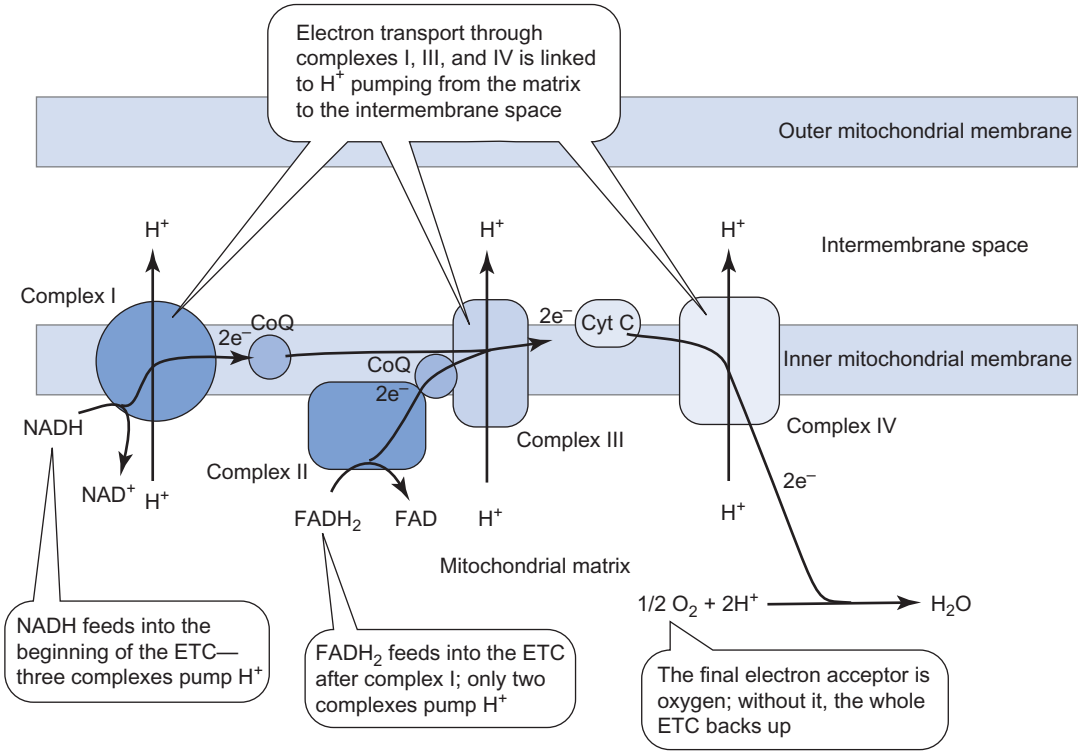

FIGURE 2.10.7 The electron transport chain (ETC). NADH feeds in reducing equivalents at the beginning of the ETC, which hands them on to proteins with progressively higher affinity until at the end of the chain the electrons are combined with oxygen. Complexes I, III, and IV use the chemical energy of oxidation to pump H^+ ions from the mitochondrial matrix to the intermembrane space. This makes an electrical current that separates charge and produces a potential difference across the mitochondrial membrane.

their free energy. The energy is released gradually, in steps, and the ETC complexes use the decrease in free energy to pump hydrogen ions from the matrix space to the intermembrane space between the inner and outer mitochondrial membranes. This pumping of hydrogen ions produces an electrochemical gradient for hydrogen ions and the energy in this gradient is used to generate ATP from ADP and Pi.

OXYGEN ACCEPTS ELECTRONS AT THE END OF THE ETC

Molecular oxygen oxidizes the last step in the ETC. This is the point at which oxygen is consumed by the mitochondria, producing water. Without oxygen to finally oxidize the ETC, the chain itself will remain reduced and no further reducing agents can be fed through it. That is, NADH cannot be converted to NAD^+ (at the far left of the chain) in the absence of oxygen. $FADH_2$ also cannot be converted to FAD in the absence of oxygen. The $\Delta E_0'$ for the redox reaction $\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$ is +0.82 volts. The cascade of electrons down the ETC is shown diagrammatically in Figure 2.10.8. Note that the electrons travel down the cascade towards ever more positive reduction potentials, as these signify increasing affinity for electrons.

PROTON PUMPING AND ELECTRON TRANSPORT ARE TIGHTLY COUPLED

If the proton gradient is at equilibrium with the free energy of electron transport, then the electrons cannot be transported through the ETC. The energy stored in the electrochemical gradient of protons across the inner mitochondrial membrane must also be drained in some way for the ETC to continue operating. **The energy in the proton electrochemical gradient is used to make ATP**. The coupling of the electrochemical gradient of H^+ across the inner mitochondrial membrane with ATP synthesis is called **chemiosmotic coupling** (because there is a concentration difference across the membrane and an electric potential). It was first proposed by Peter Mitchell in 1961, who was awarded the Nobel Prize for the work in 1978.

THE ATP SYNTHASE COUPLES INWARD H^+ FLUX TO ATP SYNTHESIS

The inner mitochondrial membrane contains many copies of a protein called the **F0F1ATPase**. This is also called **ATP synthase**. It consists of two parts: the F_0 component spans the membrane and provides a channel for protons to move into the matrix from the intermembrane space. The F_1 component is a complex of five proteins with the composition $\alpha_3\beta_3\gamma\delta\varepsilon$, with a molecular weight of about 360,000. The F_0 Part of the complex consists of an integral membrane, a subunit, a b dimer, and 8–15 small c-subunits. The structure of the ATP synthase is shown in Figure 2.10.9. This remarkable complex couples the movement of H^+ to the synthesis of ATP through mechanical intermediates. Hydrogen ions from the inner matrix access the c subunit via a channel in the a subunit, causing a rotation of the c subunit turbine. This rotates the γ subunit, which has a cam-like protrusion that deforms the α and β subunits. Each time the cam passes one of the three $\alpha\beta$ complexes, ATP is formed from ADP and Pi bound to the $\alpha\beta$ subunits. Because there are three of these $\alpha\beta$ subunits, each turn of the c-protein turbine produces 3 ATP molecules.

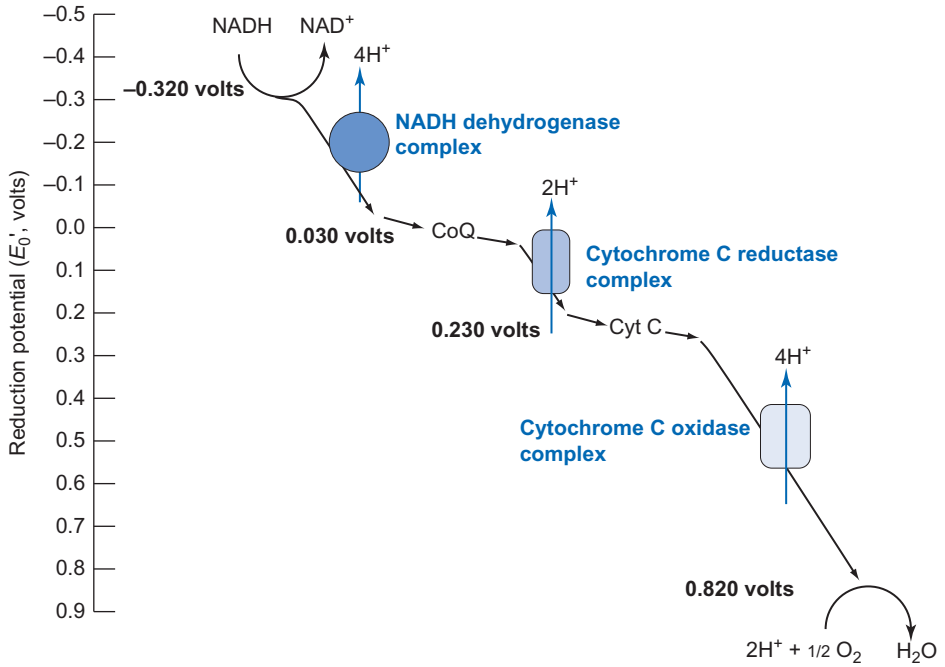

FIGURE 2.10.8 Cascade of electrons in the electron transport chain. It begins with the production of NADH by reactions in glycolysis or TCA cycle. The reduction potential of $NADH/NAD^+$ is −0.320 volts. Electrons are passed to the NADH dehydrogenase complex that pumps $4H^+$ per 2 e^- out of the mitochondrial matrix to the intermembrane space. Electrons are then passed to Coenzyme Q, with a reduction potential of 0.030 volts. Coenzyme Q passes the electrons to the cytochrome C reductase complex that pumps $2H^+$ per 2 e^-. Electrons then are transferred to cytochrome C with a reduction potential of 0.230 volts. The electrons that are taken by the Cytochrome C oxidase complex that pumps $4H^+$ per 2 e^-. Cytochrome C oxidase is finally oxidized by molecular oxygen, whose reduction potential is 0.820 volts.

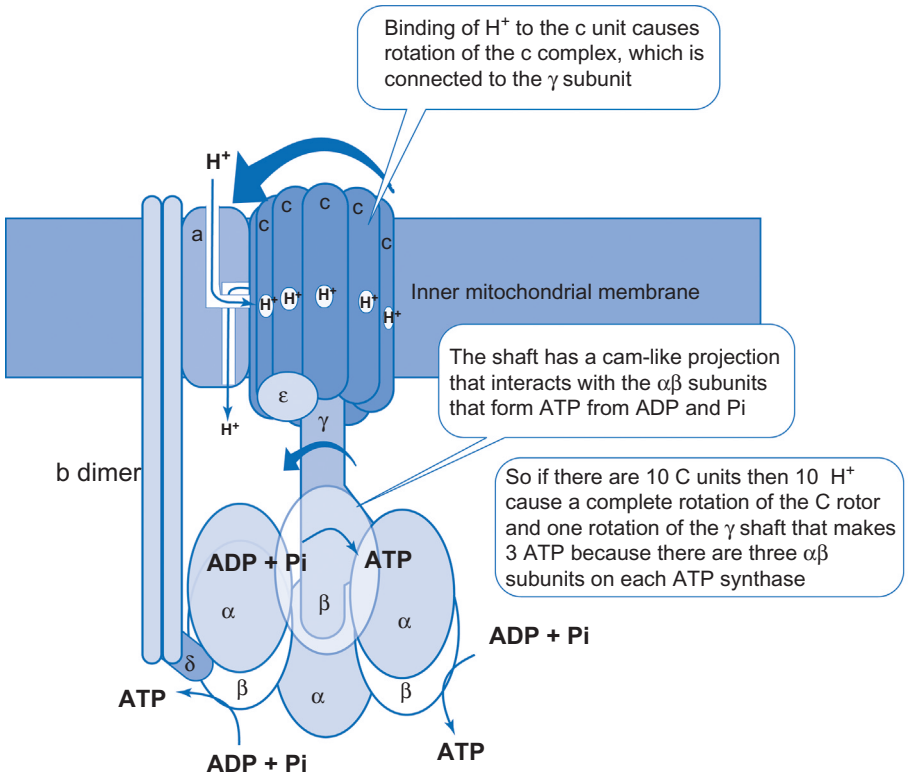

FIGURE 2.10.9 The F_0F_1ATPase or the ATP synthase of mitochondria. It consists of a complex of proteins that make up a tiny H^+-driven turbine. H^+ ions enter from the intermembrane space through protein a. They bind to a c unit in the rotator, which cause a rotation of the c-complex. After a nearly complete rotation, the H^+ ion is removed to the mitochondrial matrix. The c-complex binds the γ subunit and rotates it. The $\alpha\beta$ subunits of the head are kept steady by the stator components, δ and the b dimer. The γ subunit has a projection that interacts with each of the $\alpha\beta$ subunits, and this mechanical interaction is used to synthesize ATP from ADP and Pi. Complete rotation of the c-complex requires as many H^+ as c-subunits. For each complete rotation, the ATP synthase makes 3 ATP molecules.

THE PROTON ELECTROCHEMICAL GRADIENT PROVIDES THE ENERGY FOR ATP SYNTHESIS

The ETC pumps H^+ ions out of the matrix into the intermembrane space. The stoichiometry is about $10H^+$ ions per $2e^-$ when they originate from NADH, and about $6H^+$ when the $2e^-$ originate from $FADH_2$ (see Figure 2.10.7). Because the H^+ ions move without counter ions, this movement is an outward current that separates charge, and therefore there is a potential developed across the inner mitochondrial membrane. This potential varies depending on the state of mitochondrial activity, but a typical value is about 160 mV, negative inside. In addition to the potential, there is a concentration difference in H^+ established across the membrane. The pH of the intermembrane space is about 7.0, whereas the pH of the matrix is about 8.0. Recall that $pH = -\log [H^+]$, so that $pH = 7.0$ implies that $[H^+] = 10^{-7}$ M and $pH = 8$ means $[H^+] = 10^{-8}$ M. Thus there is a 10-fold difference in the $[H^+]$ established by the ETC. When H^+ ions travel from the intermembrane space to the matrix, they release the free energy stored in the electrochemical gradient for H^+, enabling the F_1 subunit to synthesize ATP from ADP and Pi. This **proton electrochemical gradient** is sometimes called the **proton motive force**. The free energy for H^+ transfer from the intermembrane space to the mitochondrial matrix is calculated as

$$\begin{aligned}\Delta\mu_{\text{out}\to\text{in}} &= \mu_{\text{in}} - \mu_{\text{out}} \\ &= \mu^0 + RT\ln[H^+]_{\text{in}} + \Im\psi_{\text{in}} - \mu^0 \\ &\quad - RT\ln[H^+]_{\text{out}} - \Im\psi_{\text{out}} \\ &= RT\ln\frac{[H^+]_{\text{in}}}{[H^+]_{\text{out}}} + \Im(\psi_{\text{in}} - \psi_{\text{out}})\end{aligned} \qquad [2.10.10]$$

The free energy change for ATP synthesis under the conditions of the cell varies from cell to cell and from place to place within the cell because the local concentrations of ADP, Pi, ATP, and ions that bind to them (H^+, Ca^{2+}, and Mg^{2+}) also vary from place to place. Nevertheless, we have already calculated an approximate free energy change for ATP hydrolysis under conditions of the cell to be -57.1 kJ mol^{-1}. The free energy of ATP synthesis should be the opposite of this, $+57.1$ kJ mol^{-1}.

According to the result in Example 2.10.1, there is not enough energy in one H^+ transport to synthesize ATP. If we assume integral stoichiometry, we need at least three of them. The free energy for the reaction

$$\text{ADP} + \text{Pi} + 3H^+_{\text{out}} \to \text{ATP} + 3H^+_{\text{in}} \qquad [2.10.11]$$

EXAMPLE 2.10.1 Calculate the Free Energy in the Mitochondrial H^+ Electrochemical Gradient

The free energy per mole of H^+ is given by Eqn [2.10.1] as

$$\Delta\mu_{H_{out}\rightarrow H_{in}} = RT\ \ln\left(\frac{[H^+]_{in}}{[H^+]_{out}}\right) + \Im(\psi_{in} - \psi_{out})$$

Inserting values of $R = 8.314\ J\ mol^{-1}\ K^{-1}$, $T = 310\ K$, $[H^+]_{in} = 10^{-8}\ M$, $[H^+]_{out} = 10^{-7}\ M$, we calculate the chemical part as

$$RT\ \ln\left(\frac{[H^+]_{in}}{[H^+]_{out}}\right) = 8.314\ J\ mol^{-1}\ K^{-1} \times 310\ K \times \ln\left(\frac{10^{-8}\ M}{10^{-7}\ M}\right)$$
$$= -5.93\ kJ\ mol^{-1}$$

Using $\Im = 9.649 \times 10^4\ C\ mol^{-1}$ and $\psi_{in} = -0.16\ V$ (with $\psi_{out} = 0$), the electrical part of the free energy change is

$$\Im(\psi_{in} - \psi_{out}) = 9.649 \times 10^4\ C\ mol^{-1} \times (-0.16\ V - 0)$$
$$= -15.44\ kJ\ mol^{-1}$$

Thus the total free energy change for H^+ transfer from the intermembrane space to the matrix, for this condition given here, is **$-21.37\ kJ\ mol^{-1}$**.

is the sum of the free energy of two processes:

$$\begin{aligned} &ADP + Pi \rightarrow ATP \\ &3H^+_{out} \rightarrow 3H^+_{in} \end{aligned} \qquad [2.10.12]$$

We add the two to get

$$\begin{aligned} \Delta\mu &= \Delta\mu_{ADP+Pi\rightarrow ATP} + 3\Delta\mu_{H^+_{out}\rightarrow H^+_{in}} \\ &= 57.1\ kJ\ mol^{-1} + 3(-21.37\ kJ\ mol^{-1}) \\ &= -7.01\ kJ\ mol^{-1} \end{aligned} \qquad [2.10.13]$$

The negative free energy change for this coupled reaction indicates that this process will proceed spontaneously. That is, there is enough energy in the electrochemical gradient of H^+ across the inner mitochondrial membrane to synthesize 1 ATP for every $3H^+$ ions transported. As it turns out, the stoichiometry is not integral.

NADH FORMS ABOUT 2.5 ATP MOLECULES; $FADH_2$ FORMS ABOUT 1.5 ATP MOLECULES

The amount of ATP formed from oxidative phosphorylation has been controversial but a consensus seems to have been reached. Measurements show that electron transport beginning with NADH results in $10H^+$ ions being transported from matrix to intermembrane space, and with $FADH_2$ the number is 6, because the first complex is bypassed. What happens to these H^+ ions? Most are used to drive the ATP synthase as described in Figure 2.10.9, but some are used to bring the phosphate into the mitochondria from the cytosolic compartment (see Figure 2.10.10) and some are used for other transport processes. Mitochondria from the heart of cows has 8 c-subunits in their ATP synthase, suggesting that $8H^+$ ions are needed for one complete rotation of the rotor and synthesis of 3 ATP molecules. This gives a nonintegral stoichiometry: each ATP requires $8/3 = 2.67H^+$ ions! Our calculation above indicates that the minimum number is $57.1\ kJ\ mol^{-1}/21.87\ kJ\ mol^{-1} = 2.61$. Because $1H^+$ is required to import Pi, the number of ATP produced by NADH becomes $10/(2.67 + 1) = 2.7$ ATP/NADH. If the proton motive force is used to drive other processes, the ATP yield will be lower. Recent studies suggest a relatively constant H^+/ATP ratio of 4.0, including transport processes. In this case ATP production from NADH would be $10H^+$ per NADH/$4H^+$ per ATP = 2.5 ATP per NADH. For $FADH_2$, the ratio would be $6H^+$ per $FADH_2$/$4H^+$ per ATP = 1.5 ATP per $FADH_2$. These numbers are approximate and tentative. The ATPase from different sources has different c-ring sizes that may cause differences in the H^+/ATP ratio and therefore the ATP/NADH ratio.

ATP CAN BE PRODUCED FROM CYTOSOLIC NADH

The NADH produced in the cytosol by glycolysis cannot enter the mitochondrial matrix, yet it must be oxidized back to NAD^+ to allow glycolysis to proceed. This can be accomplished by lactate dehydrogenase, as described in Chapter 2.9, but this does not extract the energy of combustion remaining in the lactic acid. Two types of shuttle mechanisms have the effect of bringing cytosolic reducing equivalents into the matrix, without NADH itself actually entering the matrix. These shuttles are the **glycerol phosphate shuttle** and the **malate/aspartate shuttle**.

In the glycerol phosphate shuttle, NADH is oxidized to NAD^+ by the cytosolic glycerol-3-phosphate dehydrogenase, while dihydroxyacetone phosphate is simultaneously reduced to glycerol-3-phosphate. Glycerol-3-phosphate then penetrates the mitochondrial outer membrane and reduces FAD to $FADH_2$ by the mitochondrial glycerol-3-phosphate dehydrogenase to form dihydroxyacetone phosphate. In this way, we start with cytoplasmic NADH and dihydroxyacetone phosphate and we end up with mitochondrial $FADH_2$ and cytoplasmic NAD^+ and dihydroxyacetone phosphate. So the reducing power of NADH is transferred to $FADH_2$, which then enters the ETC to generate 1.5 ATP molecules. Because complex I is bypassed, only 1.5 ATP are made per molecule of NADH passed on to the mitochondria by the glycerol P shuttle. Figure 2.10.11 illustrates the glycerol phosphate shuttle.

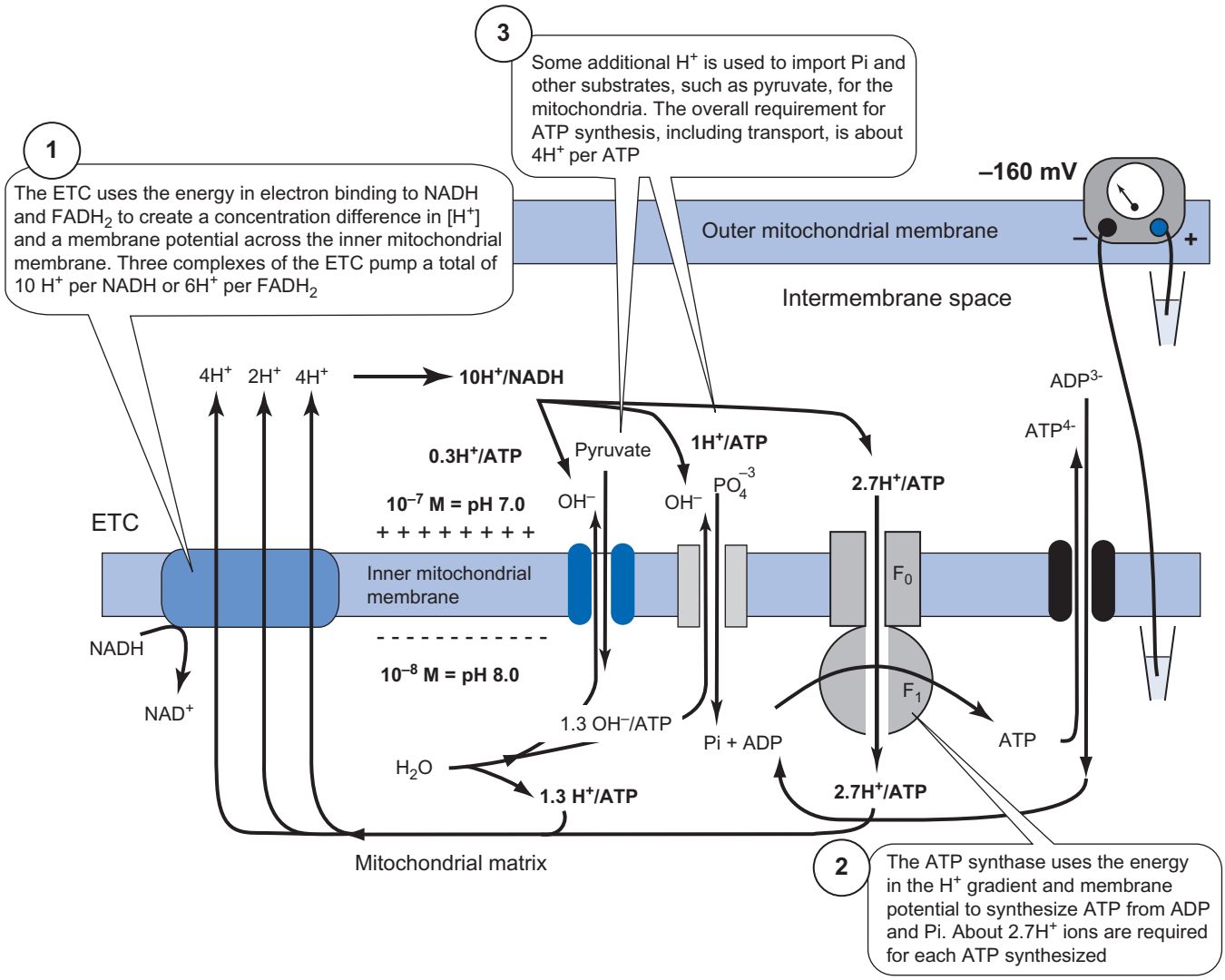

FIGURE 2.10.10 Overall coupling of the ETC to the ATP synthase. The ETC pumps electrons from the mitochondrial matrix to the intermembrane space, creating a [H^+] concentration gradient and an electrical potential difference. The ATP synthase uses the energy in this gradient to link ATP synthesis to H^+ ions going down their electrochemical gradient. Because of its mechanism (see Figure 2.10.9) in heart mitochondria $8H^+$ ions make 3 ATP molecules, for a stoichiometry of $2.7H^+$/ATP. However, the H^+ gradient is used for mitochondrial transport as well. For each Pi that enters the mitochondria, $1.0H^+$ ion is used—the exit of OH^- is equivalent to the entry of H^+. Small amounts of H^+ flow is also used for other transport processes. The total H^+ required for ATP synthesis, including transport, is about 4.0. Because each NADH causes ETC to pump $10H^+$ ions, this means that about 2.5 ATP molecules are formed per NADH. ATP exit and ADP entry do not require energy, as shown.

In the malate/aspartate shuttle, cytoplasmic NADH is used to convert cytoplasmic oxaloacetate to malate, which can be carried across the mitochondrial inner membrane by a **dicarboxylate carrier** by facilitated diffusion with no metabolic energy expenditure. Inside the matrix, the malate is converted back to oxaloacetate, generating the NADH back, which then transfers electrons to the ETC. To complete the cycle, oxaloacetate must get back outside. This is accomplished by converting oxaloacetate to aspartate (using glutamate as a substrate). The aspartate is transported out of the mitochondria where it is converted back to oxaloacetate and glutamate. The cycle is completed when glutamate goes back into the mitochondria. In this shuttle, 2.5 ATP molecules are produced for each cytosolic NADH because it is effectively transferred into the matrix as NADH. Figure 2.10.12 illustrates the malate/aspartate shuttle.

MOST OF THE ATP PRODUCED DURING COMPLETE GLUCOSE OXIDATION COMES FROM OXIDATIVE PHOSPHORYLATION

Figure 2.10.13 shows the production of ATP throughout glycolysis and the TCA cycle. Glycolysis utilizes 2 moles of ATP per mole of glucose and then produces 4 moles ATP per mole by substrate-level phosphorylation. The 2 moles of NADH produced by glyceraldehyde-3-P dehydrogenase can be converted to either 5 moles of

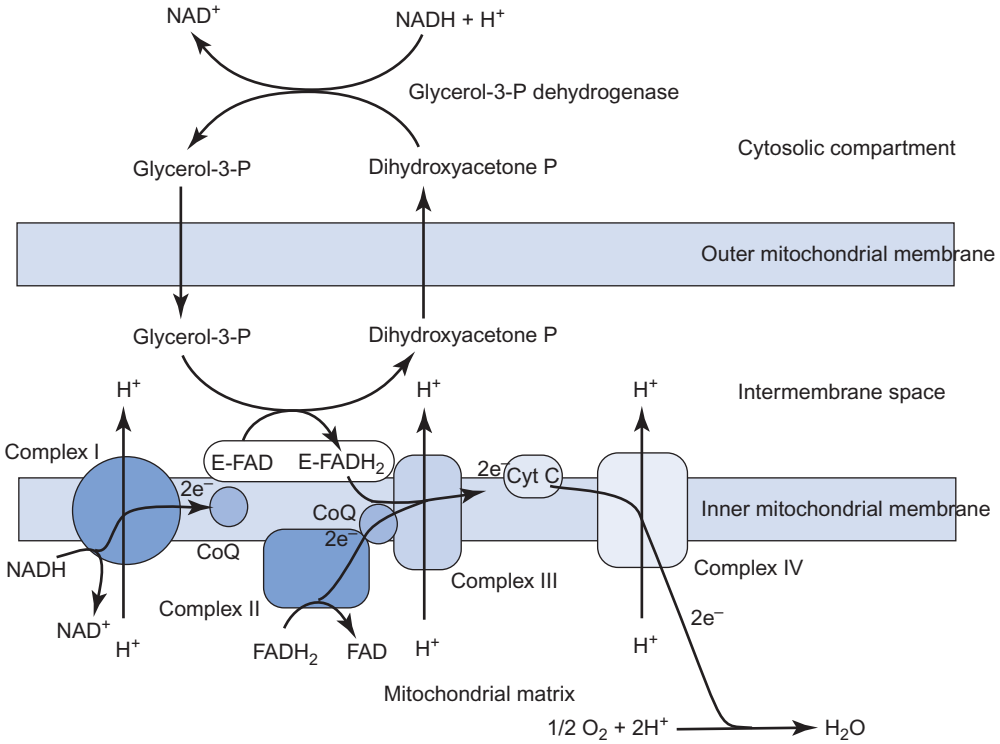

FIGURE 2.10.11 The glycerol phosphate shuttle for transferring cytosolic reducing equivalents to the mitochondria. Cytosolic NADH + H^+ is converted to NAD^+ by glycerol P dehydrogenase. Glycerol-3-P crosses the outer mitochondrial membrane and is converted back to dihydroxyacetone P by a mitochondrial glycerol-3-P dehydrogenase in the inner mitochondrial membrane. The dihydroxyacetone P goes back into the cytosol. The reduced mitochondrial glycerol-3-P dehydrogenase reduces ubiquinone in the inner membrane, which passes the reducing equivalents on to complex III of the ETC.

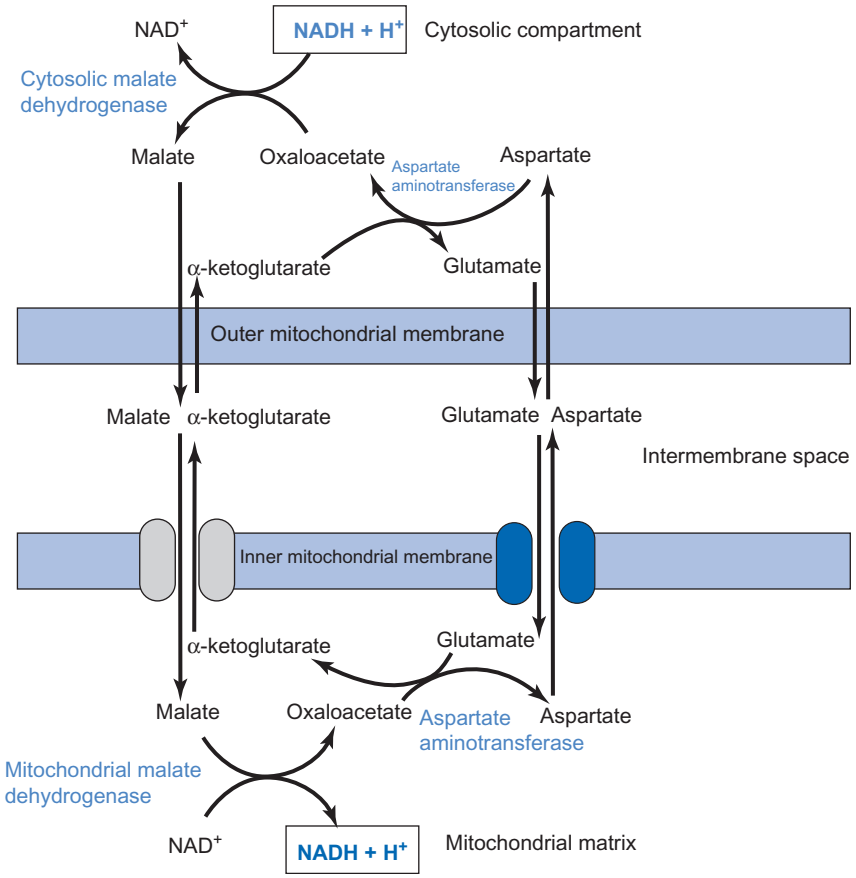

FIGURE 2.10.12 The malate shuttle. Two cycles running in opposite directions have the net effect of transferring NADH from cytosol to mitochondrial matrix. In one cycle, oxaloacetate is converted to malate while NADH is converted to NAD^+. Malate crosses the inner mitochondrial membrane where it is converted back to oxaloacetate and NADH. The oxaloacetate is then converted to aspartate, which leaves the mitochondria and passes back into the cytosol where the aspartate is converted back to oxaloacetate. A second cycle runs in the opposite direction. Malate entry into the mitochondrial matrix is accompanied by α-ketoglutarate exit. In the cytosol, the latter is converted to glutamate by aspartate amino transferase, which links the reaction α-ketoglutarate → glutamate to the reaction aspartate → oxaloacetate. The glutamate exchanges with aspartate across the inner mitochondrial membrane.

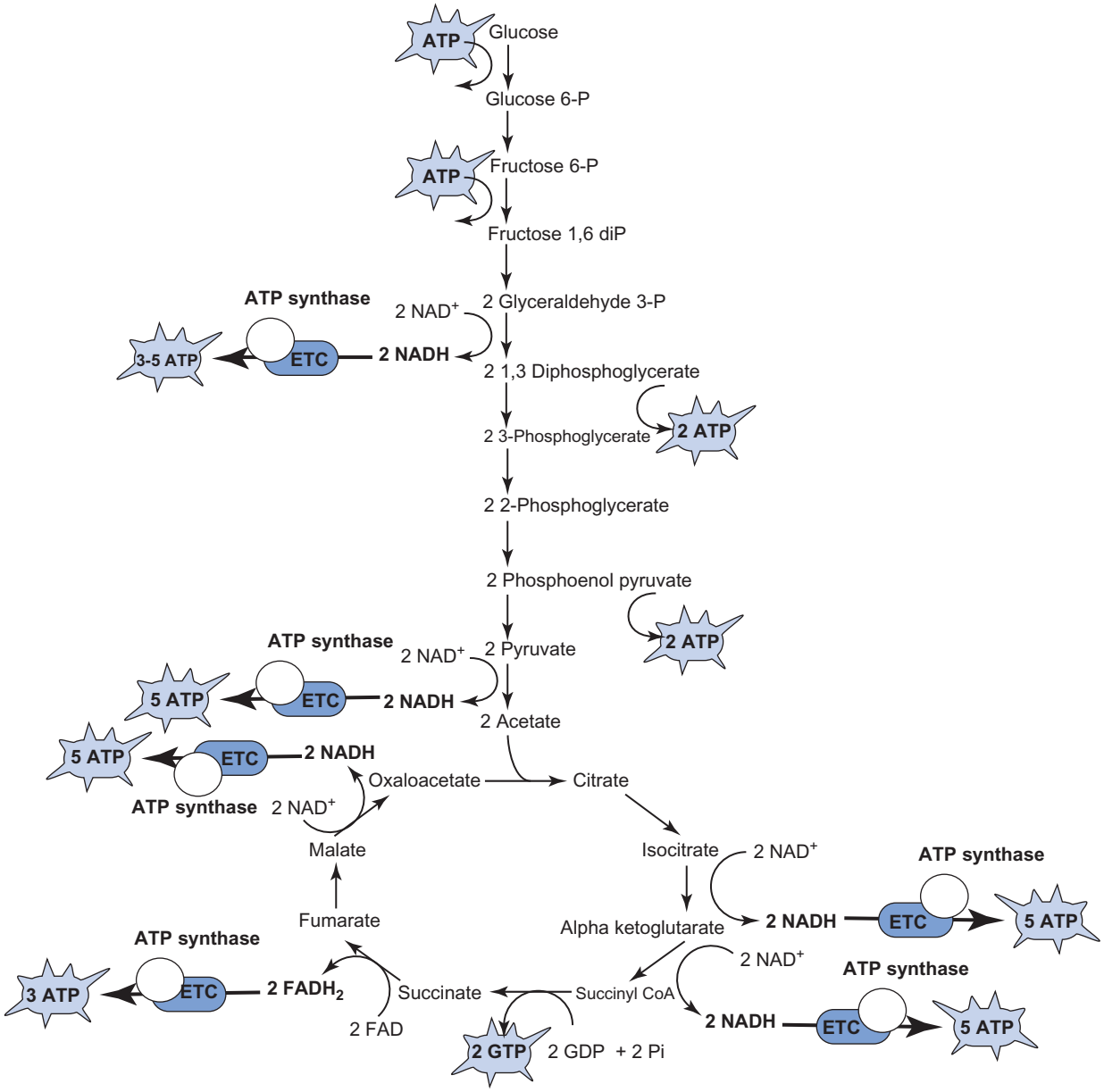

FIGURE 2.10.13 Overall ATP production from glycolysis, TCA cycle, and ETC. Glycolysis produces 4 ATP, but consumes 2 ATP molecules, per mole of glucose. It also produces 2 moles NADH per mole of glucose. These reducing equivalents are cytosolic and can result in 3–5 moles of ATP depending on how the NADH enters the mitochondria. In the mitochondria, a total of 8 moles of NADH are produced per mole of glucose, including 2 in the pyruvate dehydrogenase step and 6 in the TCA cycle. These each produce 2.5 moles of ATP per mole of NADH, so the NADH produces 8 moles NADH/mol glucose × 2.5 moles ATP/mol NADH = 20 moles ATP/mol glucose. The 2 $FADH_2$ produced at succinate dehydrogenase produce 1.5 moles of ATP per mole of $FADH_2$ for a total of 3 moles ATP/mol glucose. Succinyl CoA synthetase produces 2 moles GTP/mol glucose, which is energetically equivalent to 2 moles ATP/mol glucose. Total ATP production is thus 5–7 moles in glycolysis +25 moles in the mitochondria, for a total of 30–32 moles of ATP per mole of glucose.

ATP through the malate/aspartate shuttle or 3 moles of ATP through the glycerol phosphate shuttle.

MITOCHONDRIA HAVE SPECIFIC TRANSPORT MECHANISMS

In order for oxidative phosphorylation to work, the inner mitochondrial membrane must be impermeable to H^+ ions. H^+ ions in solution are usually bound to water as H_3O^+, the hydronium ion, which is very small. Therefore, the inner mitochondrial membrane must be relatively impermeable to most ions in order for the membrane to establish the potential difference and concentration difference in $[H^+]$. At the same time, things have to be able to get in and out. We have already discussed two such carriers—those that operate the shuttles that allow cytosolic reducing equivalents to enter the matrix. Several other carriers are shown schematically in Figure 2.10.14.

ATP produced in the matrix must leave the matrix to power cellular activities. This occurs through facilitated diffusion by the **ATP–ADP translocase**. Since ATP has

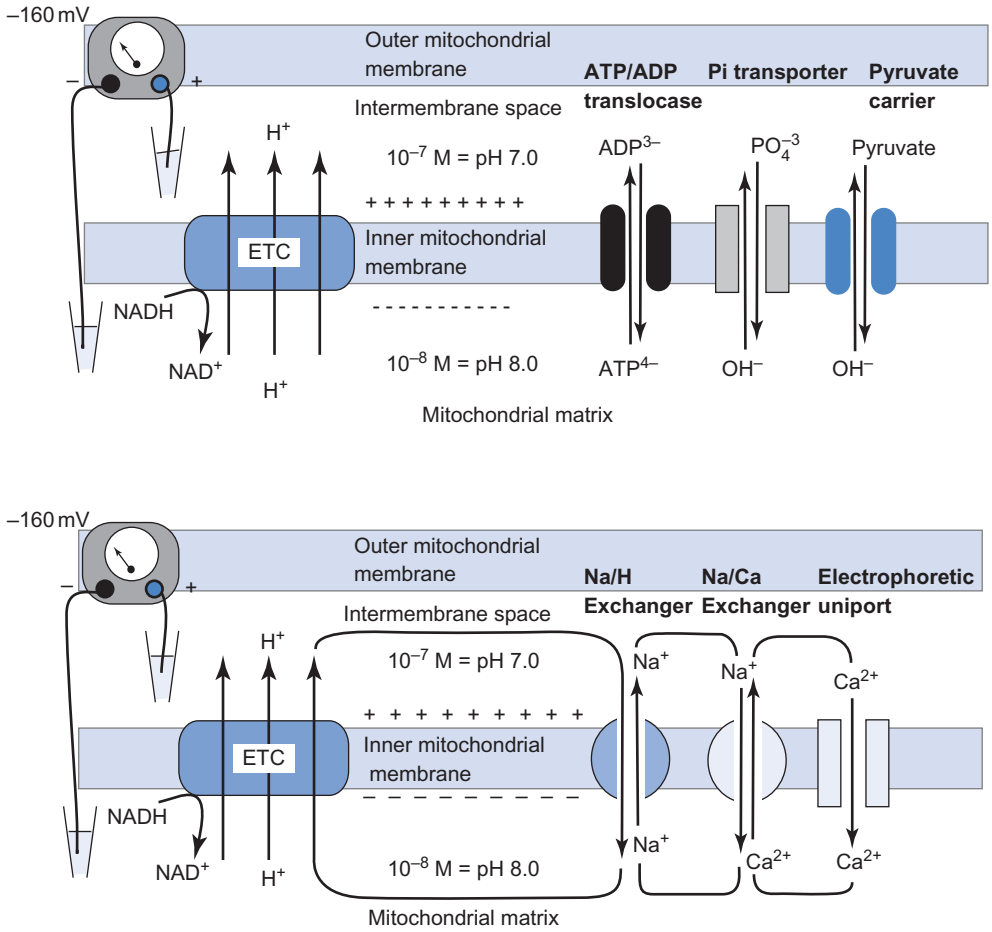

FIGURE 2.10.14 Selected transport mechanisms in mitochondria. See text for details.

more negative charges, and its concentration is higher in the mitochondria where it is produced, the transport is "downhill" in both directions. This translocase is poisoned by **atractyloside**.

Phosphate must also enter the matrix in order to be incorporated into ATP. Since phosphate is highly charged, movement across the membrane against a strong electric force is energetically unfavorable. Phosphate is carried across in exchange for OH^- ions. The outward flow of OH^- is equivalent to an inward flow of H^+. Therefore, some of the energy of the electrochemical H^+ gradient is used to transport phosphate into the mitochondria.

Mitochondria also take up Ca^{2+} because of the large negative potential inside. This uptake occurs through a channel called the **electrophoretic uniport**. This name signifies that the electrical gradient is the driving force for Ca^{2+} movement and that only one ion moves (uniport). Ca^{2+} taken up this way must be able to exit. This is accomplished by a **Na/Ca exchanger** that couples Ca^{2+} exit with Na^+ entry. This is another example of secondary active transport, in which "uphill" transport of Ca^{2+} is linked to "downhill" movement of Na^+. Of course, the Na^+ taken up by this process must also have an exit. Na^+ efflux from the mitochondria is through a **Na/H exchanger**. The Na/H exchanger is also an example of secondary active transport, in which uphill movement of Na^+ is coupled to downhill movement of H^+. In the mitochondria neither Na^+ efflux nor H^+ entry is linked to ATP hydrolysis. They are powered by the ETC pumping H^+ out of the mitochondrial matrix into the intermembrane space, thereby creating a large potential and a concentration gradient for H^+ ions.

SUMMARY

Glycolysis converts glucose into pyruvate, which enters the mitochondria by facilitated diffusion on its own carrier. The mitochondria couples the oxidation of pyruvate to the formation of the high-energy chemical bond in ATP, a process called oxidative phosphorylation. Inside the mitochondrial matrix, pyruvate enters into a series of reactions, beginning with pyruvate dehydrogenase. This series of reactions converts the three-carbon pyruvate molecule into three molecules of CO_2. In the first step, pyruvate dehydrogenase converts pyruvate into acetyl coenzyme A, producing a molecule of CO_2 and a molecule of NADH, nicotinamide adenine dinucleotide. Some of the chemical energy of oxidation of pyruvate is captured in the energy of electrons binding to NADH.

This energy is converted to ATP later on by the ATP synthase that relies on the electrochemical gradient of H^+ that is established by the ETC.

The second reaction combines acetyl CoA with oxaloacetate to form citric acid. This is the first step in the citric acid cycle, or the tricarboxylic acid cycle (TCA cycle), also called the Krebs cycle for Sir Hans Krebs. This cycle is a catalytic cycle in that it regenerates oxaloacetate and all parts of the acetate part of acetyl CoA are converted to CO_2 or H_2O. The cycle captures energy of oxidation by forming NADH or $FADH_2$, flavin adenine dinucleotide, or by forming GTP directly in the conversion of succinyl CoA to succinate by succinyl CoA synthetase. Each turn of the cycle produces 3 NADH molecules, 1 $FADH_2$ molecule, 1 molecule of GTP, and 2 molecules of CO_2.

The mitochondria make additional ATP molecules from NADH and $FADH_2$. These transfer their electrons to the ETC, which uses the energy of binding of the electrons, expressed as the reduction potential, to pump hydrogen ions from the mitochondrial matrix to the intermembrane space. This active pumping produces a concentration difference of H^+ ions and a large electrical potential. The ATP synthase in the inner mitochondrial membrane uses the energy in the electrochemical potential difference for H^+ to make ATP by a process that converts electrochemical energy to mechanical energy and back again to ATP. NADH enters the ETC early and its energy causes H^+ pumping at complexes I, III, and IV of the ETC. A total of $10H^+$ are pumped out per NADH molecule. The ATP synthase requires $4H^+$ ions to make ATP (about 3 for the direct use of the ATP synthase and 1 for transport of reactants into the mitochondria). As a consequence, each NADH produces about 2.5 ATP molecules. $FADH_2$ enters the ETC after complex I, and so it makes only 1.5 ATP molecules.

Thus complete oxidation of pyruvate to CO_2 and H_2O forms 4 NADH, 1 $FADH_2$, and 1 GTP for a total equivalent of $4 \times 2.5 + 1 \times 1.5 + 1 = 12.5$ ATP molecules. Since there are two pyruvate molecules formed from glucose, the TCA cycle accounts for 25 ATP molecules per glucose molecule.

NADH is also generated by glycolysis in the cytoplasm. This NADH is oxidized in the mitochondria, but indirectly because NADH itself cannot cross the inner mitochondrial membrane. Instead, two shuttle systems have the effect of transferring cytosolic NADH to mitochondrial matrix NADH. The glycerol phosphate shuttle converts cytosolic NADH to mitochondrial $FADH_2$, whereas the malate shuttle converts it to mitochondrial NADH. Glycolysis produces a net gain of 2 ATP and 2 NADH per glucose molecule.

REVIEW QUESTIONS

1. How does pyruvate get into the mitochondria? How does cytosolic NADH get into the mitochondria? How do ADP and Pi get into the mitochondria? How do ATP get out of the mitochondria?
2. Is NADH reduced or oxidized? Is FAD reduced or oxidized? Why does NADH make more ATP than $FADH_2$?
3. What is the TCA cycle? How many CO_2 molecules are released per pyruvate? Per acetate? In general, where is CO_2 released?
4. How many ATP molecules are produced during glycolysis per mole of glucose? Does this ATP production require oxygen?
5. What determines the direction of electron flow in the ETC? How do you calculate the energy of a reduction–oxidation reaction?
6. How many ATP molecules are produced in mitochondria during oxidative phosphorylation? Does this ATP production require oxygen?
7. Why is there a membrane potential across the inner mitochondrial membrane? If the ATP synthetase lets in H^+, why does not this current depolarize the mitochondria?
8. What is the chemiosmotic hypothesis? Some materials are proton ionophores. What effect would these have on oxidative phosphorylation?