1. Separated layers of charge create a voltage gradient

The voltage gradient across the cell membrane, or membrane potential, is created by an excess of positive charge on one side and a matching excess of negative charge on the other. The charge is concentrated in a thin (< 1 nm) layer on each side of the membrane.

6. A flow of current builds up a charge

For a squid axon membrane at the peak of the action potential, 
\[ g_{Na} = 300 \text{ pS/} \mu \text{m}^2; \]
the corresponding Na⁺ current is roughly
\[ i_{Na} = 5 \text{ pA/} \mu \text{m}^2. \]
If no other ions crossed the membrane, the charge transferred by the Na⁺ current if it were sustained for 0.2 ms at the peak value seen during the action potential would be 0.001 pC/\mu m². This charge would alter the membrane potential—see (1) and (2), above.

UNITS

| Charge: coulomb (C) (6.2 x 10⁻¹⁴ X charge on one electron) |
| Electric potential: volt (V) |
| Current: ampere (= coulombs per second) (A) |
| Capacitance: farad (= coulombs per volt) (F) |
| Conductance: siemens (= amperes per volt) (S) |
| mV: millivolt (10⁻³ V) |
| µF: microfarad (10⁻⁶ F) |
| nC: nanocoulomb (10⁻⁹ C) |
| pS: picosiemens (10⁻¹² S) |

3. The number of ions that go to form the layer of charge adjacent to the membrane is minute compared with the total number inside the cell.

One coulomb is the charge carried by roughly 6 x 10¹⁸ univalent ions, so that 0.001 pC is equivalent to 6000 univalent ions. Therefore the movement of 6000 Na⁺ ions across 1 µm² of membrane will carry sufficient charge to shift the membrane potential by about 100 mV. Because there are about 3 x 10⁷ Na⁺ ions in 1 µm³ of bulk cytoplasm, such a movement of charge will generally have a negligible effect on the ion concentration gradients across the membrane.

4. The electrochemical "driving force" is the sum of an effect of the membrane potential and an effect of the concentration gradient

For a univalent positive ion, such as Na⁺ or K⁺, at room temperature, the net "driving force" across the membrane is proportional to
\[ V = 58 \log_{10} \left( \frac{C_e}{C_i} \right), \]
where \( V \) is the membrane potential in millivolts, and \( C_e \) and \( C_i \) are, respectively, the extracellular and intracellular concentrations of the ion. The "driving force" for positive ions is zero when
\[ V = 58 \log_{10} \left( \frac{C_e}{C_i} \right) \text{ mV}. \]

This is the Nernst equation in its simplest form (see p. 315). It defines the equilibrium potential for the given positive ion. For the squid axon, the equilibrium potentials \( V_{Na} \), \( V_K \), and \( V_C \) for Na⁺, K⁺, and Cl⁻, are, respectively, about +55 mV, -75 mV, and -65 mV. The net driving force for each ion are proportional to \( V - V_e \), \( V - V_K \), and \( V - V_C \).
THE NERNST EQUATION AND ION FLOW

The flow of any ion through a membrane channel protein is driven by the electrochemical gradient for that ion. This gradient represents the combination of two influences: the voltage gradient and the concentration gradient of the ion across the membrane. When these two influences just balance each other the electrochemical gradient for the ion is zero and there is no net flow of the ion through the channel. The voltage gradient (membrane potential) at which this equilibrium is reached is called the equilibrium potential for the ion. It can be calculated from an equation that will be derived below, called the Nernst equation.

The Nernst equation is

\[ V = \frac{RT}{zF} \ln \frac{C_o}{C_i} \]

where \( V \) = the equilibrium potential in volts (internal potential minus external potential),
\( R \) = the gas constant (2 cal mol\(^{-1}\) K\(^{-1}\)),
\( T \) = the absolute temperature (K),
\( F \) = Faraday’s constant (96,485 coulombs per mole),
\( z \) = the valence (charge) of the ion,
\( \ln \) = logarithm to the base e.

The Nernst equation is derived as follows:

A molecule in solution (a solute) tends to move from a region of high concentration to a region of low concentration simply due to the pressure of numbers. Consequently, movement down a concentration gradient is accompanied by a favorable free-energy change ($\Delta G < 0$), whereas movement up a concentration gradient is accompanied by an unfavorable free-energy change ($\Delta G > 0$). (Free energy is introduced and discussed in Panel 14-1, pp. 668–669.) The free-energy change per mole of solute moved across the plasma membrane ($\Delta G_{\text{osm}}$) is equal to -$RT \ln C_o/C_i$. If the solute is an ion, moving it into a cell across a membrane whose inside is at a voltage $V$ relative to the outside will cause an additional free-energy change (per mole of solute moved) of $\Delta G_{\text{vol}} = zFV$. At the point where the concentration and voltage gradients just balance, $\Delta G_{\text{conc}} + \Delta G_{\text{vol}} = 0$ and the ion distribution is at equilibrium across the membrane. Thus,

\[ zFV - RT \ln \frac{C_o}{C_i} = 0 \]

and, therefore,

\[ V = \frac{RT}{zF} \ln \frac{C_o}{C_i} = \frac{RT}{zF} \log_{10} \frac{C_o}{C_i} \]

For a univalent ion,

\[ \frac{RT}{F} = 56 \text{ mV at } 20^\circ \text{C} \text{ and } 61.5 \text{ mV at } 37^\circ \text{C} \]

Thus, for such an ion at 37°C, $V = +61.5$ mV for $C_o / C_i = 10$, whereas $V = 0$ for $C_o / C_i = 1$.

The $K^+$ equilibrium potential ($V_K$), for example, is $61.5 \log_{10}(K^+o / K^+i)$ millivolts (~89 mV) for a typical cell, where $[K^+]o = 5 \text{ mM}$ and $[K^+]i = 140 \text{ mM}$. At $V_K$ there is no net flow of $K^+$ across the membrane. Similarly, when the membrane potential has a value of $61.5 \log_{10}(Na^+o / Na^+i)$, the Na$^+$ equilibrium potential ($V_{Na}$), there is no net flow of Na$^+$.

For any particular membrane potential, $V_m$, the net force tending to drive a particular type of ion out of the cell is proportional to the difference between $V_m$ and the equilibrium potential for the ion; hence, for $K^+$ it is $V_m - V_K$ and for Na$^+$ it is $V_m - V_{Na}$.

The number of ions that go to form the layer of charge adjacent to the membrane is minute compared with the total number inside the cell. For example, the movement of 6000 Na$^+$ ions across 1 $\mu$m$^2$ of membrane will carry sufficient charge to shift the membrane potential by about 100 mV. Because there are about $3 \times 10^7$ Na$^+$ ions in 1 $\mu$m$^2$ of bulk cytoplasm, such a movement of charge will generally have a negligible effect on the ion concentration gradients across the membrane.
GLYCOLYSIS

Whether aerobic or anaerobic, the process of glucose catabolism involves a reaction sequence that is without doubt the single most ubiquitous pathway in all energy metabolism. Glycolysis occurs in almost every living cell. It is generally regarded as a primitive process. Thus it occurs in the cytosol rather than being compartmentalized into a specific organelle within the eukaryotic cell. Furthermore, it is thought to have arisen early in biological history, before the advent of eukaryotic organelles and perhaps even before oxygen was a prominent component in the atmosphere.

The glycolytic pathway was the first major metabolic sequence to be elucidated. Most of the decisive work was done in the 1930s by the German biochemists G. Embden, O. Meyerhof, and O. Warburg, two of whom gave the sequence its alternative name, the Embden-Meyerhof pathway.

The glycolytic pathway appears in detail in Figure 14–1 (also see Table 14–1) and is shown in the context of overall chemotrophic energy metabolism in Figure 15–1. The essence of the process is suggested by the name, since glycolysis comes from the Greek roots glykos, meaning sweet, and lysis, meaning loosing. Literally, then, glycolysis is the loosing or splitting of something sweet, which is, of course, the starting sugar. From Figure 14–1 it is clear that the actual splitting occurs at the aldolase step. It is at this point that a six-carbon sugar is cleaved to yield two three-carbon compounds, one of which, glyceraldehyde-3-phosphate, is the only oxidizable molecule in the whole pathway. Subsequent to the cleavage, two successive ATP-generating steps occur. These represent the energy payoff of the process, since they are the only ATP-yielding reactions of the pathway under anaerobic conditions.

### Table 14-1

Readings, Enzymes, and Free Energies for Steps in the Glycolytic Pathway

<table>
<thead>
<tr>
<th>Step</th>
<th>Reaction</th>
<th>Enzyme</th>
<th>ΔG**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose + ATP → glucose-6-phosphate + ADP + H⁺</td>
<td>Hexokinase</td>
<td>-4.0</td>
</tr>
<tr>
<td>2</td>
<td>Glucose-6-phosphate → fructose-6-phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP + H⁺</td>
<td>Phosphofructokinase</td>
<td>-3.4</td>
</tr>
<tr>
<td>4</td>
<td>Fructose-1,6-bisphosphate → dihydroxyacetone phosphate + glyceraldehyde-3-phosphate</td>
<td>Aldolase</td>
<td>+5.7</td>
</tr>
<tr>
<td>5</td>
<td>Dihydroxyacetone phosphate → glyceraldehyde-3-phosphate</td>
<td>Triose phosphate isomerase</td>
<td>+1.8</td>
</tr>
<tr>
<td>6</td>
<td>Glyceraldehyde-3-phosphate + Pᵢ + NAD⁺ → glyceraldehyde-1,3-bisphosphate + NADH + H⁺</td>
<td>Phosphoglycerate dehydrogenase</td>
<td>+1.5</td>
</tr>
<tr>
<td>7</td>
<td>Glyceraldehyde-3-phosphate + ADP → glyceraldehyde-3-phosphate + ATP</td>
<td>3-Phosphoglycerate kinase</td>
<td>-4.5</td>
</tr>
<tr>
<td>8</td>
<td>Glyceraldehyde-3-phosphate → glyceraldehyde-2-phosphate</td>
<td>Phosphoglyceromutase</td>
<td>+1.1</td>
</tr>
<tr>
<td>9</td>
<td>Glyceraldehyde-2-phosphate → phosphoenolpyruvate + H₂O</td>
<td>Enolase</td>
<td>+0.4</td>
</tr>
<tr>
<td>10</td>
<td>Phosphoenolpyruvate + ADP + H⁺ → pyruvate + ATP</td>
<td>Pyruvate kinase</td>
<td>-7.5</td>
</tr>
</tbody>
</table>

Net reaction: 

\[
C₆H₁₂O₆ + 2 NAD⁺ + 2 ADP + 2 Pᵢ → 2 C₃H₄O₃ + 2 NADH + 2 H⁺ + 2 ATP + 2 H₂O
\]
EATP

Glucose by means of acetyl-CoA and oxaloacetate (y) to CO₂ by transferring electrons to 

Cytochrome is converted to cytochrome the TCA cycle, leading to the oxidation of glucose into the TCA cycle. The components of respiratory metabolism include glycolysis, the TCA cycle.
of the most interesting reactions. III can be resolved into two components (Section 5.8). Redox potentialities can also be employed for the Fe/S proteins, in which case the redox state of the components is monitored by ESR.

![Diagram of mitochondrial respiratory chain](image)

**Figure 5.9** $E_m$ values for components of the mitochondrial respiratory chain and $E_n$ values for mitochondria respiring in state 4. Values are consensus values for mammalian mitochondria. (→) $E_{m,7}$ values obtained with de-energized mitochondria, (→) $E_{n,7}$ values for mitochondria in state 4.

\[
NAD^+ + H^+ + 2e^- \rightarrow NADH \\
\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O
\]

\[
\frac{1}{2}O_2 + NADH + H^+ \rightarrow H_2O + NAD^+ \quad \Delta \varepsilon = 1.130 \text{V}
\]

\[
\Delta G = -nF\Delta \varepsilon' = -\left(2 \text{ mole}^{-1} \text{ mol}^{-1}\right) \times 23 \left(\text{kcal mol}^{-1}\right) (1.13 \text{V}) = 521 \text{ kcal mol}^{-1}
\]
Table 14-2  Typical ion concentrations in invertebrates and vertebrates

<table>
<thead>
<tr>
<th></th>
<th>Cell (mM)</th>
<th>Blood (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SQUID AXON</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>400</td>
<td>20</td>
</tr>
<tr>
<td>Na⁺</td>
<td>50</td>
<td>440</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>40–150</td>
<td>560</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.0003</td>
<td>10</td>
</tr>
<tr>
<td>X⁻</td>
<td>300–400</td>
<td></td>
</tr>
<tr>
<td><strong>MAMMALIAN CELL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>139</td>
<td>4</td>
</tr>
<tr>
<td>Na⁺</td>
<td>12</td>
<td>145</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>4</td>
<td>116</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>12</td>
<td>29</td>
</tr>
<tr>
<td>X⁻</td>
<td>138</td>
<td>9</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>&lt;0.0005</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*The large nerve axon of the squid is chosen as an example of an invertebrate cell because it has been used widely in studies of the mechanism of conduction of electric impulses.

'X⁻' represents proteins, which have a net negative charge at the neutral pH of blood and cells.

ions even if the cells are cultured in very dilute salt solutions. The generation and maintenance of such ion gradients require a great deal of energy.

Another important property of the plasma membrane is that it is selectively permeable to different cations and anions, including the principal cellular ions Na⁺, K⁺, and Cl⁻. The membrane contains specific types of transport proteins, called ion channel proteins, that allow certain ions to move through it at different rates down their concentration gradients. Selective permeability and ion concentration gradients together create a difference in electric potential between the inside and the outside of the cell. The magnitude of this potential—~70 millivolts (mV) with the inside of the cell negative with respect to the outside—does not seem like much until we realize that the plasma membrane is only about 3.5 nm thick. Thus the voltage gradient across the plasma membrane is 0.07 V per 3.5 × 10⁻⁷ cm, or 200,000 volts per centimeter!

The ion gradients and electric potential across the plasma membrane drive many biological processes. Variations in the permeability of Na⁺ and K⁺ ions are essential to the conduction of an electric impulse down the axon of a nerve cell. In many animal cells, the concentration gradient of Na⁺ ions and the membrane electric potential power the transport of other molecules into the cells against their concentration gradient; amino acids frequently enter cells in this manner. In most cells, a rise in the concentration of Ca²⁺ ions in the cytosol is an important regulatory signal. In muscle cells, for instance, it initiates contraction; in the exocrine cells of the pancreas, it triggers secretion of digestive enzymes.

First, we will turn to the question of how ion gradients and specific ion transport proteins generate a membrane electric potential. Then we will examine the proteins that generate ion concentration gradients.

Simple Models Explain the Electric Potential across the Cell Membrane

The situation outlined in Figure 14-7 is similar to the distribution of Na⁺ and Cl⁻ ions between an animal cell and its aqueous environment. A membrane separates a 15 mM solution of NaCl on the right side (representing the "outside" of the cell) from a 15-mM solution of NaCl on the left side (the "inside"). A potentiometer (voltmeter) connected to the solution on each side to measure the difference in electric potential across the membrane. If the membrane is impermeable to both Na⁺ and Cl⁻ ions, ions will flow across it (there will be no electric potential difference across it; see Figure 14-7a).

Now suppose the membrane is permeable only to Na⁺ ions; it contains channel proteins that accommodate Na⁺ ions but exclude Cl⁻ ions. Na⁺ ions then tend to move down their concentration gradient from the right side of the left, leaving an excess of negative Cl⁻ ions compared with Na⁺ ions on the right side and generating an excess of positive Na⁺ ions compared with Cl⁻ ions on the left side. There is now a separation of charge across the membrane, which a potentiometer can measure as an electric potential, or voltage. The right side is negative with respect to the left (see Figure 14-7b). As more and more Na⁺ ions move across the membrane, the magnitude this charge difference increases. However, continuous movement of the Na⁺ ions eventually is inhibited by the excess of positive charges accumulated on the left side of the membrane and by the attraction of Na⁺ ions to the excess negative charge built up on the right side. The system soon reaches an equilibrium point at which the opposing factors that determine the movement of Na⁺ ions—the membrane electric potential and the ion concentration gradient—balance each other out. At equilibrium, no net movement of Na⁺ ions occurs across the membrane.

The magnitude of the resulting sodium equilibrium potential in volts (the electric potential across a membrane permeable only to Na⁺ ions) is given by the Nernst equation, which is derived from basic principles of physical chemistry:

\[
E_{Na} = \frac{RT}{ZF} \ln \frac{N_{A}}{N_{Na}}
\]

where \( R \) (the gas constant) = 1.987 cal/(degree-mol), 8.28 joules/(degree-mol), \( T \) (the absolute temper
tured) = 293°K at 20°C, $Z$ (the valency) = +1, $F$ (the Faraday constant) = 23,062 cal/(mol·V), or 96,000 coulombs/(mol·V), and $Na^+$ and $Na^+$, are the $Na^+$ equilibrium concentrations on the left and right sides, respectively. The Nernst equation is similar to the equations used to calculate the voltage change associated with oxidation or reduction reactions, which also involve movement of electric charges. At 20°C, equation 14-7 reduces to

$$E_{Na^+} = 0.059 \log \frac{Na^+}{Na^+}$$  \hspace{1cm} (14-8)

In this example, $Na^+/Na^+ = 0.1$ and $E_{Na^+} = -0.059$ V, or $-59$ mV, with the right side negative with respect to the left.

If the membrane is permeable only to $Cl^-$ ions and not to $Na^+$ ions, the calculation is the same:

$$E_{Cl^-} = \frac{RT}{ZF} \ln \frac{Cl^-}{Cl^-}$$  \hspace{1cm} (14-9)

except that $Z = -1$. The magnitude of the membrane electric potential is the same (59 mV), except that the right side is now positive with respect to the left (see Figure 14-7c). This is precisely the opposite polarity to that obtained with selective $Na^+$ permeability.

If the membrane is permeable to $Na^+$ and $Cl^-$ ions to the same degree, then $Na^+$ and $Cl^-$ ions can move together down their concentration gradients from the right side to the left. In this case, no membrane electric potential is expected and none is observed. An intermediate situation between these two extremes can also occur. When the membrane is permeable to both $Na^+$ and $Cl^-$ ions but more permeable to $Na^+$ ions, the right side initially has a negative potential relative to the left, but the magnitude of the potential is somewhat less than $E_{Na^+} = -59$ mV. Eventually, due to the diffusion of $Na^+$ and $Cl^-$

\textbf{Figure 14-7}  A voltage potential is created by the selective permeability of a membrane to different ions. (a) An impermeable membrane separates a 150-mM NaCl solution from a 15-mM NaCl solution. No ions move across the membrane, and no difference in electric potential is registered on the potentiometer connecting the two solutions. (b) The membrane is selectively permeable only to $Na^+$ ions, which diffuse from right to left down the concentration gradient. The $Cl^-$ anion cannot cross the membrane, so a net positive charge builds up on the left side and a negative charge builds up on the right side. At equilibrium, the membrane potential caused by the charge separation becomes equal to the Nernst potential $E_{Na^+}$ registered on the potentiometer, and the movement of $Na^+$ ions in the two directions becomes equal. (c) The membrane is selectively permeable only to $Cl^-$ ions, which diffuse from right to left down the concentration gradient. The $Na^+$ ion cannot cross the membrane, creating a net negative charge on the left side and a net positive charge on the right side. At equilibrium, the membrane electric potential is equal to $E_{Cl^-}$. 
ions, there will be an equal concentration of ions on both sides of the membrane and no membrane electric potential.

Thus two forces govern the movement of such ions as K⁺, Cl⁻, and Na⁺ across selectively permeable membranes: the membrane electric potential, and the ion concentration gradient. These forces may act in the same direction or in opposite directions. The free-energy change $\Delta G$ required to transport 1 mol of Na⁺ ions from the outside (exterior) to the inside (cytosol) of a typical mammalian cell is about $-3 \text{kcal/mol}$ (Figure 14-8). Since $\Delta G < 0$, this reaction is thermodynamically favored. About one-half of this $\Delta G$ value is contributed by the membrane electric potential and one-half is contributed by the Na⁺ ion concentration gradient. It is important to understand these forces in some detail, since the inward movement of Na⁺ ions is used to power the uphill movement of several ions and small molecules into animal cells.

The free-energy change generated from the Na⁺ ion concentration gradient is

$$\Delta G_c = RT \ln \frac{\text{Na}_\text{in}}{\text{Na}_\text{out}}$$  (14-10)

At the Naᵢn and Naᵢout values in Figure 14-8, which are typical for many cells, $\Delta G_c = -1.45 \text{kcal/mol}$, the change in free energy that would be required to transport 1 mol of Na⁺ ions from outside to inside the cell if there were no membrane electric potential. The free-energy change generated from the membrane electric potential is

$$\Delta G_m = \mathcal{F}E$$  (14-11)

where $\mathcal{F}$ is the Faraday constant and $E$ is the membrane electric potential. If $E = -70 \text{mV}$, then $\Delta G_m = -1.6 \text{kcal mol}$, the change in free energy that would be required to transport 1 mol of Na⁺ ions from outside to inside the cell if there were no Na⁺ ion concentration gradient. Given both forces acting on Na⁺ ions, the total $\Delta G$ will be the sum of the two partial values:

$$\Delta G = \Delta G_c + \Delta G_m = (-1.45) + (-1.61) = -3.06 \text{kcal/mol}$$

In this typical example, the Na⁺ ion concentration gradient and the membrane electric potential contribute almost equally to total $\Delta G$ for Na⁺ ion transport.

**Active Ion Transport and ATP Hydrolysis**

When the aerobic production of adenosine triphosphate (ATP) in a cell is inhibited experimentally by 2,4-dinitrophenol, the ion concentration inside the cell gradually approaches that of the exterior environment. This is caused by a slow leak of ions across the membrane down their electric and concentration gradients. Eventually the cell dies, in part because many intracellular enzymes are specialized to function in a solution of low Na⁺ ions, neutral pH, and high K⁺ ions. A significant fraction of available energy in every cell is required to maintain the concentration gradients of such ions as Na⁺, K⁺, H⁺, and Ca²⁺ across the plasma and intracellular membranes. In the human erythrocyte, up to 50 percent of the energy stored in ATP molecules is used for this purpose. Thus a central issue of cellular metabolism is how permeation systems use energy.

There are three principal classes of enzymes in which ATP hydrolysis is directly coupled to ion transport against an electrochemical gradient (Table 14-3). In the P class of ATPases, a single transmembrane polypeptide is phosphorylated during the transport process. Included in this class is the Na⁺-K⁺ ATPase, which transports Na⁺ ions out of and K⁺ ions into an animal cell, and several Ca²⁺ ATPases, which transport Ca²⁺ ions out of the cell or, in muscle cells, from the cytosol to the sarcoplasmic reticulum. A third member of the P class transports protons, in some cases together with K⁺. The V and F classes are always proton-transporting proteins; V ATPases maintain the low pH of plant vacuoles and of lysosomes and other vesicles in animal cells.

In each case, an enzyme system that can split ATP into adenosine diphosphate (ADP) and inorganic phosphate
tors about 1958, and it still goes under two different names, ubiquinone and coenzyme Q. The former name indicates the almost ubiquitous nature of the quinone in aerobic cells ranging from bacteria to plants and mammals. The length of the side chain varies from six prenyl (five-carbon) units in some bacteria to ten in mammals, but the different ubiquinones are functionally interchangeable. Some bacteria contain a naphthoquinone with a similar side chain (menaquinone, or vitamin K) in place of or in addition to UQ. With either UQ or the menaquinones, the hydrocarbon tail makes the molecule strongly hydrophobic. In bacteria, the UQ is found in the cytoplasmic membrane; in eukaryotes it is found mainly in the mitochondrial inner membrane. Compared with the concentrations of the cytochromes in these membranes, the concentration of UQ is relatively large. In heart mitochondria, for example, the concentration of UQ is about seven times that of cytochrome a₃. Although the mitochondrial inner membrane contains several different proteins that bind UQ, at any given time most of the UQ probably is not bound to proteins, but rather moves freely in the phospholipid bilayer of the membrane.

Ubiquinone undergoes a two-electron reduction to the dihydroquinone or quinol, UQH₂ (Figure 16-9). It also can accept a single electron and stop at the semiquinone, which can be either anionic (UQ⁻) or neutral (UQH⁻), depending on the pH and on the nature of the binding site when the semiquinone is bound to a protein. The • in UQ•⁻ or UQH• indicates that the semiquinone contains an unpaired electron.

The reduction of UQ can be measured by the disappearance of an absorption band at 275 nm. Using this technique, it was shown that the addition of a substrate such as succinate caused a rapid reduction of essentially all the UQ present in the mitochondrial membrane, and that the UQH₂ could be reoxidized by the cytochrome system in the presence of O₂. To determine whether UQ is a necessary participant in electron transport from succinate to O₂, the quinone was removed from the mitochondria by extraction with an organic solvent. The depleted mitochondria were incapable of respiration but recovered this activity when they were reconstituted with UQ. Similar results were obtained with electron donors other than succinate, including glycerol-3-phosphate and substrates that transfer their electrons initially to NADH, such as malate, α-ketoglutarate, and β-hydroxybutyrate (CH₃—CHOH—CH₂—CO₂⁻). These results suggest that the large pool of UQ in the mitochondrial inner membrane accepts electrons from several different dehydrogenases, and is responsible for passing these electrons on to the cytochrome system. The kinetics of UQ reduction and oxidation are consistent with this view.

Redox Potentials Are a Measure of Oxidizing Strength

We have seen that the respiratory system includes a variety of molecules—cytochromes, flavins, ubiquinone, and iron-sulfur proteins—all of which can act as electron carriers. Our goal now is to understand how these carriers are organized to transport electrons from reduced substrates to O₂. To proceed, we first need to have a way of characterizing the relative tendencies of these different molecules to give off or accept electrons.

Consider the lactate dehydrogenase reaction, in which NADH reduces pyruvate to lactate:

\[ \text{NADH} + \text{H}^+ + \text{pyruvate} \rightarrow \text{NAD}^+ + \text{lactate} \]

Conceptually, we can imagine the reaction as occurring in two steps. In the first step, NADH undergoes oxidation to NAD⁺, releasing two electrons and
a proton; in the second, pyruvate undergoes reduction by accepting two electrons and two protons:

\[
\text{NADH} \rightarrow \text{NAD}^+ + 2e^- + H^+ \quad (1)
\]

\[
\text{Pyruvate} + 2e^- + 2H^+ \rightarrow \text{lactate} \quad (2)
\]

Actually, it is a hydride ion (H\(^-\)) that is transferred from NADH to pyruvate (see Chapter 11), but the details of the mechanism do not concern us for the moment. Each of the steps involves a redox couple of two molecules that are interconvertible by the transfer of electrons and protons.

The standard free energy change (\(\Delta G^\circ\)) for the overall reaction between NADH and pyruvate is \(-6.22\) kcal/mole. The negative sign implies that, under standard conditions (1 M NADH, NAD\(^+\), lactate, and pyruvate, pH 7, and 25°C), the reaction proceeds spontaneously in the direction of lactate. This means that NADH must be a stronger electron donor than lactate. Now how does NADH compare with FMNH\(_2\) or with cytochrome c? To deal quantitatively with questions like these, it is useful to define a parameter called the standard redox potential, \(E^\circ\), which expresses the relative tendency of a pair of molecules like the NAD\(^+\)/NADH couple to release or accept electrons. Like the electrical potentials that govern the direction of electron flow from one physical region to another, redox potentials are specified in units of volts. Because electron-transfer reactions frequently involve protons also, an additional symbol is used to indicate that the \(E^\circ\) value applies to a particular pH; an \(E^\circ\) thus refers to an \(E^\circ\) at pH 7. (People who work in bioenergetics commonly refer to \(E^\circ\) as "the midpoint" potential and use the symbols \(E_m\) and \(E_m^\circ\) in place of \(E^\circ\) and \(E^\circ\).)

Suppose we have two redox couples \(D_{ox}/D_{red}\) and \(A_{ox}/A_{red}\), where the subscripts ox and red indicate the oxidized and reduced forms of the molecules. Consider the reaction in which \(D_{red}\) acts as an electron donor (reductant) and \(A_{ox}\) as the electron acceptor (oxidant). We take the difference between the \(E^\circ\) values of two couples (\(\Delta E^\circ\)) to be proportional to the standard free energy change for the reaction (see Box 12–C):

\[
D_{red} + A_{ox} \rightarrow D_{ox} + A_{red} \quad (3)
\]

\[
\Delta E^\circ = E_{A_{ox}}^\circ - E_{D_{ox}}^\circ = -\Delta G^\circ/nF \quad (4)
\]

or

\[
\Delta G^\circ = -nF \Delta E^\circ \quad (5)
\]

Here \(n\) is the number of electrons transferred from the donor to the acceptor (two in the case of the lactate dehydrogenase reaction), and the proportionality constant \(F\) is the Faraday constant (23,060 cal per volt per mole of electrons). Note that these expressions specify a convention concerning the signs of \(E^\circ\) values: the overall reaction is spontaneous (\(\Delta G^\circ\) negative) if \(\Delta E^\circ\) is positive, that is, if electrons are transferred from a couple with a more negative \(E^\circ\) value to one with a more positive value. In other words, relatively negative \(E^\circ\) values are associated with strong reductants, and relatively positive values with strong oxidants. With \(n = 2\), a difference of 0.1 V between the \(E^\circ\) values corresponds to a \(\Delta G^\circ\) of \(-4.61\) kcal/mole.

The relationship between \(\Delta G^\circ\) and \(\Delta E^\circ\) is simply the expression that describes electron flow between two physical regions that are at different electrical potentials, such as the two terminals of a battery. Electrons tend to flow spontaneously in the direction of more positive potential, and the maximum amount of work that can be obtained from such flow is proportional to the number of electrons that move and to the potential difference. In fact, one can measure \(\Delta E^\circ\) values by separating the two redox couples physically into different compartments connected by a salt bridge and a voltmeter (Figure 16–10). Electrons can flow from one solution to the other through the
 meter. If the solutions in the two compartments are both under standard conditions, the meter will sense a voltage difference equal to the $\Delta E^\circ$.

So far, we have defined only the difference between two $E^\circ$ values. To set the individual values, it is merely necessary to choose a particular redox couple as a reference. The reference that is used most commonly by biochemists is the standard hydrogen half-cell, in which protons at 1 M [H$^+$] are reduced to H$\_2$ at a pressure of 1 atm (2H$^+$ + 2e$^- \rightarrow H_2$). This half-cell is arbitrarily assigned an $E^\circ$ value of zero. (Note that the standard condition for this half-cell is pH 0; this does not restrict one's choice of conditions for the cell in the other compartment of the apparatus.) Relative to the standard hydrogen half-cell, the NAD$^+$/NADH couple has an $E^\circ$ of $-0.32$ V, and the pyruvate/lactate couple has an $E^\circ$ of $-0.19$ V.

Table 16-1 gives the $E^\circ$ values of a number of biochemical redox couples, including some of the components of the respiratory chain. These are listed in order of increasing $E^\circ$, which means that under standard conditions a given couple will reduce any of the couples below it in the table.

As mentioned above, quinones and flavins can undergo one-electron reduction to semiquinone forms, or can be fully reduced by the addition of two electrons. The $E^\circ$ values for the individual one-electron steps can be very different. In the case of UQ, the one-electron redox couples UQ/UQH$^-$ and UQH$^+$/UQH$_2$ have $E^\circ$ values of approximately $0.03$ and $0.19$ V. Since the $E^\circ$ of the second couple is more positive than that of the first, the second electron is thermodynamically easier to add than the first. When the first electron is put on, a second usually follows, as long as two electrons are available from the donor. The $E^\circ$ for the overall two-electron reduction of UQ to

<table>
<thead>
<tr>
<th>Redox Couple</th>
<th>$E^\circ$(V)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate + CO$_2$ + 2H$^+$ + 2e$^-$ $\rightarrow$ α-ketoglutarate + H$_2$O</td>
<td>$-0.67$</td>
<td>2</td>
</tr>
<tr>
<td>Glycerate-3-phosphate + 2H$^+$ + 2e$^-$ $\rightarrow$ glyceraldehyde-3-phosphate + H$_2$O</td>
<td>$-0.55$</td>
<td>2</td>
</tr>
<tr>
<td>α-Ketoglutarate + CO$_2$ + 2H$^+$ + 2e$^-$ $\rightarrow$ isocitrate</td>
<td>$-0.38$</td>
<td>2</td>
</tr>
<tr>
<td>NAD$^+$ + H$^+$ + 2e$^-$ $\rightarrow$ NADH</td>
<td>$-0.32$</td>
<td>2</td>
</tr>
<tr>
<td>Glycerate-1,3-bisphosphate + 2H$^+$ + 2e$^-$ $\rightarrow$ glyceraldehyde-3-phosphate + P$_i$</td>
<td>$-0.29$</td>
<td>2</td>
</tr>
<tr>
<td>Lipoic acid + 2H$^+$ + 2e$^-$ $\rightarrow$ dihydrolipoic acid</td>
<td>$-0.29$</td>
<td>2</td>
</tr>
<tr>
<td>FMN + 2H$^+$ + 2e$^-$ $\rightarrow$ FMNH$_2$</td>
<td>$-0.22^b$</td>
<td>2</td>
</tr>
<tr>
<td>FAD + 2H$^+$ + 2e$^-$ $\rightarrow$ FADH$_2$</td>
<td>$-0.22^b$</td>
<td>2</td>
</tr>
<tr>
<td>Acetaldehyde + 2H$^+$ + 2e$^-$ $\rightarrow$ ethanol</td>
<td>$-0.20$</td>
<td>2</td>
</tr>
<tr>
<td>Pyruvate + 2H$^+$ + 2e$^-$ $\rightarrow$ lactate</td>
<td>$-0.19$</td>
<td>2</td>
</tr>
<tr>
<td>Oxaloacetate + 2H$^+$ + 2e$^-$ $\rightarrow$ malate</td>
<td>$-0.17$</td>
<td>2</td>
</tr>
<tr>
<td>Fumarate + 2H$^+$ + 2e$^-$ $\rightarrow$ succinate</td>
<td>$-0.03$</td>
<td>2</td>
</tr>
<tr>
<td>Cytochrome-b$_1$(Fe$^{3+}$) + e$^-$ $\rightarrow$ cytochrome-b$_1$(Fe$^{2+}$)</td>
<td>$-0.03$</td>
<td>1</td>
</tr>
<tr>
<td>UQ + H$^+$ + e$^-$ $\rightarrow$ UQH$^-$</td>
<td>$+0.03^c$</td>
<td>1</td>
</tr>
<tr>
<td>Cytochrome-b$_5$(Fe$^{3+}$) + e$^-$ $\rightarrow$ cytochrome-b$_5$(Fe$^{2+}$)</td>
<td>$+0.05$</td>
<td>1</td>
</tr>
<tr>
<td>UQ + 2H$^+$ + 2e$^-$ $\rightarrow$ UQH$_2$</td>
<td>$+0.11^c$</td>
<td>2</td>
</tr>
<tr>
<td>UQH$^-$ + H$^+$ + e$^-$ $\rightarrow$ UQH$_2$</td>
<td>$+0.19^c$</td>
<td>1</td>
</tr>
<tr>
<td>Cytochrome-c$_1$(Fe$^{3+}$) + e$^-$ $\rightarrow$ cytochrome-c$_1$(Fe$^{2+}$)</td>
<td>$+0.23$</td>
<td>1</td>
</tr>
<tr>
<td>Cytochrome-c(Fe$^{3+}$) + e$^-$ $\rightarrow$ cytochrome-c(Fe$^{2+}$)</td>
<td>$+0.24$</td>
<td>1</td>
</tr>
<tr>
<td>Cytochrome-c(Fe$^{3+}$) + e$^-$ $\rightarrow$ cytochrome-c(Fe$^{2+}$)</td>
<td>$+0.25$</td>
<td>1</td>
</tr>
<tr>
<td>Rieske Fe-S(Fe$^{3+}$) + e$^-$ $\rightarrow$ Fe-S(Fe$^{2+}$)</td>
<td>$+0.28$</td>
<td>1</td>
</tr>
<tr>
<td>Cytochrome-c(Fe$^{3+}$) + e$^-$ $\rightarrow$ cytochrome-c(Fe$^{2+}$)</td>
<td>$+0.35$</td>
<td>1</td>
</tr>
<tr>
<td>O$_2$ + 4H$^+$ + 4e$^-$ $\rightarrow$ 2H$_2$O</td>
<td>$+0.82$</td>
<td>4</td>
</tr>
</tbody>
</table>

$^a$ is the number of electrons transferred.

$^b$This value is for the free coenzyme. $E^\circ$ values for flavoproteins range from $-0.3$ to 0 V.

$^c$For UQ in aqueous ethanol.
UQH₂ is the mean of the $E^o$ values for the two one-electron steps, 0.11 V. ($E^o$ values for UQ depend strongly on the solvent; these values are for aqueous ethanol.) If only one electron is available, the reduction has to stop at the semiquinone.

$E^o$ values are called standard potentials because they refer to conditions under which the oxidant and reductant of the couple are at standard concentrations. (Standard conditions are chosen usually to be 1 M concentrations of all reactants and products except for $H^+$, $OH^-$, and $H₂O$.) By the principle of mass action, a solution containing a redox couple $D_{ox}/D_{red}$ can be made more oxidizing by increasing the concentration ratio $[D_{ox}]/[D_{red}]$, or more reducing by decreasing this ratio. The voltage measured with the apparatus shown in Figure 16–10 will reflect such changes. Suppose that the couple in one of the compartments is the standard hydrogen half-cell, and that the concentrations here are held constant while those of $D_{ox}$ and $D_{red}$ in the other compartment are varied. The voltage difference measured between the two compartments can be defined as the redox potential, $E$, in the solution containing $D_{ox}$ and $D_{red}$. Again, what actually is measured is the difference between the redox potentials in the two compartments ($\Delta E$), and the redox potential of the standard half-cell is set equal to zero by convention. The relationship between $E$ and $E^o$ is the same as that between the free energy change in a reaction ($\Delta G$) and the standard free energy change ($\Delta G^o$). Considering how $\Delta G$ depends on $\Delta G^o$ and on the concentrations of the reactants and products (Chapter 12), one can see that $E$ must depend on the concentration ratio as follows:

$$E = E^o + (2.303 \frac{RT}{nF}) \log \left( \frac{[D_{ox}]}{[D_{red}]} \right)$$

(6)

This is one of two expressions that go under the name of the Nernst equation. If $[D_{ox}]/[D_{red}]$ is increased by a factor of 10, $E$ increases by an amount

![Figure 16–10](image)

Apparatus for measuring the difference between the $E^o$ values of two redox couples. The cell on the left contains equimolar concentrations of NADH and NAD⁺; that on the right, equimolar concentrations of FMNH₂ and FMN. If both solutions are at pH 7, the voltmeter will sense the difference between the two $E^o$ values (0.10 V, negative on the left, in this example). To determine the $E^o$ value of one of the redox couples, the other couple is replaced by a standard redox couple such as the standard hydrogen half-cell ($H_2/H^+$ at pH 0 and 1 atm $H_2$). The dependence of one of the $E^o$ values on pH can be measured by changing the pH in the experimental cell and holding that in the reference cell constant. Experimentally, the calomel redox couple ($Hg/HgCl_2$) is often used as a working reference instead of the hydrogen half-cell, and the results are corrected by subtracting the $E^o$ value of the calomel couple (0.24 V). It may be necessary to add a catalytic amount of another organic redox couple as a mediator to facilitate the transfer of electrons to and from the metallic electrodes.
equal to 2.303 RT/nF. At 25°C, 2.303 RT/nF is equal to 0.060 V for n = 1, and 0.030 V for n = 2.

Another way to view the situation is that if we impose a redox potential of E on a solution containing Dox and Dred—for example, by replacing the voltmeter in Figure 16–10 by a power supply, or by adding a large amount of some other reductant or oxidant to the solution—Dox will accept electrons or Dred will release them, until the ratio [Dox]/[Dred] is in accord with the Nernst equation. The Nernst equation is analogous to the Henderson-Hasselbach equation (see Chapter 1), which describes how the ratio of base and acid concentrations depends on the pH and the pKa of an acid. Figure 16–11 shows how the [Dox]/[Dred] ratio depends on E for several redox couples with different values of E°. Note that, because of the way n enters into Equation (6), the curves shown in Figure 16–11 are steeper if n = 2 than if n = 1. [Dox]/[Dred] increases by a factor of 10 for every 0.06 V increase in E when n = 1, and for every 0.03 V when n = 2.

Let us now return to the point that E° values can depend on pH. When UQ, for example, is reduced to UQH2, two protons are added to the molecule along with each pair of electrons. By mass action, increasing the proton concentration (lowering the pH) should favor the reduction. Lowering the pH thus must make UQ a stronger oxidant, which means that it must increase UQ’s E°. This relation can be expressed quantitatively by including the proton concentration along with the concentrations of UQ and UQH2 in the equation for the free energy change associated with the oxidation-reduction reaction. The result is that, if the pH is decreased by one pH unit, the E° value becomes more positive by (2.303 RT/F)(nH+ /n), where nH+ is the number of protons that are taken up (two in the case of the UQ/UQH2 couple) and n again is the number of electrons transferred (two in this case). At 25°C, (2.303 RT/F)(nH+ /n) is 0.060 V per pH unit when nH+ is equal to n:

$$\frac{\Delta E^°}{\Delta pH} = -(2.303 \frac{RT}{F})(n_{H^+}/n) = -(0.060 \text{ V})(n_{H^+}/n)$$

Note that it is the ratio of nH+ to n that is important, and not the absolute value of either of these quantities.

E° Values of Electron Carriers. Given equal concentrations of reactants and products, electrons will tend to flow spontaneously from a carrier with a more negative E° value to one with a more positive value. The sequence of carriers in the respiratory chain should, therefore, be consistent with the relative E° values of the carriers. The linear sequence of b-, c-, and α-type cytochromes that was proposed above agrees with this expectation. This can be seen by plotting the E° values as a function of the carriers’ suggested positions in the chain (Figure 16–12). There are, however, difficulties in using the E° values to determine the exact organization of the respiratory chain, particularly in regions where there are numerous carriers with similar values of E°. First, the effective E° values of the respiratory carriers are difficult to measure precisely, because they can depend on the local pH and electrical potential in the interior of the membrane. The E° of cytochrome c decreases by about 0.05 V when the soluble cytochrome binds to a phospholipid membrane, and the E° value of UQ in a membrane or on a UQ-binding protein could be very different from the value measured in ethanol. In addition, interactions between neighboring carriers can make it difficult to determine the E° of one carrier in isolation from the other.

Keep in mind also that because the concentrations of reactants and products are rarely equal, it is not really ΔE° that determines the direction of electron flow, but the difference between the operating redox potentials, ΔE°.

*In Equation (7), ΔE° and ΔpH refer to small changes in E° and pH.
Electrons can move in the direction of more negative $E^\circ$ if the reactants (the reduced form of the electron donor and the oxidized form of the electron acceptor) are present at sufficiently high concentrations relative to the products (the oxidized donor and the reduced acceptor). This is simply an application of the law of mass action.

Finally, even if electron transfer from a particular carrier to another is thermodynamically favorable, it may not be able to occur unless the two carriers are sufficiently close together. Contacts between carriers in the respiratory chain will depend on how the hemes, flavins, quinones, and iron-sulfur centers are positioned in the proteins that bind them, and on how the proteins are arranged in the membrane.

The Sequence of Carriers Can Be Determined with Spectral Analysis and Inhibitors

Because the sequence of electron carriers in the respiratory chain cannot be determined simply on the basis of the $E^\circ$ values, it is necessary to bring additional experimental techniques to bear on the problem. Spectrophotometric techniques have been particularly helpful, because they make it possible to examine the rates at which the different electron carriers accept and release electrons in intact, functioning mitochondria. Other approaches that have played key roles are the use of specific inhibitors of the electron-transfer reactions, and the isolation of protein complexes that represent small sections of the respiratory chain.

Difference Spectra. Extremely sensitive and rapid spectrophotometric techniques for examining the redox states of the respiratory carriers in intact mitochondria were developed by Britton Chance in the 1950s. Chance measured the differences between the optical absorption spectra of two samples...