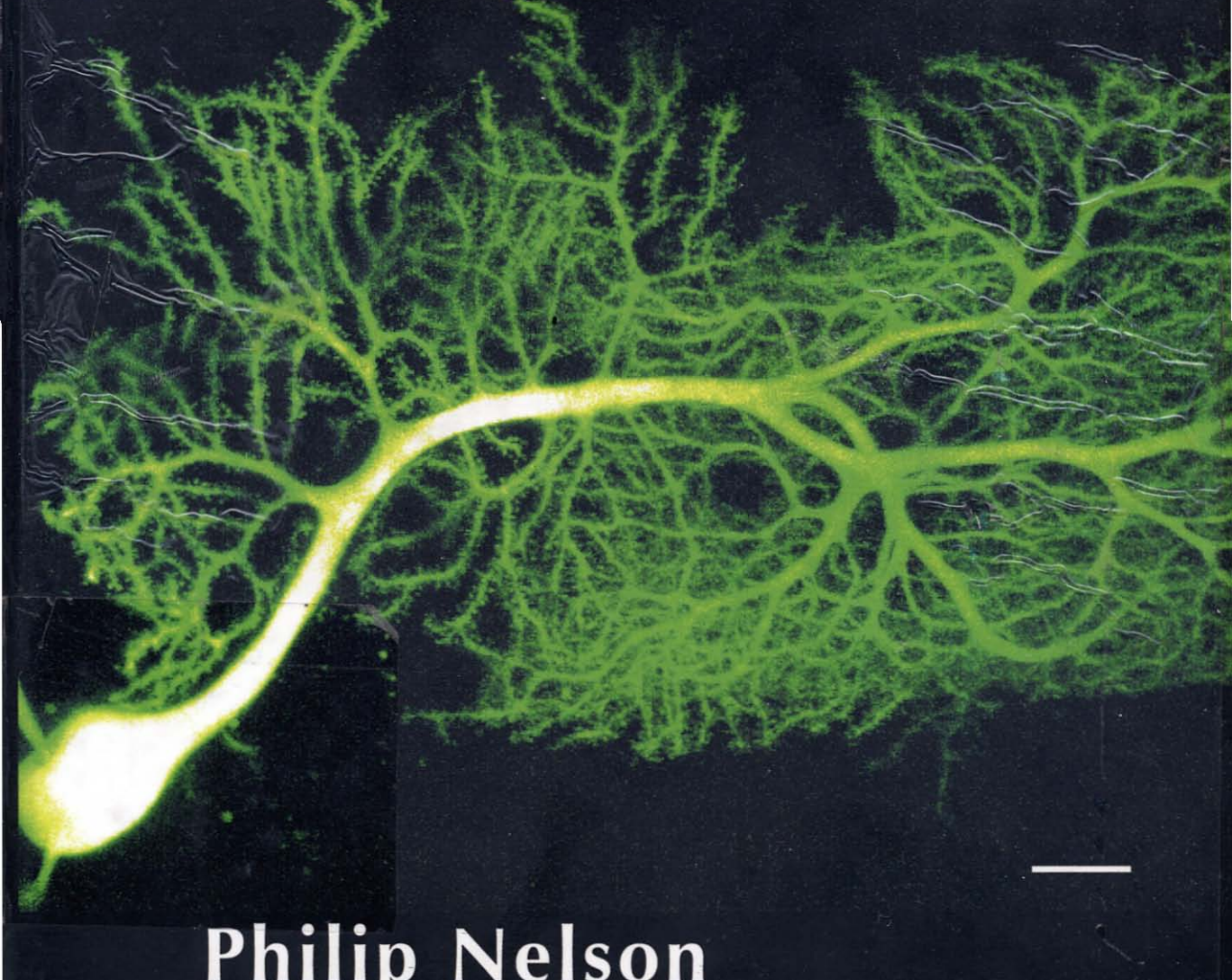
BIOLOGICAL PHYSICS

Energy, Information, Life



Philip Nelson

Chapter 11

Machines in membranes

> In going on with these Experiments how many pretty Systems do we build which we soon find ourselves oblig'd to destroy! If there is no other Use discover'd of Electricity this however is something considerable, that it may *help to make a vain man humble.* – B. Franklin to P. Collinson, 1747

Chapter 12 will discuss the question of nerve impulses, the electrical signals running along nerve fibers that make up the ghostly fabric of thought. Before we can discuss nerve impulses, however, this penultimate chapter must look at how living cells generate electricity in the first place. Chapter 4 skirted this question in the discussion of the Nernst formula; we are now ready to return to this question as a matter of free energy transduction, armed with a general understanding of molecular machines. We will see how indirect, physical arguments led to the discovery of a remarkable class of molecular machines, the *active ion pumps,* long before the precise biochemical identity of these devices was known. The story may remind you of how Muller, Delbrück, and their colleagues characterized the nature of the genetic molecule, using physical experiments and ideas, many years before others identified it chemically as DNA (Section 3.3.3). The interplay of physical and biochemical attack on life-science problems will continue to bear fruit as long as both sets of researchers know about each others' work.

The Focus Question for this chapter is:
Biological question: The cytosol's composition is very different from that of the outside world. Why doesn't osmotic flow through the plasma membrane burst (or shrink) the cell?
Physical idea: Active ion pumping by molecular machines can maintain a nonequilibrium, osmotically regulated state.

11.1 Electro-osmotic effects

11.1.1 Before the ancients

The separation of the sciences into disciplines is just a modern aberration. Historically there was a lively interplay between the study of bioelectric phenomena and the great project of understanding

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physically what electricity really was. For example Benjamin Franklin's famous demonstration in 1752 that lightning was just a very big electrical spark led to much speculation and experimentation on electricity in general. Lacking sophisticated measurement devices, it was natural for the scientists of the day to focus on the role of electricity in living organisms, in effect using them as their instruments. The physicians Albrecht von Haller and Luigi Galvani found that electricity, generated by physical means and stored in a capacitor, could stimulate strong contraction in animal muscles. Galvani published his observations in 1791, and speculated that muscles were also a *source* of electricity. After all, he reasoned, even without the capacitor he could evoke muscle twitches just by inserting electrodes between two points.

Alessandro Volta did not accept this last conclusion. He regarded muscles as electrically passive, receiving electrical signals but not generating any electricity themselves. He explained Galvani's no-capacitor experiment by suggesting that an electrical potential could develop between two dissimilar metals in any electrolyte, alive or not. To prove his point, in 1800 he invented a purely nonliving source of electricity, merely placing two metal plates in an acid bath. Volta's device—the "Voltaic cell"—led to decisive advances in our understanding of physics and chemistry. As technology, Volta's device also wins the longevity award: The batteries in your car, flashlight, and so on are Voltaic cells.

But Volta was too quick to dismiss Galvani's idea that life processes could also generate electricity directly. Sections 11.1.2–11.2.3 will show how this can happen. Our discussion will rest upon many hard-won experimental facts. For example, after Galvani decades would pass before E. DuBois Reymond, another physician, showed in the 1850s that living frog skin maintained a potential difference of up to 100 mV between its sides. And the concept of the cell membrane as an electrical insulator only a few nanometers thick remained a speculation until 1927, when H. Fricke measured quantitatively the capacitance of a cell membrane and thus estimated its thickness, essentially using Equation 7.26 on page 236.

To understand the origin of resting membrane potentials, we first return to the topic of ions permeating membranes, a story begun in Chapter 4.

11.1.2 Ion concentration differences create Nernst potentials

Figure 4.14 on page 125 shows a container of solution with two charged plates outside supplying a fixed external electric field. Section 4.6.3 calculated the concentration profile in equilibrium, and from this the change in concentration of charged ions between the two ends of the container (Equation 4.25). We then noted that the potential drop needed to get a significant concentration jump across the container was roughly comparable to the difference in electrical potential across the membrane of most living cells. We're now in a position to see *why* the results of Section 4.6.3 should have anything to do with cells, starting with some ideas from Section 7.4.

Figure 11.1 shows the physical situation of interest. An uncharged membrane, shown as a long cylinder, separates the world into two compartments, #1 and #2. Two electrodes, one inside and one outside, measure the electrical potential across the membrane. The figure is meant to evoke the long, thin tube, or axon, emerging from the body of a nerve cell. Indeed experimentally one can literally insert a thin needle-like electrode into living nerve axons, essentially as sketched here, and connect them to an amplifier. Historically the systematic study of nerve impulses opened up only when a class of organisms was found with large enough axons for this delicate procedure (the cephalopods). For example, the **"giant" axon** of the squid *Loligo forbesi* has a diameter of about a

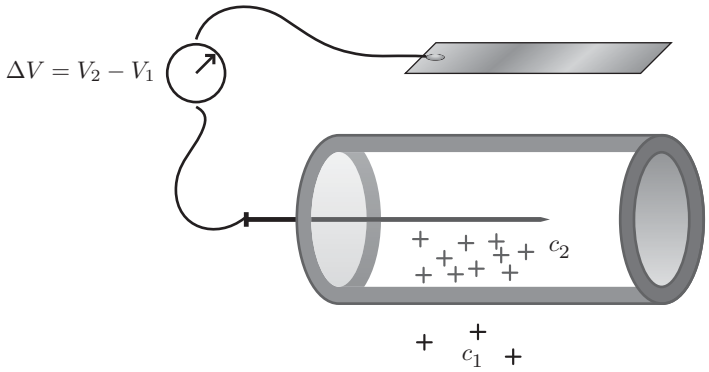

Figure 11.1: (Schematic.) Measurement of membrane potential. The bulk concentration c_2 of interior cations is greater than the exterior concentration, c_1, as shown; the corresponding bulk concentrations of negative charges follow the same pattern (not shown), as required by charge neutrality. The symbol on the left represents a voltmeter.

millimeter, much bigger than the typical axon diameter in your body, about 5–20 μm.

Each compartment contains a salt solution, which for simplicity we'll take to be monovalent—say potassium chloride. Imagine that the membrane is slightly permeable to K^+ ions, but not at all to Cl^- (actually, squid axon membranes are only about twice as permeable to K^+ as they are to Cl^-). For now we will also ignore the osmotic flow of water (see Section 11.2.1). We imagine initially preparing different salt solutions on the inside and outside of the cell: Far from the membrane, the salt concentration in each compartment is uniform and equals c_2 on the inside, and c_1 on the outside. Suppose that $c_2 > c_1$ as shown in Figure 11.1.

After the system reaches equilibrium, the concentration $c_+(r)$ of potassium ions will not be uniform near the membrane, and neither will be the chloride concentration, $c_-(r)$ (see Figure 11.2a). To understand the origin of membrane potential, we must first explain these equilibrium concentration profiles.

The permeant K^+ ions face a dilemma: They could increase their entropy by crossing the membrane to erase the imposed concentration difference. Indeed they will do this, up to a point. But their impermeant partners, the Cl^- ions, keep calling them back by electrostatic attraction. Thus, far from the membrane on both sides the concentrations of K^+ and Cl^- will be equal, as required by overall charge neutrality. Only a few K^+ ions will actually cross the membrane, and even these won't travel far: They deplete a thin layer just inside the membrane, and cling in a thin layer just outside (see the c_+ curve in Figure 11.2a).

The behavior shown in Figure 11.2 is just what we could have expected from our study of electrostatic interactions in Section 7.4.3 on page 233. To see the connection, first consider the region to the right of point C in Figure 11.2. This region is a salt solution in contact with an "object" of net negative charge. The "object" consists of the membrane plus the interior of the cylinder in Figure 11.1; it's negatively charged because some of its positive ions have permeated the membrane and escaped. But a solution in contact with a negatively charged object develops a neutralizing positive layer, just as in Figure 7.8a on page 233. This layer is shown in Figure 11.2 as the region between points C and D. Its thickness λ is roughly analogous to x_0 in our discussion

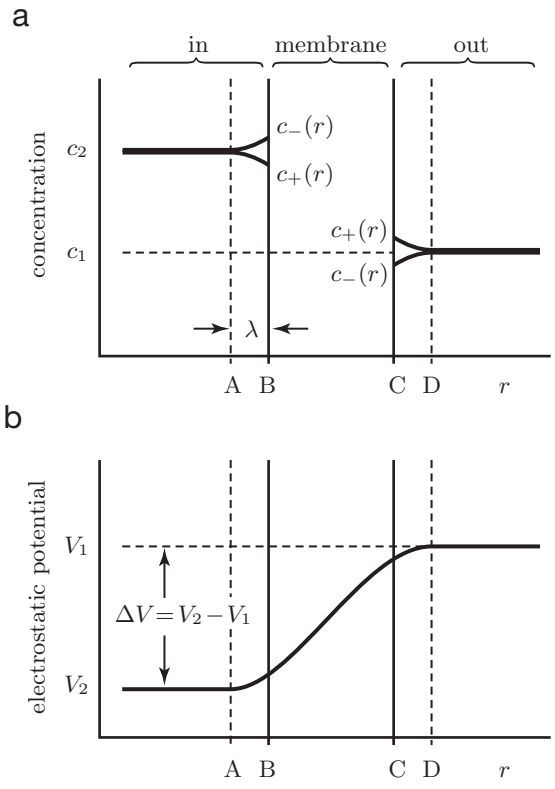

Figure 11.2: (Sketch graphs.) (a) Concentration profiles near a membrane, for the situation sketched in Figure 11.1. Far outside the membrane the concentrations $c_\pm$ of positive and negative ions must be equal, by charge neutrality; their common value c_1 is just the exterior salt concentration. Similarly, deep inside the cell $c_+ = c_- = c_2$. The situation shown assumes that only the positive ions are permeant. Thus some positive ions leak out, enhancing c_+ in a layer of thickness λ just outside the membrane and depleting it just inside. c_- drops just outside the membrane, because negative ions move away from the negatively charged cell. The concentrations in the membrane's hydrophobic interior (the region between B and C) are nearly zero. (b) The corresponding electrical potential V created by the charge distribution in (a). In equilibrium, ΔV equals the Nernst potential of the permeant species (in this case the positive ions).

of the electrical double layer (Equation 7.25 on page 236).[1] Unlike Figure 7.8a, however, we now have both positive and negative mobile charges in the solution. Hence, the layer of enhanced K^+ concentration is also *depleted* of Cl^-, since the negative region to the left of point C in the figure *repels* anions. The effect of both these disturbances is to create a layer of net positive charge just outside the membrane.

Just inside the membrane the situation is reversed. Here we have a salt solution facing a *positive* object, namely everything to the right of point B in the figure. Thus there is a region relatively depleted of K^+, and enriched in Cl^-, a layer of net negative charge just inside the membrane.

We can now turn to the question of finding the electrical potential jump across the membrane. One way to find it would be to solve the Gauss Law (Equation 7.20 on page 232) for the electric field $\mathcal{E}(x)$ given the charge density shown in Figure 11.2a, then integrate to find $V(x)$. Let's instead think physically (see Figure 11.2b). Suppose we bring a positively charged test object in from

[1] T2 Or more appropriately, to the Debye length λ_D (Equation 7.34 on page 250).

outside (from the right of the figure). At first, everything to the left of our test object has net charge zero, and so the net force on it is also zero and its potential energy is a constant. Once the test object enters the outer charge cloud, at point D, however, it starts to feel and be attracted to the net negative object to the left of point C. Its potential thus begins to decrease. The deeper it gets into the cloud, the more charge it sees: The slope of its potential curve increases.

The membrane itself was assumed to be uncharged. There will be a few permeant ions inside it, in transit, but typically very few. Thus while traversing the membrane the test charge feels a *constant* force attracting it toward the interior, from the charge of the region to the left of point B. Its potential thus falls linearly until it crosses point B, then levels off in the neutral interior of the cylinder.

The potential curve $V(r)$ sketched in Figure 11.2b summarizes the narrative in the preceding two paragraphs.

Your Turn 11a

Arrive at the same conclusion for the potential $V(r)$ by describing qualitatively the solution to the Gauss law with the charge density $\rho_{\rm q}(r) = e(c_+(r) - c_-(r))$, where $c_\pm(r)$ are as shown in Figure 11.2a.

Your Turn 11b

Repeat the discussion, again assuming that $c_2 > c_1$, but this time considering a fictitious membrane permeable to Cl^- but not to K^+. What changes?

To determine the potential drop $\Delta V = V_2 - V_1$ quantitatively, imagine replacing the voltmeter in Figure 11.1 by a battery of adjustable voltage, and cranking the voltage until the current through the system just stops. The permeant ion species is then in equilibrium throughout the system. Writing its charge q as the proton charge e times an integer z (the ion's valence), its concentration must obey the Boltzmann distribution: $c(x) = \text{const} \times \mathrm{e}^{-zeV(x)/k_{\rm B}T}$. Taking the logarithm and evaluating on the inside and outside reproduces the Nernst relation:

$$\Delta V \;=\; \mathcal{V}^{\rm Nernst} \text{ in equilibrium, where}$$
$$\Delta V \equiv V_2 - V_1 \text{ and } \mathcal{V}^{\rm Nernst} \equiv -\frac{k_{\rm B}T}{ze}\ln\frac{c_2}{c_1}. \tag{11.1}$$

In the language of Section 8.1.1, the Nernst relation says that in equilibrium the electrochemical potential of any permeant ion species must be everywhere the same.

Notice that z in Equation 11.1 is the valence of the permeant species only (in our case it's +1). In fact the other, impermeant species in the problem doesn't obey the Nernst relation at all, nor should it, since it's not at all in equilibrium. If we suddenly punched a hole through the membrane, the impermeant Cl^- would begin to rush out, while K^+ would not, since we adjusted the battery to exactly balance its electric force (to the left) against its entropic, diffusive force (to the right). Similarly, you just found in Your Turn 11b that switching the roles of the two species actually *reverses* the sign of the membrane's equilibrium potential drop.

T2 *Section 11.1.2′ on page 437 gives some further comments involving ion permeation through membranes.*

11.1.3 Donnan equilibrium can create a resting membrane potential

Section 11.1.2 arrived at a simple conclusion:

> *The Nernst relation gives the potential arising when a permeant species reaches equilibrium. Equivalently, it gives the potential that must be applied to* ***stop*** *the net flux of that species, given the concentration jump across a membrane.* (11.2)

In this subsection we begin to explore a slightly more complicated problem, in which there are more than two ion species. The problem is relevant to living cells, where there are several important small permeant ions. We will simplify our discussion by considering only three species of small ions, with concentrations c_i, where the label i runs over Na^+, K^+, Cl^-.

Cells are also full of proteins and nucleic acids, huge macromolecules carrying net negative charge. The macromolecules are practically impermeant, so we expect a situation analogous to Figure 11.2, and a resulting membrane potential. Unlike the simpler case with just two species, however, the bulk concentrations are no longer automatically fixed by the initial concentrations and by the condition of charge neutrality: The cell can import some more Na^+ while still remaining neutral, if at the same time it expels some K^+ or pulls in some Cl^-. Let's see what happens.

A typical value for the total charge density $\rho_{q,macro}$ of the trapped (impermeant) macromolecules is the equivalent of 125 mM of excess electrons. Just as in Section 11.1.2, small ions can and will cross the cell membrane, in order to reduce the total free energy of the cell. We will suppose that our cell sits in an infinite bath with exterior ion concentrations $c_{1,i}$. (It could be an algal cell in the sea, or a cell in your blood.) These concentrations, like $\rho_{q,macro}$, are fixed and given; some illustrative values are $c_{1,Na^+} = 140\,\text{mM}$, $c_{1,K^+} = 10\,\text{mM}$, and $c_{1,Cl^-} = 150\,\text{mM}$. These values make sense, since they imply that the exterior solution is neutral:

$$c_{1,Na^+} + c_{1,K^+} - c_{1,Cl^-} = 0.$$

The cell's interior is not infinite, and so the concentrations there, $c_{2,i}$, are *not* fixed. Instead they are all unknowns, for which we must solve. Moreover, the membrane potential drop $\Delta V = V_2 - V_1$ is a fourth unknown. We therefore need to find four equations, in order to solve for these four unknowns. First, charge neutrality in the bulk interior requires

$$c_{2,Na^+} + c_{2,K^+} - c_{2,Cl^-} + \rho_{q,macro}/e = 0. \tag{11.3}$$

(Section 12.1.2 will discuss neutrality in greater detail.) The other three equations reflect the fact that the same electrostatic potential function affects every ion species. Thus in equilibrium each permeant species must separately be in Nernst equilibrium at the same value of ΔV:

$$\Delta V = -\frac{k_B T}{e} \ln \frac{c_{2,Na^+}}{c_{1,Na^+}} = -\frac{k_B T}{e} \ln \frac{c_{2,K^+}}{c_{1,K^+}} = -\frac{k_B T}{-e} \ln \frac{c_{2,Cl^-}}{c_{1,Cl^-}}. \tag{11.4}$$

To solve Equation 11.3–11.4, we first notice that the latter can be rewritten as the **Gibbs–Donnan relations**

$$\frac{c_{1,Na^+}}{c_{2,Na^+}} = \frac{c_{1,K^+}}{c_{2,K^+}} = \frac{c_{2,Cl^-}}{c_{1,Cl^-}} \quad \text{in equilibrium.} \tag{11.5}$$

Example

a. Why is the chloride ratio in these relations inverted relative to the others?
b. Finish the calculation using the illustrative values for $c_{1,i}$ and $\rho_{q,macro}$ listed above. That is, find $c_{2,i}$ and ΔV.

Solution: a. The charge on a chloride ion is opposite to that on potassium or sodium, leading to an extra minus sign in Equation 11.4. Upon exponentiating the formula, this minus sign turns into an inverse.
b. Let $x = [\mathrm{Na}^+] = c_{2,\mathrm{Na}^+}/1\,\mathsf{M}$. Use Equation 11.5 and the given values of $c_{1,i}$ to express c_{2,K^+} and c_{2,Cl^-} in terms of x. Substitute into Equation 11.3 and multiply the equation by x to get

$$\left(1 + \frac{0.01}{0.14}\right) x^2 - 0.15 \times 0.14 - 0.125x = 0.$$

Solving with the quadratic formula gives $x = 0.21$, or $c_{2,\mathrm{Na}^+} = 210\,\mathsf{mM}$, $c_{2,\mathrm{K}^+} = 15\,\mathsf{mM}$, $c_{2,\mathrm{Cl}^-} = 100\mathsf{mM}$. Then Equation 11.4 gives $\Delta V = -10\,\mathsf{mV}$. (Appendix B gives $k_\mathrm{B}T_\mathrm{r}/e = (1/40)\,\mathsf{volt}$.)

The equilibrium state you just found is called the **Donnan equilibrium**; ΔV is called the **Donnan potential** for the system.

So we have found one realistic way in which a cell can maintain a permanent (resting) electrical potential across its membrane, simply as a consequence of the fact that some charged macromolecules are sequestered inside it. Indeed the typical values of such potentials are in the tens of millivolts. No energy needs to be spent maintaining the Donnan potential—it's a feature of an equilibrium state, a state of minimum free energy. Notice that we could have arranged for charge neutrality by having only c_{2,Na^+} greater than the exterior value, with the other two concentrations the same inside and out. But that state is not the minimum of free energy; instead all available permeant species share in the job of neutralizing $\rho_{\mathrm{q,macro}}$.

11.2 Ion pumping

11.2.1 Observed eukaryotic membrane potentials imply that these cells are far from Donnan equilibrium

The sodium anomaly Thus Donnan equilibrium appears superficially to be an attractive mechanism for explaining resting membrane potentials. But a little more thought reveals a problem. Let us return to the question of osmotic flow through our membrane, which we postponed at the start of Section 11.1.2. The macromolecules are not very numerous; their contribution to the osmotic pressure will be negligible. The small ions, however, greatly outnumber the macromolecules and pose a serious osmotic threat. To calculate the osmotic pressure in the Example on page 414, we add the contributions from all ion species:

$$\Delta c_\mathrm{tot} = c_{2,\mathrm{tot}} - c_{1,\mathrm{tot}} \approx 25\,\mathsf{mM}. \tag{11.6}$$

The sign of our result indicates that small ions are more numerous inside the model cell than outside. To stop inward osmotic flow, the membrane thus would have to maintain an interior pressure of $25\,\mathsf{mM} \cdot k_\mathrm{B}T_\mathrm{r} \approx 6 \cdot 10^4\,\mathsf{Pa}$. But we know from Section 7.2.1 on page 219 that eukaryotic cells lyse (burst) at much smaller pressures than this!

Certainly our derivation is very rough. We have completely neglected the osmotic pressure of other, uncharged solutes (like sugar). But the point is still valid: The equations of Donnan equilibrium give a unique solution for electro-osmotic equilibrium and neutrality. There is no reason why that solution should *also* coincidentally give small osmotic pressure! To maintain Donnan

Table 11.1: Approximate ion concentrations inside and outside the squid giant axon. The second line illustrates the "sodium anomaly": The Nernst potential of sodium is nowhere near the actual membrane potential of $-60\,\mathsf{mV}$.

Ion	Valence z	Interior $c_{2,i}$ (mM)	Relation	Exterior $c_{1,i}$ (mM)	Nernst potential $\mathcal{V}_i^{\text{Nernst}}$ (mV)
K^+	$+1$	400	$>$	20	-75
Na^+	$+1$	**50**	$<$	**440**	**$+54$**
Cl^-	-1	52	$<$	560	-59

equilibrium you've got to be strong. In fact, plant, algal, and fungal cells, as well as bacteria, surround their bilayer plasma membrane with a rigid wall; thus they can withstand significant osmotic pressures. Indeed plant tissue actually uses the rigidity resulting from turgor for structural support, and becomes limp when the plant dehydrates. (Think about eating old celery.) But your own body's cells lack a strong wall. Why they don't burst under osmotic pressure?

Table 11.1 shows the actual (measured) concentration differences across one particular cell's membrane. Donnan equilibrium predicts that the presence of trapped, negative macroions will give $c_{2,\mathrm{Na}^+} > c_{1,\mathrm{Na}^+}$, $c_{2,\mathrm{K}^+} > c_{1,\mathrm{K}^+}$, $c_{2,\mathrm{Cl}^-} < c_{1,\mathrm{Cl}^-}$, and $\Delta V < 0$. These predictions make sense intuitively: The trapped negative macroions tend to push out negative permeant ions and pull in positive ones. But the table shows that of these four predictions, *the first one proves to be very wrong.* In thermodynamic equilibrium all the entries in the last column would have to be the same, according to the Gibbs–Donnan relations. In fact both the potassium and chloride ions roughly obey this prediction, and moreover the measured membrane potential $\Delta V = -60\,\mathsf{mV}$ really is similar to each of their Nernst potentials. But the Gibbs–Donnan relation fails for sodium, and even for K^+ the quantitative agreement is not very successful.

To summarize:

> *1. The Nernst potential of potassium is slightly more negative than the actual membrane potential ΔV.*
> *2. The Nernst potential of sodium is **much** more positive than ΔV.* (11.7)

All animal cells (not just the squid axon) have a **sodium anomaly** of this type.[2]

One interpretation for these results might be that the sodium and other discrepant ions simply cannot permeate on the time scale of the experiment, so they need not obey the equilibrium relations. However, we are discussing the steady-state, or **resting**, potential; the "time scale" of this measurement is infinity. Any permeation at all would eventually bring the cell to Donnan equilibrium, contrary to the actual observed concentrations. More importantly, it's possible to measure directly the ability of sodium ions to pass through the axon membrane; the next subsection will show that this permeability, although small, is not negligible.

We are forced to conclude that the ions in a living cell are not in equilibrium. But why should they be? Equilibrium is not life, it's death. Cells at rest are constantly burning food, precisely to *combat* the drive toward equilibrium! If the metabolic cost of maintaining a nonequilibrium ion concentration is reasonable compared to the rest of the cell's energy budget, then there's no reason not to do it. After all, the benefits can be great. We have already seen how maintaining

[2]Many bacteria, plants, and fungi instead show a similar anomaly involving the concentration of protons; see Section 11.3.

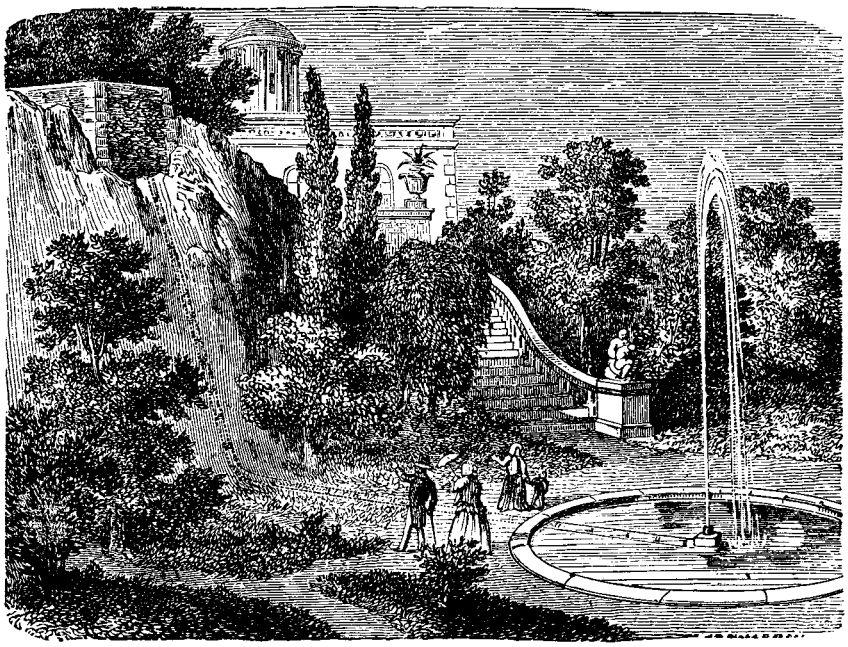

Figure 11.3: (Metaphor.) If water is continuously pumped to the upper reservoir, the fountain will come to a nonequilibrium steady state. If not, it will come to a quasisteady state, which lasts until the reservoir is empty.

electrostatic and osmotic equilibrium could place a cell under large internal pressure, bursting or at least immobilizing it.

We get a big clue that we're finally on the right track when we put our nerve cell in the refrigerator. Chilling a cell to just above freezing doesn't change the absolute temperature very much. But it does shut down the cell's metabolism. Suddenly the cell loses its ability to maintain a nonequilibrium sodium concentration difference. Moreover, the shut-down cell also loses its ability to control its interior volume, or **osmoregulate**. When normal conditions are restored, the cell's metabolism starts up again and the interior sodium falls.

Certain genetic defects can also interfere with osmoregulation. For example, patients with hereditary spherocytosis have red blood cells whose plasma membrane is much more permeable to sodium than that of normal red cells. The affected cells must work harder than normal cells to pump sodium out. Hence they are prone to osmotic swelling, which in turn triggers their destruction by the spleen. Entropic forces can kill.

A look ahead This section raised two puzzles: Eukaryotic cells maintain a far-from-equilibrium concentration drop of sodium, and they don't suffer from the immense osmotic pressure predicted by Donnan equilibrium. In principle both of these problems could be solved if, instead of being in equilibrium, cells could constantly *pump* sodium across their membranes, using metabolic energy. Such active pumping could create a nonequilibrium, but steady, state.

Here is a mechanical analogy: Suppose you visit your friend and see in his garden a fountain. The fountain could be supplied by a high tank of water (Figure 11.3). In that case it flows, converting the gravitational potential energy of the water in the tank to kinetic energy (and ultimately heat), until the tank is empty; that is, it drives to equilibrium. But if you watch the fountain for many

hours and it never stops, you may begin to suspect that your friend instead recirculates the water with a *pump,* using some external source of energy. In that case the fountain is in a steady, but nonequilibrium, state. Similarly, Section 10.4.1 discussed the steady state of an enzyme presented with nonequilibrium concentrations of its substrate and product.[3]

In the context of cells, we are exploring the hypothesis that that cells must somehow be using their metabolism to maintain resting ion concentrations far from equilibrium. To make this idea quantitative (that is, to see if it's right) we now return to the topic of transport across membranes (introduced in different contexts in Sections 4.6.1 and 7.3.2).

11.2.2 The Ohmic conductance hypothesis

To begin exploring nonequilibrium steady states, first note that *the Nernst potential need not equal the actual potential jump* across a membrane, just as we found that the quantity $(\Delta c)k_{\mathrm{B}}T$ need not equal the actual pressure jump Δp (Section 7.3.2 on page 228). If the actual pressure jump across a membrane differs from $(\Delta c)k_{\mathrm{B}}T$, we found there would be a *flux* of water across the membrane. Similarly, if the potential drop differs from the Nernst potential for some ion species, that species will be out of equilibrium and will permeate, giving a net electric current. In this case, the potentials obtained from Equation 11.1 for different kinds of ions need not agree with each other.

To emphasize the distinction, Equation 11.1 on page 413 introduced $\mathcal{V}_i^{\mathrm{Nernst}}$ (read "the Nernst potential of ion species i") to mean precisely $-\frac{k_{\mathrm{B}}T}{ez_i}\ln(c_{2,i}/c_{1,i})$, reserving the symbol ΔV for the *actual* potential drop $V_2 - V_1$. Our sign convention is thus that a positive Nernst potential represents an entropic force driving positive ions into the cell.

Prior experience (Sects. 4.6.1 and 4.6.4) leads us to expect that the flux of ions through a membrane will be dissipative, and hence proportional to a net driving force, at least if the driving force is not too large. Furthermore, according to Idea 11.2 on page 414, the net driving force on ions of type i vanishes when $\Delta V = \mathcal{V}_i^{\mathrm{Nernst}}$. Thus the net force is given by the sum of an energetic term, $z_i e \Delta V$ (from the electric fields) plus an entropic term, $-z_i e \mathcal{V}_i^{\mathrm{Nernst}}$ (from the tendency of ions to diffuse to erase any concentration difference).[4] This is just the behavior we have come to expect from our studies of osmotic flow (Section 7.3.2) and of chemical forces (see the Example on page 261).

In short we expect that

$$\boxed{j_{\mathrm{q},i} = z_i e j_i = (\Delta V - \mathcal{V}_i^{\mathrm{Nernst}}) g_i. \qquad \text{Ohmic conductance hypothesis}} \tag{11.8}$$

Here as usual the number flux j_i is the number of ions of type i per area per time crossing the membrane; the electric charge flux $j_{\mathrm{q},i}$ is this quantity times the charge $z_i e$ on one ion. We choose the sign convention that j is positive if the net flux is outward. The constant of proportionality g_i appearing in Equation 11.8 is called the **conductance per area** of the membrane to ion species i. It's always positive, and has units[5] $\mathsf{m}^{-2} \cdot \Omega^{-1}$. A typical magnitude for the overall conductance per area of a resting squid axon membrane is around $5\,\mathsf{m}^{-2} \cdot \Omega^{-1}$.

[3] We also encountered steady or quasi-steady nonequilibrium states in Sections 4.6.1, 4.6.2, 10.2.3, 10.4.3, and 10.4.4.

[4] Equivalently, the net driving force acting on ions is the difference in electrochemical potential $\Delta\mu_i$ (see Equation 8.3 on page 261).

[5] Neuroscientists use the synonym **siemens** (symbol S) for inverse ohm; an older synonym is the "mho" (symbol $\mho$). We won't use either notation, instead writing Ω^{-1}. Note that conductance per area has different units from the *conductivity* of a bulk electrolyte (Section 4.6.4 on page 127); the latter has units $\mathsf{m}^{-1}\Omega^{-1}$.

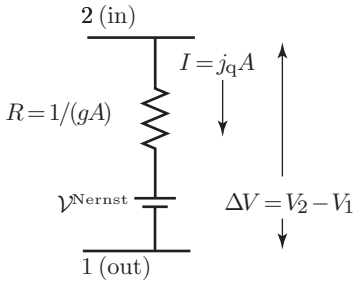

Figure 11.4: (Circuit diagram.) Equivalent circuit model for the electrical properties of a small patch of membrane of area A and conductance per area g, assuming the Ohmic hypothesis (Equation 11.8). The membrane patch is equivalent to a battery with potential drop $\mathcal{V}^{\text{Nernst}}$, in series with resistor with resistance $R = 1/(gA)$. For a positive ion species ($z > 0$), a positive Nernst potential means that the ion concentration is greater outside the cell; in this case an entropic force pushes ions upward in the diagram (into the cell). A positive applied potential ΔV has the opposite effect, pushing positive ions downward (out of the cell). Equilibrium is the state where these forces balance, or $\mathcal{V}^{\text{Nernst}} = \Delta V$; then the net current I equals zero. The electric current is deemed positive when it is directed outward.

Equation 11.8 is just another form of Ohm's law. To see this, note that the electric current I through a patch of membrane of area A equals $j_q A$. If only one kind of ion can permeate, Equation 11.8 gives the potential drop across the membrane as $\Delta V = IR + \mathcal{V}^{\text{Nernst}}$. The first term is the usual form of Ohm's law, where $R = 1/(gA)$. The second term corresponds to a battery of fixed voltage $\mathcal{V}^{\text{Nernst}}$ connected in series with the resistor, as shown in Figure 11.4. The voltage across the terminals of this virtual battery is the Nernst potential of ion species i.

We must bear in mind, though, that a membrane's regime of Ohmic behavior, where Equation 11.8 applies, may be very limited. First, Equation 11.8 is just the first term in a power series in $\Delta V - \mathcal{V}_i^{\text{Nernst}}$. Since we have seen that sodium is far from its equilibrium concentration difference (Table 11.1), we can't expect Equation 11.8 to give more than a qualitative guide to the resting electrical properties of cells. Moreover, the "constant" of proportionality g_i need not be constant at all; it may depend on environmental variables such as ion concentrations and ΔV itself. Thus, we can only use Equation 11.8 if both ΔV and the concentration of ion species i are close to their resting values. From now on, the unadorned symbol g_i will refer specifically to the conductance per area of a membrane to ion species i at resting external conditions. For other conditions we'll have to allow for the possibility that the conductance per area changes, for example writing $g_i(\Delta V)$. This subsection will consider only small deviations from the resting conditions; Section 12.2.4 will explore more general situations.

The conductance per area, g_i, is related to the ion's permeability $\mathcal{P}_s$ (see Equation 4.20 on page 121):

Your Turn 11c

Find the relation between the conductance per area and the permeability of a membrane to a particular ion species, assuming that the inside and outside concentrations are nearly equal. Discuss why your result is reasonable. [*Hint:* Remember that $c_{1,i} - c_{2,i}$ is small, and use the expansion $\ln(1 + \epsilon) \approx \epsilon$ for small ϵ.]

Notice that the conductances per area for various ion species, g_i, need not all be the same. Different ions have different diffusion constants in water; they have different radii and so encounter different obstructions passing through different channels, and so on. Just as a membrane can be permeable

to water but not to ions, so the conductances to different ions can differ. If a particular ion species is impermeant, then its concentration needn't obey the Nernst formula, just like the Cl^- ions in the example of Section 11.1.2. The impermeant species *are* important to the problem, however: They enter the system's overall charge neutrality condition.

T2 *Section 11.2.2′ on page 437 mentions nonlinear corrections to the Ohmic behavior of membrane conductances.*

11.2.3 Active pumping maintains steady-state membrane potentials while avoiding large osmotic pressures

We can now return to the sodium anomaly in Table 11.1. To investigate nonequilibrium steady states using Equation 11.8, we need separate values of the conductances per area, g_i, of membranes to various ions. Several groups made such measurements around 1948 using radioactively labeled sodium ions on one side of a membrane and ordinary sodium on the other side. They then measured the leakage of radioactivity across the membrane under various conditions of imposed potentials and concentrations. This technique yields the sodium current, separated from the contributions of other ions.[6] The result of such experiments was that in general nerve and muscle cells indeed behave Ohmically (see Equation 11.8) under nearly resting conditions. The corresponding conductances are appreciable for potassium, chloride, *and sodium*; A. Hodgkin and B. Katz found for the squid axon that

$$g_{K^+} \approx 25 g_{Na^+} \approx 2 g_{Cl^-}. \qquad \text{(resting)} \tag{11.9}$$

Thus the sodium conductance is small, but not negligible and certainly not zero.

Section 11.2.1 argued that a nonzero conductance for sodium implies that the cell's resting state is not in equilibrium. Indeed, in 1951 Ussing and K. Zehran found that living frog skin, with identical solutions on both sides, and membrane potential ΔV maintained at zero, nevertheless transported sodium ions, even though the net force in Equation 11.8 was zero. Apparently Equation 11.8 must be supplemented with an additional term describing the active **ion pumping** of sodium. The simplest modification we could entertain is

$$j_{Na^+} = \frac{g_{Na^+}}{e}(\Delta V - \mathcal{V}^{\text{Nernst}}_{Na^+}) + j^{\text{pump}}_{Na^+}. \tag{11.10}$$

The new, last term in this modified Ohm's law corresponds to a current source in parallel with the elements shown in Figure 11.4. This current source must do work if it's to push sodium ions "uphill" (against their electrochemical potential gradient). The new term distinguishes between the inner and outer sides of the membrane: It's positive, indicating that *the membrane pumps sodium outward.* The source of free energy needed to do that work is the cell's own metabolism.

A more detailed study in 1955 by Hodgkin and R. Keynes showed that sodium is not the only actively pumped ion species: Part of the *inward* flux of potassium through a membrane also depends on the cell's metabolism. Intriguingly, Hodgkin and Keynes (and Ussing, a year earlier) found that the outward sodium-pumping action stopped even in normal cells, when they were deprived of any exterior potassium, suggesting that the pump couples its action on one ion to the other. Hodgkin and Keynes also found that metabolic inhibitors (such as dinitrophenol) reversibly stop the active pumping of both sodium and potassium in individual living nerve cells (Figure 11.5), leaving the

[6] An alternative approach is to shut down the permeation of other ions using specific **neurotoxins** (a class of poisons).

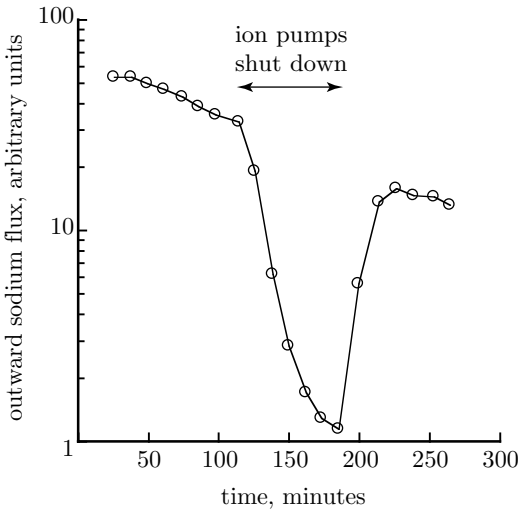

Figure 11.5: (Experimental data.) Flux of sodium ions out of a cuttlefish axon after electrical stimulation. At the beginning of the experiment the axon was loaded with radioactive sodium, then placed in ordinary sea water; the loss of radioactivity was then monitored. During the interval represented by the arrow, the axon was exposed to the toxin dinitrophenol (DNP), temporarily shutting down sodium pumping. Later the toxin was washed away with fresh seawater, and ion pumping spontaneously resumed. The horizontal axis gives the time after end of electrical stimulation; the vertical scale gives the rate at which radioactively labeled sodium left the axon. [Data from Hodgkin & Keynes, 1955.]

passive, Ohmic part of the fluxes unchanged. Moreover, even with the cell's metabolism shut down, pumping resumes when one injects the cellular energy-storing molecule ATP into the cell.

To summarize, the results described above pointed to a hypothesis:

> *A specific molecular machine embedded in cell membranes hydrolyzes ATP, then uses some of the resulting free energy to pump sodium ions out of the cell. At the same time the pump imports potassium, partially offsetting the loss of electric charge from the exported sodium.* (11.11)

The pump operates only when sodium and ATP are available on its inner side and potassium is available on its outer side. If any of these are cut off, the cell slowly reverts to the ion concentrations appropriate for equilibrium.

Idea 11.11 amounts to a remarkably detailed portrait of the membrane pump, considering that in 1955 no specific membrane constituent was even known to be a candidate for this job. Clearly *something* was pumping those ions, but there are thousands of transmembrane proteins in a living cell membrane, and it was hard to find for the right one. But in 1957, the physiologist J. Skou isolated a single membrane protein with ATPase activity from crab leg neurons. Carefully controlling the ion content of his solutions, Skou found that to hydrolyze ATP, his enzyme required both sodium and potassium, the same behavior Hodgkin, Katz, and Ussing had found for whole nerve axons (Figure 11.6). Skou concluded that his enzyme must have separate binding sites for both sodium and potassium. For this and other reasons, he correctly guessed that it was the missing sodium pump.

Additional experiments confirmed Skou's hypotheses: Remarkably, it is possible to prepare a pure lipid bilayer, introduce the purified pump protein, the necessary ions, and ATP, then watch as

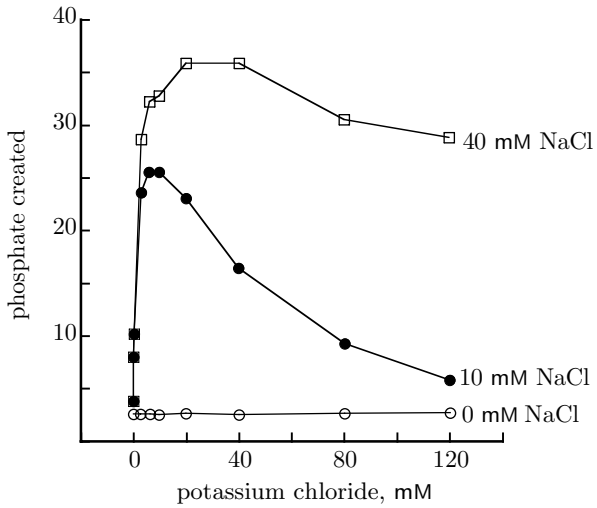

Figure 11.6: (Experimental data.) The rate of ATP hydrolysis catalyzed by the sodium–potassium pump, as a function of the available interior sodium and exterior potassium. The vertical axis gives the quantity of inorganic phosphate (arbitrary units) generated in a certain time interval. The data show that if either sodium or potassium is missing, ATP consumption, and hence P_i production, stop. [Data from Skou, 1957.]

the protein self-assembles in the membrane and begins to function in this totally artificial system.

The fact that the pump's ATPase activity depends on the presence of the pumped ions has an important implication: The pump is a *tightly coupled* molecular machine, wasting very little ATP on futile cycles. Later work showed that in fact the magnitude of the potassium current is always 2/3 as large as that of the sodium ions, maintaining this relation across a range of different ATP concentrations. In other words, the pump carries out **coupled transport** of sodium and potassium ions. We can think of the machine as a special kind of revolving door, which waits for three Na^+-binding sites to be occupied on its interior face. Then it pushes these ions out (or **translocates** them), releases them, and waits for two K^+-binding sites on the outer face to be occupied. Finally it translocates the potassiums, releases them on the interior, and begins its cycle anew. Thus each cycle of this machine causes the net transport of one unit of charge out of the cell; we say that the pump is **electrogenic**.[7] Specific membrane pumps, or **active transporters**, of this sort are among the most important molecular machines in a cell.

Before concluding that the ATPase enzyme discovered by Skou really is (in part) responsible for resting membrane potentials, we should verify that the proposed pumping process is energetically reasonable.

Example Compare the free energy gain from hydrolyzing one ATP molecule to the cost of running the pump through a cycle.

Solution: To pump one sodium ion out of the cell costs both electrostatic potential energy $-e\Delta V$ and the free energy cost of enhancing the world's order (by incrementally increasing the difference in sodium concentration across the membrane). This entropy is what the Nernst potential measures. Consulting Table 11.1 on page 416,

[7] Figure 2.30 on page 58 simplified the sodium–potassium pump, sketching only one of each kind of binding site. A "nonelectrogenic" pump has $j^{\text{pump}}_{K^+} + j^{\text{pump}}_{Na^+} = 0$. An example of this sort of behavior is the H^+/K^+ exchanger, found in the cells lining your stomach. In each cycle it transports two protons out of the cell, helping make your gastric juices acidic, while importing two potassium ions.

the total free-energy cost to pump one sodium ion out is thus

$$-e(\Delta V - \mathcal{V}^{\text{Nernst}}_{\text{Na}^+}) = e(60\,\text{mV} + 54\,\text{mV}) = e \times 114\,\text{mV}.$$

For inward pumping of potassium, the corresponding calculation gives

$$+e(\Delta V - \mathcal{V}^{\text{Nernst}}_{\text{K}^+}) = e(-60\,\text{mV} - (-75\,\text{mV})) = e \times 15\,\text{mV}.$$

which is also positive. The total cost of one cycle is then $3(e \times 114\,\text{mV}) + 2(e \times 15\,\text{mV}) = 372\,\text{eV} = 15 k_{\text{B}} T_{\text{r}}$. (The unit eV, or "electron volt," is defined in Appendix A.) ATP hydrolysis, on the other hand, liberates about $19 k_{\text{B}} T_{\text{r}}$ (see Problem 10.3). The pump is fairly efficient; only $6 k_{\text{B}} T_{\text{r}}$ is lost as thermal energy.

Let us see how the discovery of ion pumping helps make sense of the data presented in Table 11.1 on page 416. Certainly the sodium–potassium pump's net effect of pushing one unit of positive charge out of the cell will drive the cell's interior potential down, away from the sodium Nernst potential and toward that of potassium. The net effect of removing one osmotically active ion from the cell per cycle also has the right sign to reduce the osmotic imbalance we found in Donnan equilibrium (Equation 11.6 on page 415).

To study pumping quantitatively, we first note that a living cell is in a steady state, since it maintains its potential and ion concentrations indefinitely (as long as it remains alive). Thus there must be no net flux of any ion; otherwise some ion would pile up somewhere, eventually changing the concentrations. Every ion must either be impermeant (like the interior macromolecules), or in Nernst equilibrium, or actively pumped. Those ions that are actively pumped (Na^+ and K^+ in our simplified model) must separately have their Ohmic leakage exactly matched by their active pumping rates. Our model assumes that $j^{\text{pump}}_{\text{K}^+} = (-2/3) j^{\text{pump}}_{\text{Na}^+}$ and that $j^{\text{pump}}_{\text{Na}^+} > 0$, since our convention is that j is the flux directed outward. Summarizing this paragraph, in steady state we must have $j_{\text{Na}^+} = j_{\text{K}^+} = 0$, or

$$j^{\text{pump}}_{\text{K}^+} = -j^{\text{Ohmic}}_{\text{K}^+} = -\tfrac{2}{3} j^{\text{pump}}_{\text{Na}^+} = -\tfrac{2}{3}(-j^{\text{Ohmic}}_{\text{Na}^+}).$$

In this model chloride is permeant and not pumped, so its Nernst potential must agree with the resting membrane potential. Indeed from Table 11.1, its Nernst potential really is in good agreement with the actual membrane potential $\Delta V = -60\,\text{mV}$. Turning to sodium and potassium, the previous paragraph implies that the Ohmic part of the corresponding ion fluxes must be in the ratio $-2/3$. The Ohmic hypothesis (Equation 11.8) says that

$$-\tfrac{2}{3}\big(\Delta V - \mathcal{V}^{\text{Nernst}}_{\text{Na}^+}\big) g_{\text{Na}^+} = \big(\Delta V - \mathcal{V}^{\text{Nernst}}_{\text{K}^+}\big) g_{\text{K}^+}.$$

Solving for ΔV gives

$$\Delta V = \frac{2 g_{\text{Na}^+} \mathcal{V}^{\text{Nernst}}_{\text{Na}^+} + 3 g_{\text{K}^+} \mathcal{V}^{\text{Nernst}}_{\text{K}^+}}{2 g_{\text{Na}^+} + 3 g_{\text{K}^+}}. \tag{11.12}$$

We now substitute the Nernst potentials appearing in Table 11.1 on page 416, and the measured relation between conductances (Equation 11.9), finding $\Delta V = -72\,\text{mV}$. We can then compare our prediction to the actual resting potential, about $-60\,\text{mV}$.

Our model is thus moderately successful at explaining the observed membrane potential. In part the inaccuracy stemmed from our use of the Ohmic (linear) hypothesis for membrane conduction, Equation 11.8, when at least one permeant species (sodium) was far from equilibrium. Nevertheless,

we *have* qualitatively answered our paradox: The membrane potential predicted by Equation 11.12 lies *between* the Nernst potentials of sodium and potassium, and is much closer to the latter, as observed in experiments. Indeed Equation 11.12 shows that

> *The ion species with the greatest conductance per area gets the biggest vote in determining the steady-state membrane potential. That is, the resting membrane potential ΔV is closer to the Nernst potential of the most permeant pumped species (here $\mathcal{V}_{K^+}^{\text{Nernst}}$), than it is to that of the less permeant ones (here $\mathcal{V}_{Na^+}^{\text{Nernst}}$).* (11.13)

Our prediction for ΔV also displays experimentally verifiable, and correct, trends as we change the ion concentrations on either side of the membrane.

Even more interestingly, if our membrane could suddenly *switch* from conducting potassium better than sodium to the other way round, then Idea 11.13 predicts that its transmembrane potential would change drastically, switching suddenly from a negative value close to $\mathcal{V}_{K^+}^{\text{Nernst}}$ to a *positive* value closer to $\mathcal{V}_{Na^+}^{\text{Nernst}}$. And in fact, Chapter 12 will show that the measured membrane potential during a nerve impulse really does reverse sign and come close to $\mathcal{V}_{Na^+}^{\text{Nernst}}$. But this is idle speculation—isn't it? Surely the permeabilities of a membrane to various dissolved substances are fixed forever by its physical architecture and chemical composition—aren't they? We will come back to this point in the next chapter.

T2 *Section 11.2.3′ on page 437 gives some more comments about active ion pumping.*

11.3 Mitochondria as factories

Like kinesin, studied in Chapter 10, the sodium-potassium pump runs on a fuel, the molecule ATP. Other molecular motors also run on ATP (or in some cases other NTPs). It takes a lot of ATP to run your body—some estimates are as high as $2 \cdot 10^{26}$ ATP molecules per day, all ultimately derived from the food you eat. That much ATP would weigh 160 kg, but you don't need to carry such a weight around: Each ATP molecule gets recycled many times per minute. That is, ATP is a *carrier* for free energy.

ATP synthesis in eukaryotic cells also involves active ion pumping, though not of sodium or potassium. Instead, the last step in oxidizing your food (called **respiration**) pumps *protons* across a membrane.

11.3.1 Busbars and driveshafts distribute energy in factories

Chapter 10 used the term "machine" to denote a relatively simple system, with few parts, doing just one job. Indeed the earliest technology was of this sort: Turn a crank, and a rope lifts water out of the well.

As technology developed, it became practical to combine machines into a factory, a loose collection of several machines with specialized subtasks. The factory was flexible: It could be reconfigured as needed, individual machines could be replaced, all without disrupting the overall operation. In particular, some of the machines could specialize in importing energy and converting it into a common currency to be fed into the other machines. The latter then made the final product, or perhaps yet another form of energy currency for export.

The graphic on page 1 shows such a factory, circa 1820. The waterwheel converts the weight of the incoming water to a torque on the driveshaft. The driveshaft runs through the mill, distributing

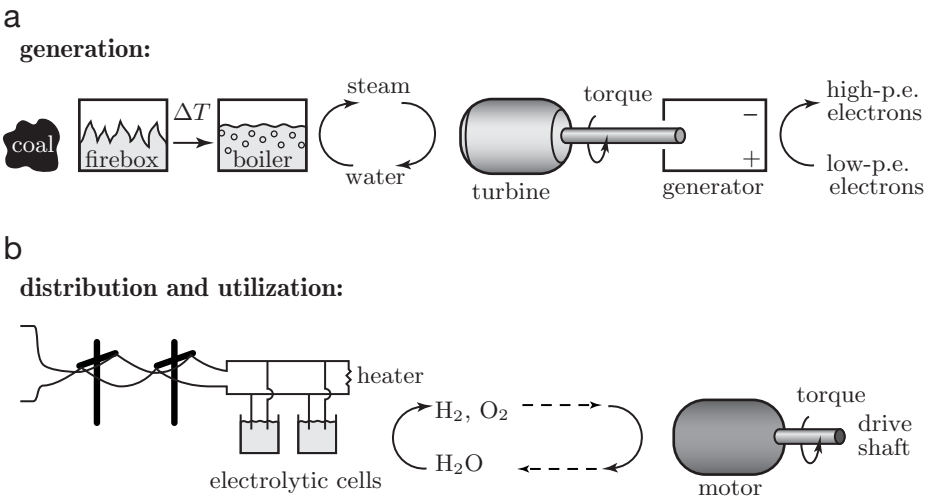

Figure 11.7: (Schematic.) An imagined industrial process. (a) Chemical fuel is burned, ultimately creating a difference in the electrical potential of electrons across two wires. The difference is maintained by electrical insulation (in this case air) between the wires on the far right. (b) Inside a factory, the electrons are used to drive an uphill chemical process, converting low-energy molecules to ones with high stored chemical energy. The latter can then be loaded into an automobile to generate torque and do useful work. If desired, some of the electrons' potential energy can be converted directly to thermal form by placing a resistor (the "heater") across the power lines.

mechanical energy to the various machines attached to it. Later, the invention of electrical technology allowed a more flexible energy currency, the potential energy of electrons in a conductor. With this system, the initial conversion of chemical energy (for example, in coal) to electricity could occur many kilometers away from the point of use in the factory. Within the factory, distribution could be accomplished using a **busbar**, a large conducting bar running through the building, with various machines attached to it.

Figure 11.7 sketches a factory of a sort that may one day supply hydrogen-powered automobiles. Some high-energy substrate, like coal or uranium, comes in at the left. A series of transductions converts the incoming free energy to the potential energy of electrons; the electrons themselves are recirculated. In the factory, a busbar distributes the electricity to a series of electrolytic cells, which convert low-energy water molecules to high-energy hydrogen and oxygen. The hydrogen gets packaged and delivered to cars, which burn it (or convert it directly to electricity) and generate useful work. In winter, some of the electricity can instead be sent through a resistor, doing no mechanical work but warming up the factory for the comfort of those working inside it.

The next subsections will discuss the close parallels between the simple industrial process just described and the activity of mitochondria.

11.3.2 The biochemical backdrop to respiration

The overall biochemical process we wish to study is one of **oxidation**. Originally this term referred to the chemical addition of oxygen to something else, and indeed you breathe in oxygen, attach it to high-energy compounds with carbon and hydrogen, and exhale low-energy H_2O and CO_2. Chemists have found it useful, however, to generalize the concept of oxidation, in order to identify individual subreactions as oxidation or the opposite process, **reduction**. According to this generalization,

the key fact about oxygen is the tremendous lowering of its internal energy when it acquires an additional electron. Thus, as mentioned in Chapter 7, in a water molecule the hydrogen atoms are nearly stripped of their electrons, having given them almost entirely to the oxygens. Burning molecular hydrogen in the reaction $2H_2 + O_2 \rightarrow 2H_2O$ thus oxidizes it in the sense of removing electrons.

More generally, any reaction removing an electron from an atom or molecule is said to "oxidize" it. Because electrons are not created or destroyed in chemical reactions, any oxidation reaction must be accompanied by another reaction effectively *adding* an electron to something—a reduction reaction. For example, oxygen itself gets reduced when we burn hydrogen.

With this terminology in place, let us examine what happens to your food. The early stages of digestion break complex fats and sugars down to simple molecules, for example the simple sugar glucose, which then get transported to the body's individual cells. Once inside the cell, glucose undergoes **glycolysis** in the cytoplasm. We will not study glycolysis in detail, though it does generate a small amount of ATP (two molecules per glucose). Of greater interest to us is that glycolysis splits glucose to two molecules of **pyruvate** (CH_3–CO–COO^-), another small, high-energy molecule.

In anærobic cells, this is essentially the end of the story. The pyruvate is a waste product, which typically gets converted to ethanol or lactate and excreted by the cell, leaving only the two ATP molecules per glucose as the useful product of metabolism. Prior to about 1.8 billion years ago, Earth's atmosphere lacked free oxygen, and living organisms had to manage with this "anærobic metabolism." Even today, intense exercise can locally exhaust your muscle cells' oxygen supply, switching them to anærobic mode, with a resulting buildup of lactate.

With oxygen, however, a cell can synthesize *about thirty more* molecules of ATP per glucose. E. Kennedy and A. Lehninger found in 1948 that the site of this synthesis is the mitochondrion (Figure 2.7 on page 37). The mitochondrion carries out a process called **oxidative phosphorylation**: That is, it imports and oxidizes the pyruvate generated by glycolysis, coupling this energetically favorable reaction to the unfavorable one of attaching a phosphate group to ADP ("phosphorylating" it).

The mitochondrion is surrounded by an outer membrane, which is permeable to most small ions and molecules. Inside this membrane lies a convoluted inner membrane, whose interior is called the **matrix**. The matrix contains closed loops of DNA and its transcriptional apparatus, similarly to a bacterium. The inner side of the inner membrane is densely studded with spherical buttons visible in electron microscopy and sketched in Figure 2.7b. These are ATP synthase particles, to be discussed below.

Figure 11.8 shows in very rough form the steps involved in oxidative phosphorylation, discussed in this subsection and the next one. The figure has been drawn in a way intended to stress the parallels between the mitochondrion and the simple factory in Figure 11.7.

Decarboxylation of pyruvate The first step in oxidative phosphorylation takes place in the mitochondrion's matrix. It involves the removal of the carboxyl (CO) group from pyruvate and its oxidation to CO_2, via a giant enzyme complex called pyruvate dehydrogenase (see Figure 2.4m on page 33). The remainder of the pyruvate is an acetyl group, CH_3–CO–; it gets attached to a carrier molecule called "coenzyme A" (abbreviated CoA) via a sulfur atom, forming "acetyl-CoA." As mentioned above, a reduction must accompany the oxidation of the carbon. The pyruvate dehydrogenase complex couples the oxidation tightly to one *particular* reduction, that of the carrier

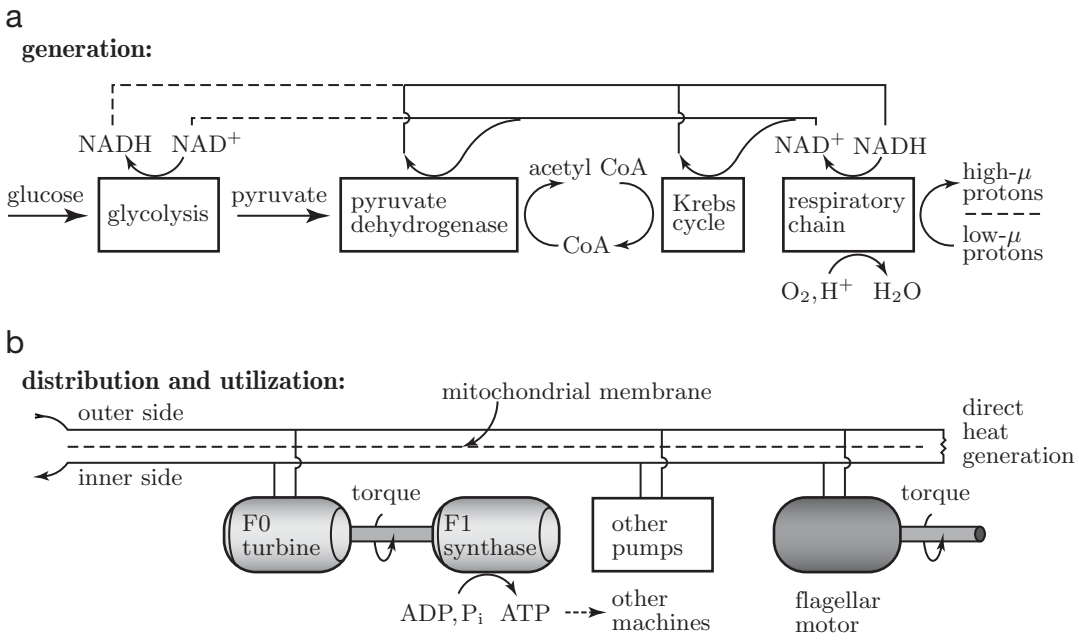

Figure 11.8: (Schematic.) Outline of the activity of a mitochondrion, emphasizing the parallels to Figure 11.7. (a) Metabolism of sugar generates a difference in the electrochemical potential of protons across the inner mitochondrial membrane. For simplicity, "NAD" represents both the carrier molecules NADH and $FADH_2$ (the dashed line represents an indirect process of import into the mitochondrion). (b) The protons in turn drive a number of molecular machines. (Although mitochondria do not have flagella, bacteria such as *E. coli* have a similar arrangement, which does drive their flagellar motor.)

molecule nicotinamide adenine dinucleotide (or **NAD^+**). The net reaction,

$$CH_3\text{–}CO\text{–}COO^- + HS\text{–}CoA + NAD^+ \rightarrow CH_3\text{–}CO\text{–}S\text{–}CoA + CO_2 + NADH, \tag{11.14}$$

adds two electrons (and a proton) to NAD^+, yielding NADH. (Glycolysis also generates another molecule of NADH per pyruvate; this NADH enters the respiratory chain indirectly.)

Krebs cycle The second step also occurs in the mitochondrial matrix. A cycle of enzyme-catalyzed reactions picks up the acetyl-CoA generated in the previous step, oxidizing further the acetyl group and recovering coenzyme A. Corresponding to this oxidation, three more molecules of NAD^+ are reduced to NADH; in addition a second carrier molecule, flavin adenine dinucleotide (abbreviated **FAD**), gets reduced to $FADH_2$. The net reaction,

$$\begin{aligned} &CH_3\text{–}CO\text{–}S\text{–}CoA + 2H_2O + FAD + 3NAD^+ + GDP^{3-} + P_i^{2-} \\ &\quad \rightarrow 2CO_2 + FADH_2 + 3NADH + 2H^+ + GTP^{4-} + HS\text{–}CoA, \end{aligned} \tag{11.15}$$

thus adds eight electrons (and three protons) to the carriers FAD and NAD^+. It also generates one GTP, which is energetically equivalent to an ATP. Different authors refer to this part of the reaction as the **Krebs cycle**, or the **tricarboxylic acid cycle**.

Summary Reactions Equations 11.14 and 11.15 oxidize pyruvate completely: Pyruvate's three carbon atoms each end up as molecules of carbon dioxide. Conversely, four molecules of the carrier

NAD^+ and one of FAD get reduced to NADH and $FADH_2$. Since glycolysis generates two molecules of pyruvate and two of NADH, the overall effect is to generate ten NADH and two $FADH_2$ per glucose. Two ATP per glucose have been formed so far from glycolysis, and the equivalent of two more from the citric acid cycle.

11.3.3 The chemiosmotic mechanism identifies the mitochondrial inner membrane as a busbar

How does the chemical energy stored in the reduced carrier molecules gets harnessed to synthesize ATP? Early attempts to solve this puzzle met with a frustrating inability to pin down the exact stoichiometry of the reaction: Unlike, say, Equation 11.14, where each incoming pyruvate yields exactly one NADH, the number of ATP molecules generated by respiration did not seem to be any definite, integral number. This difficulty dispersed with the discovery of the **chemiosmotic mechanism**, proposed by Peter Mitchell in 1961.

According to the chemiosmotic mechanism, ATP synthesis is *indirectly* coupled to respiration via a power transmission system. Thus we can break the story down into the generation, transmission, and utilization of energy, just as in a factory (Figure 11.8).

Generation The final oxidation reaction in a mitochondrion (respiration) is

$$\mathrm{NADH} + \mathrm{H^+} + \tfrac{1}{2}\mathrm{O_2} \rightarrow \mathrm{NAD^+} + \mathrm{H_2O}. \tag{11.16}$$

(and a similar reaction for $FADH_2$). This reaction has a standard free energy change of[8] $\Delta G'^0_{\mathrm{NAD}} = -88 k_{\mathrm{B}} T_{\mathrm{r}}$, but the enzyme complex that facilitates Reaction 11.16 couples it to the pumping of ten protons across the inner mitochondrial membrane. The net free energy change of the oxidation reaction is thus partially offset by the difference in the electrochemical potential of a proton across the membrane (see Section 8.1.1 on page 260), times ten.

Your Turn 11d

a. Adapt the logic of the Example on page 422 to find the difference in electrochemical potential for protons across the mitochondrial inner membrane. Use the following experimental input: The pH in the matrix minus that outside is $\Delta\mathrm{pH} = 1.4$, while the corresponding electrical potential difference equals $\Delta V \approx -0.16\,\mathsf{volt}$.

b. The difference you just found is often expressed as a "proton-motive force," or "p.m.f.," defined as $(\Delta\mu_{\mathrm{H^+}})/e$. Compute it, expressing your answer in volts.

c. Compute the total $\Delta G'^0_{\mathrm{NAD}} + 10\Delta\mu_{\mathrm{H^+}}$ for the coupled oxidation of one molecule of NADH and transport of ten protons. Is it reasonable to expect this reaction to go forward? What information would you need in order to be sure?

Transmission Under normal conditions, the inner mitochondrial membrane is impermeable to protons. Thus by pumping protons out, the mitochondrion creates an electrochemical potential difference that spreads all over the surface of its inner membrane. The impermeable membrane plays the role of the electrical insulation separating the two wires of an electrical power cord: It maintains the potential difference between the inside and outside of the mitochondrion. Any other

[8] The actual ΔG is even greater in magnitude than $\Delta G'^0$, because the concentrations of the participating species are not equal to their standard values. We will nevertheless use the above value as a rough guide.

machine embedded in the membrane can utilize the excess free energy represented by this $\Delta\mu$ to do useful work, just as any machine can tap into the busbar along a factory.

Utilization The chemiosmotic mechanism requires a second molecular machine, the **ATP synthase**, embedded in the inner membrane. These machines allow protons back inside the mitochondrion, but couple their transport to the synthesis of ATP. Under cellular conditions, the hydrolysis of ATP yields a $\Delta G_{\rm ATP}$ of about $20k_{\rm B}T_{\rm r}$ (see Appendix B). This is about 2.1 times as great as the value you found for the proton's $|\Delta\mu|$ in Your Turn 11d, so we conclude that at least 2.1 protons must cross back into the mitochondrion per ATP synthesis. The actual value is thought to be closer to 3.[9] Another proton is thought to be used by the active transporters that pull ADP and P_i into, and ATP out of, the mitochondrion. As mentioned earlier, each NADH oxidation pumps ten protons out of the mitochondrion. Thus we expect a maximum of about 10/(3+1), or *roughly 2.5 ATP molecules synthesized per NADH.* This is indeed the approximate stoichiometry measured in biochemical experiments. The related molecule $FADH_2$ generates another 1.5 ATP on average from its oxidation. Thus the ten NADH and two $FADH_2$ generated by the oxidation of one glucose molecule ultimately give rise to $10 \times 2.5 + 2 \times 1.5 = 28$ ATP molecules.

Adding to these the two ATP generated directly from glycolysis, and the two GTP from the citric acid cycle, yields a rough total of about *32 molecules of ATP or GTP from the oxidation of a single glucose molecule.* This figure is only an upper bound, assuming high efficiency (small dissipative losses) throughout the respiration/synthesis system. Remarkably, the actual ATP production is close to this limit: The machinery of oxidative phosphorylation is quite efficient. The schematic Figure 11.9 summarizes the mechanism presented in this section.

T2 *Section 11.3.3′ on page 437 gives some more comments about ATP production.*

11.3.4 Evidence for the chemiosmotic mechanism

Several elegant experiments confirm the chemiosmotic mechanism.

Independence of generation and utilization Several of these experiments were designed to demonstrate that oxidation and phosphorylation proceed almost independently, linked only by the common value of the electrochemical potential difference, $\Delta\mu$, across the inner mitochondrial membrane. For example, artificially changing $\Delta\mu$ by preparing an acidic exterior solution was found to induce ATP synthesis in mitochondria without any source of food. Similar results were obtained with chloroplasts in the absence of *light.* (In fact, an external electrical potential can be directly applied across a cell membrane to operate other proton-driven motors—see this chapter's Excursion.)

In a more elaborate experiment, E. Racker and W. Stoeckenius assembled a totally artificial system, combining artificial lipid bilayers with a light-driven proton pump (bacteriorhodopsin) obtained from a bacterium. The resulting vesicles generated a pH gradient when exposed to light. Racker then added an ATP synthase from beef heart to his preparation. Despite the diverse origins of the components, the combined system synthesized ATP when exposed to light, again emphasizing the independence of ATP synthase from any aspect of the respiratory cycle other than the electrochemical potential jump $\Delta\mu$.

[9] T2 The precise stoichiometry of the ATP synthase is still under debate. Thus the numbers here are subject to revision.

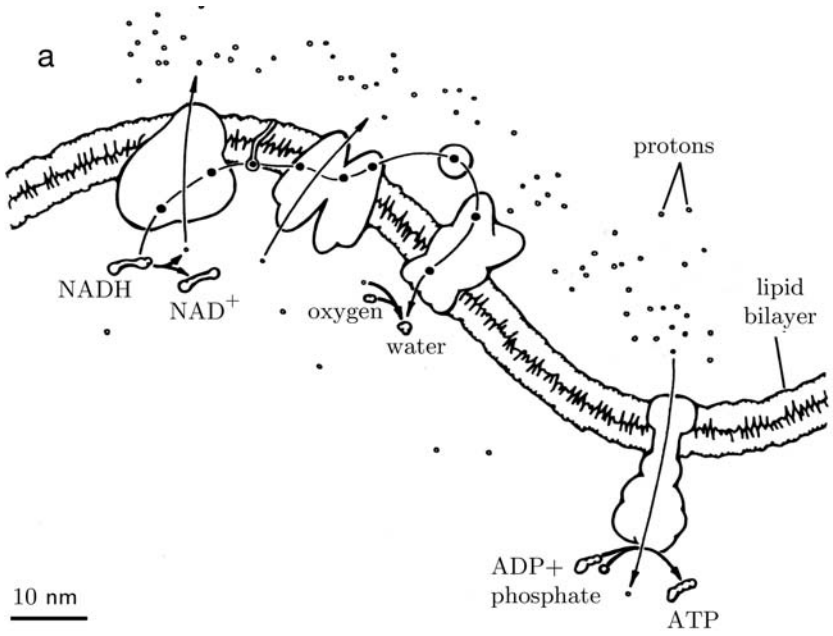

Figure 11.9: (Schematic.) Mechanism of oxidative phosphorylation. Electrons are taken from NADH molecules and transferred down a chain of carriers (*black dots*), ultimately ending up on an oxygen atom in water. Two of the membrane-bound enzymes shown couple this process to the pumping of protons across the inner mitochondrial membrane, seen in cross section. Protons then flow back through the F0F1 complex (*right*), which synthesizes ATP. See also the more realistic depiction of these objects in Figure **??** on page **??**. [From Goodsell, 1993.]

Membrane as electrical insulation It is possible to rip apart the mitochondrial membrane into fragments (using ultrasound), without damaging the individual proteins embedded in it. Ordinarily these fragments would reassemble into closed vesicles, because of the high free energy cost of a bilayer membrane edge (see Section 8.6.1), but this reassembly can be prevented by adding a detergent. The detergent, a one-chain amphiphile, protects the membrane edges by forming a micelle-like rim (Figure 8.8 on page 285). When such fragments were made from the mitochondrial inner membrane, they continued to oxidize NAD^+, *but lost the ability to synthesize ATP.* The loss of function is easy to understand in the light of the chemiosmotic mechanism: In a membrane fragment, the electrical transmission system is "short-circuited"; protons pumped to one side can simply diffuse to the other side.

Similarly, introducing any of a class of membrane channel proteins, or other lipid-soluble compounds known to transport protons short-circuits the mitochondrion, cutting ATP production. Analogous to the electric heater shown in Figure 11.7, such short-circuiting converts the chemical energy of respiration directly into heat. Some animals engage this mechanism in the mitochondria of "brown fat" cells, when they need to turn food directly into heat (for example, during hibernation).

Operation of the ATP synthase We have seen that an elaborate enzymatic apparatus accomplishes the oxidation of NADH and the associated proton pumping. In contrast, the ATP synthase turned out to be remarkably simple. As sketched in Figure 11.10a, the synthase consists of two major units, called **F0** and **F1**. The F0 unit (shown as the elements a, b, and c in the figure) is normally embedded in the inner mitochondrial membrane, with the F1 unit (shown as the elements

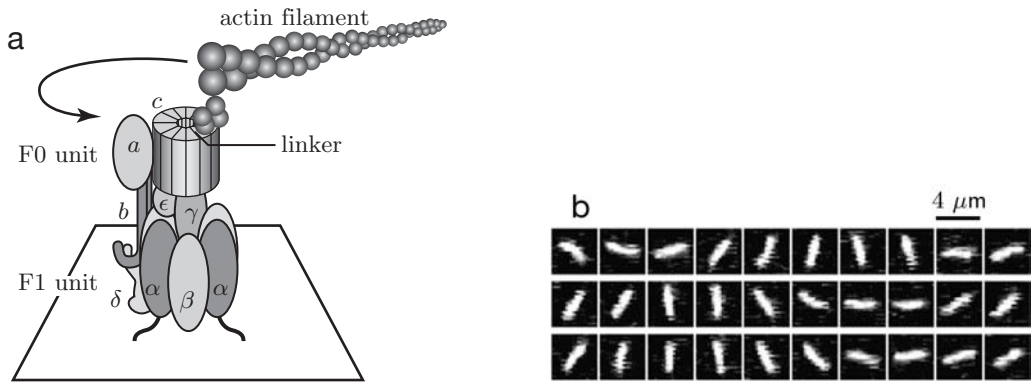

Figure 11.10: (Schematic; video micrograph frames) Direct observation of the rotation of the "c" ring of the F0 proton turbine. (a) A complete ATP synthase from *E. coli* (both F0 and F1 units) is attached to a coverslip, and a long, fluorescently labeled filament of actin is attached. (b) Successive video frames showing the rotation of the actin filament in the presence of 5 mM ATP. The frames are to be read from left to right, starting with the first row; they show a counterclockwise rotation of the actin filament. [From Wada et al., 2000.]

α, β, γ, δ, and ϵ in the figure) projecting into the matrix. Thus the F1 units are the round buttons (often called "lollipops") seen projecting from the inner side of the membrane in electron micrographs. They were discovered and isolated in the 1960s by H. Fernandez–Moran and by Racker, who found that in isolation they catalyzed the *breakdown* of ATP. This result seemed paradoxical: Why should the mitochondrion, whose job is to *synthesize* ATP, contain an ATPase?

To answer the paradox, we first must remember that an enzyme cannot alter the direction of a chemical reaction (see Ideas 8.14 on page 267 and 10.13 on page 374). ΔG sets the reaction's direction, regardless of the presence of enzyme. The only way an enzyme can implement an uphill chemical reaction ($\Delta G_{\mathrm{F1}} > 0$ for ATP synthesis) is by coupling it to some downhill process ($\Delta G_{\mathrm{F0}} < 0$), with the net process being downhill ($\Delta G_{\mathrm{F1}} + \Delta G_{\mathrm{F0}} < 0$). It was easy to guess that the F1 unit is somehow coupled to the F0 unit, and that F0, being embedded in the membrane, is driven by the electrochemical potential difference of protons across the membrane. By isolating the F1 unit, the experimenters had inadvertently removed this coupling, converting F1 from a synthase to an ATPase.

P. Boyer proposed in 1979 that both F0 and F1 are *rotary* molecular machines, mechanically coupled by a driveshaft. According to Boyer's hypothesis, we may think of F0 as a proton "turbine," driven by the chemical potential difference of protons and supplying *torque* to F1. Boyer also outlined a mechanochemical process by which F1 could convert rotary motion to chemical synthesis. Fifteen years later, J. Walker and coauthors gave concrete form to Boyer's model, finding the detailed atomic structure for F1 (sketched in Figure 11.10a). The elements labeled a, b, α, β, and δ in the figure remain fixed with respect to each other, while c, γ, and ϵ rotate relative to them. Each time the driveshaft γ passes a β subunit, the F1 unit catalyzes the interconversion of ATP with ADP; the direction of rotation determines whether synthesis or hydrolysis takes place.

Although static atomic structures such as the one in Figure 11.10a can be highly suggestive, nevertheless they do not actually establish that one part moves relative to another. The look-and-see proof that F1 is a rotary machine came from an ingenious direct experiment by K. Kinosita, Jr., M. Yoshida, and coauthors. Figure 11.10 shows a second-generation version of this experiment.

With a diameter of less than 10 nm, F1 is far too small to observe directly by light microscopy. To overcome this problem, the experimenters attached a long, stiff actin filament to the c element,

as sketched in Figure 11.10a. They labeled the filament with a fluorescent dye and anchored the α and β elements to a glass slide, so that relative rotary motion of the c element would crank the entire actin filament. The resulting motion pictues showed that the motor took random (Brownian) steps, with no net progress, until ATP was added. With ATP, it moved in one direction at speeds up to about six revolutions per second. The motion was not uniform; slowing the F1 motor by using low ATP levels showed discrete, 120° steps. Such steps are just what we would expect on structural grounds: The structure of F1 shows three β subunits, each one-third of a revolution from the others. (Compare the steps taken by kinesin, Figure 10.22 on page 385.) A later experiment used the entire F0F1 complex, not just F1, to confirm that the F0 really is rigidly connected to F1 (see Figure 11.10).

The experiments just described also allow an estimate of the torque generated by ATP hydrolysis (or the torque required for ATP synthesis), using ideas from low Reynolds-number flow. The experimenters found that an actin filament $1\,\mu$m long rotated at about 6 revolutions per second, or an angular velocity of $2\pi \times 6$ radians per second, when ATP was supplied. Section 5.3.1 on page 153 claimed that the viscous drag force on a thin rod, dragged sideways through a fluid, is proportional to its speed, v, and to the viscosity of water, η. The force should also be proportional to the rod's length. Detailed calculation for a rod of length $1\,\mu$m, with the thickness of an actin filament, gave Kinosita and coauthors the constant of proportionality:

$$f \approx 3.0\eta L v. \tag{11.17}$$

Your Turn 11e

Equation 11.17 gives the force needed to drag a rod at a given speed v. But we want the *torque* needed to crank a rod pivoted at one end at *angular* velocity ω.
a. Work this out from Equation 11.17. Evaluate your answer for a rod of length $1\,\mu$m rotating at 6 revolutions per second.
b. How much work must the F1 motor do for every one-third revolution of the actin filament?

More precisely, the rotation rate just quoted was achieved when ATP was supplied at a concentration $c_{\mathrm{ATP}} = 2\,$mM, along with $c_{\mathrm{ADP}} = 10\,\mu$M and $c_{\mathrm{P_i}} = 10\,$mM.

Your Turn 11f

a. Find ΔG for ATP hydrolysis under these conditions (recall Section 8.2.2 and Problem 10.3).
b. If each ATP hydrolysis cranks the γ element by one-third of a revolution, how efficiently does F1 transduce chemical free energy to mechanical work?

Thus F1 is a highly efficient transducer, when operated in its ATPase mode. Under natural conditions, F1 operates in the opposite direction (converting mechanical energy supplied by F0 to ATP production) with a similarly high efficiency, contributing to the overall high efficiency of ærobic metabolism.

11.3.5 Vista: Cells use chemiosmotic coupling in many other contexts

Section 11.2 introduced ion pumping across membranes as a practical necessity, reconciling

- The need to segregate macromolecules inside a cellular compartment, so they can do their jobs in a controlled chemical environment,

- The need to give macromolecules an overall net negative charge, in order to avert a clumping catastrophe (Section 7.4.1 on page 229), and
- The need to maintain osmotic balance, or osmoregulate, in order to avoid excessive internal pressure (see Section 11.2.1).

This chain of logic may well explain why ion pumps evolved in the first place: to meet a challenge posed by the physical world.

But evolution is a tinkerer. Once a mechanism evolves to solve one problem, it's available to be pressed into service for some totally different purpose. Ion pumping implies that the resting, or steady, state of the cell is not in equilibrium, and so is not a state of minimal free energy. That is, the resting state is like a charged battery, with available (free) energy distributed all over the membrane. We should think of the ion pumps as a "trickle charger," constantly keeping the battery charged despite "current leaks" tending to discharge it. Section 11.3.3 showed one useful cellular function that such a setup could perform: the transmission of useful energy among machines embedded in the mitochondrial membrane. In fact, the chemiosmotic mechanism is so versatile that it appears over and over in cell biology.

Proton pumping in chloroplasts and bacteria Chapter 2 mentioned a second class of ATP-generating organelles in the cell, the chloroplasts. Chloroplasts capture sunlight and use its free energy to pump protons across their membrane. From this point on, the story is similar to that in Section 11.3.3: The proton gradient drives a "CF0CF1" complex similar to F0F1 in mitochondria.

Bacteria, too, maintain a proton gradient across their membranes. Some ingest and metabolize food, driving proton pumps related to, though simpler than, the ones in mitochondria. Others, for example the salt-loving *Halobacterium salinarium* contain a light-driven pump, bacteriorhodopsin. Again, whatever the source of the proton gradient, bacteria contain F0F1 synthases quite similar to those in mitochondria and chloroplasts. This high degree of homology, found at the molecular level, lends strong support to the theory that both mitochondria and chloroplasts originated as free-living bacteria. At some point in history, they apparently formed symbiotic relations with other cells. Gradually the mitochondria and chloroplasts lost their ability to live independently, for example losing some of their genomes.

Other pumps Cells have an array of active pumps. Some are powered by ATP: For example, the calcium ATPase, which pumps Ca^{++} ions out of a cell, plays a role in the transmission of nerve impulses (see Chapter 12). Others pull one molecule against its gradient by coupling its motion to the transport of a second species *along* its gradient. Thus for example the lactose permease allows a proton to enter a bacterial cell, but only at the price of bringing along a sugar molecule. Such pumps, where the two coupled motions are in the same direction, are generically called symports. A related class of pumps, coupling an inward to an outward transport, are called antiports. An example is the sodium–calcium exchanger, which uses sodium's electrochemical potential gradient to force calcium out of animal cells (see Problem 11.1).

The flagellar motor Figure 5.9 on page 157 shows the flagellar motor, another remarkable molecular device attached to the power busbar of *E. coli*. Like F0, the motor converts the electrochemical potential jump of protons into a mechanical torque; Section 5.3.1 on page 153 described how this torque turns into directed swimming motion. The flagellar motor spins at up to 100 revolutions per

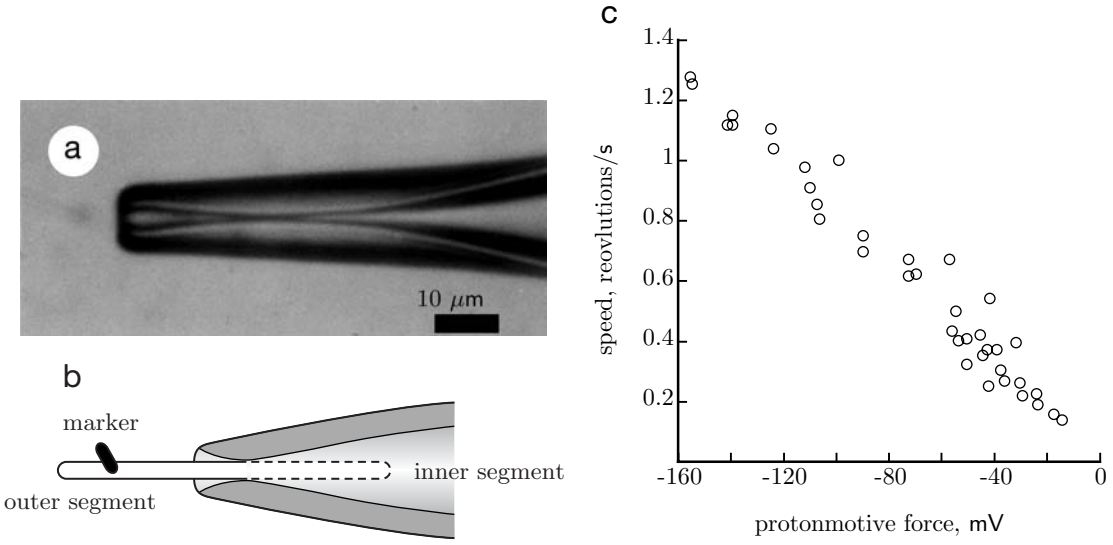

Figure 11.11: (Photomicrograph; schematic; experimental data.) Experiment to show that the flagellar motor runs on proton-motive force. (a) Micropipette tip used to study the bacterial flagellar motor. (b) Micropipette with a partially inserted bacterium. Dashed lines represent the part of the cell wall permeabilized by cephalexin. [Image kindly supplied by H. C. Berg; see Fung & Berg, 1995.] (c) Flagellar motor speed versus the proton-motive force across the part of the membrane containing the motor.

second; each revolution requires the passage of about 1000 protons. The Excursion to this chapter describes a remarkable experiment showing directly the relation between proton-motive force and torque generation in this motor.

11.4 Excursion: "Powering up the flagellar motor" by H. C. Berg and D. Fung

Flagellar rotary motors are driven by protons or sodium ions that flow from the outside to the inside of a bacterial cell. *E. coli* uses protons. If the pH of the external medium is lower than that of the internal medium, protons move inward by diffusion. If the electrical potential of the external medium is higher than that of the internal medium, they are driven in by a transmembrane electric field. We thought that it would be instructive to power up the flagellar motor with an external voltage source, for example a laboratory power supply.[10] *E. coli* is rather small, less than $1\,\mu$m in diameter by about $2\,\mu$m long. And its inner membrane, the one that needs to be energized, is enclosed by a cell wall and porous outer membrane. Thus, it is difficult to insert a micropipette into a cell. But one can put a cell into a micropipette.

First, we grew cells in the presence of a penicillin analog called cephalexin: This suppresses septation (formation of new cell walls between the halves of a dividing cell). The cells then just grow longer without dividing—they become filamentous, like snakes. Then we attached inert markers (dead cells of normal size) to one or more of their flagella. We learned how to make glass micropipettes with narrow constrictions (Figure 11.11a). Then by suction we pulled a snake about half way into the pipette, as shown schematically in panel b of the figure. The pipette contained

[10] Actually, we used a voltage clamp; see Section 12.3.1 on page 465.

an ionophore, a chemical that made the inner segment of the cell permeable to ions, as indicated by the dashed lines. One electrode from the voltage clamp was placed in the external medium and the other was placed inside the pipette. At the beginning of the experiment, the largest resistance in the circuit between the electrodes was the membrane of the outer segment: The resistances of the fluid in the pipette and the membrane of the inner segment were relatively small. Therefore, nearly all of the voltage drop was across the membrane of the outer segment, as desired. However, a substantial fraction of the current flowing between the electrodes leaked around the outside of the cell, so we could not measure the current flowing through the flagellar motors (or other membrane ion channels). The job of the voltage clamp was to supply whatever current was necessary to maintain a specified difference in potential.

When we turned up the control knob of the voltage clamp, the marker spun faster. When we turned it down, the marker spun more slowly. If we turned it up too far (beyond about 200 mV), the motor burned out (the membrane suffered dielectric breakdown). In between, the angular speed of the motor proved to be linearly proportional to the applied voltage, a satisfying result. When we reversed the sign of the voltage, the motor spun backward for a few revolutions and then stopped. When we changed the sign back again, the motor failed to start for several seconds, and then sped up in a stepwise manner, gaining speed in equally spaced increments. Evidently, the different force-generating elements of the motor—we think there are eight, as in a V-8 automobile engine—either were inactivated or came off of the motor when exposed to the reverse potential. They were reactivated or replaced, one after another, when the initial potential was restored! We did not expect to see this self-repair phenomenon.

The main difficulty with this experiment was that the ionophore used to permeabilize the inner segment soon found its way to the outer segment, destroying the preparation. Correction could be made for this, but only for a few minutes. We are still trying to find a better way to permeabilize the inner segment.

For more details See Blair & Berg, 1988 and Fung & Berg, 1995.

Howard Berg is Professor of Molecular and Cellular Biology, and of Physics, at Harvard University. Having studied chemistry, medicine, and physics, he began looking for a problem involving all these fields—and settled upon the molecular biology of behavior. David Fung did his doctoral work on several aspects of the bacterial flagellar motor. He currently works on technology transfer at Memorial Sloan–Kettering Cancer Center in New York.

The big picture

Returning to the Focus Question, this chapter gave a glimpse of how cells actively regulate their interior composition, and hence their volume. We followed a trail of clues that led to the discovery of ion pumps in the cell membrane. In some ways the story is reminiscent of the discovery of DNA (Chapter 3): A tour de force of indirect reasoning left little doubt that some kind of ion pump existed, years before the direct isolation of the pump enzyme. We then turned to a second use for ion pumping, the transmission of free energy from the cell's respiration pathway to its ATP synthesis machinery. The following chapter will develop a third use: Ion pumps create a nonequilibrium state, in which excess free energy is distributed over the cell's membrane. We will see how another class of molecular devices, the voltage-gated ion channels, has evolved to turn this "charged" membrane into an excitable medium, the resting state of a nerve axon.

Key formulas

- *Gibbs–Donnan:* If several ion species can all permeate a membrane, then in order to have equilibrium their Nernst potentials must all agree with each other (and with the externally imposed potential drop, if any). For example, suppose the ions are sodium, potassium, and chloride, and let $c_{1,i}$ and $c_{2,i}$ be the exterior and interior concentrations, respectively, of species i. Then (Equation 11.5)
$$\frac{c_{1,\mathrm{Na}^+}}{c_{2,\mathrm{Na}^+}} = \frac{c_{1,\mathrm{K}^+}}{c_{2,\mathrm{K}^+}} = \frac{c_{2,\mathrm{Cl}^-}}{c_{1,\mathrm{Cl}^-}}.$$

- *Pumps:* The effect of active ion pumping is to add an ATP-dependent current source to the membrane. Making the Ohmic hypothesis gives $j_{\mathrm{Na}^+} = \frac{g_{\mathrm{Na}^+}}{e}(\Delta V - \mathcal{V}_{\mathrm{Na}^+}^{\mathrm{Nernst}}) + j_{\mathrm{Na}^+}^{\mathrm{pump}}$ (Equation 11.10). Here j_{Na^+} is the flux of sodium ions, g_{Na^+} is the membrane's conductance, $\mathcal{V}_{\mathrm{Na}^+}^{\mathrm{Nernst}}$ is the Nernst potential, and ΔV is the actual potential difference across the membrane.

Further reading

Semipopular:
History: Hodgkin, 1992.

Intermediate:
Section 11.2 follows in broad outline the approach of Benedek & Villars, 2000c. See also Katz's classic book, Katz, 1966.
Osmoregulation: Keener & Sneyd, 1998, §2.8
Electroosmotic aspects of kidney function: Hoppensteadt & Peskin, 2002; Benedek & Villars, 2000c.
Many biochemistry and cell-biology texts describe the biochemical aspects of respiration, for example Berg et al., 2002; Nelson & Cox, 2000; Voet & Voet, 2003; Karp, 2002.
Chemiosmotic mechanism: Atkins, 2001; Alberts et al., 1997.
Modeling of ion transport, cell volume control, and kidney function: Hoppensteadt & Peskin, 2002; Keener & Sneyd, 1998.

Technical:
Ion Pumps: Läuger, 1991; Skou, 1989.
F0F1: Noji et al., 1997; Boyer, 1997; Oster & Wang, 2000.

T2 Track 2

11.1.2′

1. To see why the charge density in the membrane is small, think of how permeation works:
a. Some permeation occurs through channels; the volume of these channels is a small fraction of the total volume occupied by the membrane.
b. Some permeation occurs by dissolving the ions in the membrane material; the corresponding partition coefficient (see Section 4.6.1 on page 121) is small. That's because the ions have a large Born self-energy in the membrane interior, whose permittivity is low (see Section 7.4.1 on page 229).

2. We can get Equation 11.1 more explicitly if we imagine membrane permeation literally as diffusion through a channel in the membrane. Applying the argument in Section 4.6.3 on page 124 to the channel gives

$$V_2' - V_1' = -\frac{k_B T}{ze} \ln \frac{c_2'}{c_1'}.$$

Here V' and c' refer to the potential and density at the mouth of the channel (at lines B or C in Figure 11.2). But we can write similar formulas for the potential drops across the charge layers themselves, for example $V_2 - V_2' = -\frac{k_B T}{ze} \ln \frac{c_2}{c_2'}$. Adding these three formulas again gives Equation 11.1.

Actually, we needn't be so literal. The fact that the permeation constant of the membrane drops out of the Nernst relation means that any diffusive transport process will give the same result.

11.2.2′ Section 11.2.2 on page 418 mentioned that there will be nonlinear corrections to Ohmic behavior when $\Delta V - \mathcal{V}_i^{\text{Nernst}}$ is not small. Indeed, each of the many ion conductances has its own characteristic current-versus-potential relation, some of them highly nonlinear (or "rectifying"), others not. One simple model for a nonlinear current–voltage relation is the "Goldman–Hodgkin–Katz" formula; see for example Appendix C of Berg, 1993.

11.2.3′

1. Adding up the columns of Table 11.1 seems to show that even with ion pumping there is a big osmotic imbalance across the cell membrane. We must remember, however, that while the list of ions shown in the table is fairly complete for the extracellular fluid (essentially seawater), still the cytosol has many other osmotically active solutes, not listed in the table. The total of *all* interior solute species just balances the exterior salt, as long as active pumping keeps the interior sodium level small. If active pumping stops, the interior sodium level rises, and an inward flow of water ensues.

2. The sodium–potassium pump can be artificially driven by external electric fields, instead of by ATP. Even an oscillating field, which averages to zero, will induce a directed net flux of sodium in one direction and potassium in the other: The pump uses the nonequilibrium, externally imposed field to rectify the thermally actived barrier crossings of these ions, like the diffusing ratchet model of molecular motors (Section 10.4.4 on page 389). See Astumian, 1997; Läuger, 1991.

11.3.3′ The discussion in Section 11.3.3 did not mention how pyruvate and ADP enter the mitochondrial matrix, nor how ATP exits. Specialized transporters in the mitochondrial membrane

accomplish these tasks. Some of these transporters themselves require ATP, reducing the net production per glucose. For more details see Berg et al., 2002; Hinkle et al., 1991.

Problems

11.1 *Heart failure*

A muscle cell normally maintains a very low interior calcium concentration; Section 12.4.2 will discuss how a small increase in the interior $[Ca^{2+}]$ causes the cell to contract. To maintain this low concentration, muscle cells actively pump out Ca^{2+}. The pump used by cardiac (heart) muscle is an antiport (Section 11.3.5): It couples the extrusion of calcium ions to the entry into the cell of sodium.

The drug oubain suppresses the activity of the sodium–potassium pump. Why do you suppose this drug is widely used to treat heart failure?

11.2 *Electrochemical equilibrium*

Suppose we have a patch of cell membrane stuck on the end of a pipette (tube). The membrane is permeable to bicarbonate ions, HCO_3^-. On side A we have a big reservoir with bicarbonate ions at a concentration of 1 M; on side B there's a similar reservoir with a concentration of 0.1 M. Now we connect a power supply across the two sides of this membrane, to create a fixed potential difference $\Delta V = V_A - V_B$.

a. What should ΔV be in order to maintain equilibrium (no net ion flow)?

b. Suppose $\Delta V = 100\,\mathsf{mV}$. Which way will bicarbonate ions flow?

11.3 *Vacuole equilibrium*

Here are some data for the marine alga *Chætomorpha*. The extracellular fluid is seawater; the "plasmalemma" (outer cell membrane) separates the outside from the cytoplasm; a second membrane ("tonoplast membrane") separates the cytoplasm from an interior organelle, the vacuole (Section 2.1.1 on page 34). (In this problem we pretend that there are no other small ions than the ones listed here.)

Ion	Vacuole (mM)	Cytoplasm (mM)	Extracellular (mM)	$\mathcal{V}^{\text{Nernst}}$ (plasmalemma) (mV)	$\mathcal{V}^{\text{Nernst}}$ (tonoplast) (mV)
K^+	530	425	10	?	−5.5
Na^+	56	50	490	+57	?
Cl^-	620	30	573	−74	+76

a. The table gives some of the Nernst potentials across the two membranes. Fill in the missing ones.

b. The table does not list the charge density $\rho_{\text{q,macro}}$ arising from impermeant macroions in the cytoplasm. What is $-\rho_{\text{q,macro}}/e$ in mM?

c. The actual measured membrane potential difference across the tonoplast membrane is $+76\,\mathsf{mV}$. Suppose all the quoted numbers are accurate to about 2%. Which ion(s) must be actively pumped across the tonoplast membrane, and in which direction(s)?

d. Suppose we selectively shut down the ion pumps in the tonoplast membrane, but the cell metabolism continues to maintain the listed concentrations in the cytoplasm. The system then relaxes to a Donnan equilibrium across the tonoplast membrane. What will be the approximate ion concentrations inside the vacuole, and what will be the final Donnan potential?

11.4 $\boxed{T_2}$ *Relaxation to Donnan equilibrium*

Explore what happens to the resting steady state (see Section 11.1.3) after the ion pumps are suddenly turned off, as follows.

a. Table 11.1 on page 416 shows that sodium ions are far from equilibrium in the resting state. Find the conductance per area for these ions, using the value $5\,\Omega^{-1}\mathsf{m}^{-2}$ for the total membrane conductance per area and the ratios of individual conductances given in Equation 11.9 on page 420.
b. Using the Ohmic hypothesis, find the initial charge flux carried by sodium ions just after the pumps have been shut off. Reexpress your answer as charge per time per unit length along a giant axon, assuming its diameter to be $1\,\mathsf{mm}$.
c. Find the total charge per unit length carried by all the sodium ions inside the axon. What would the corresponding quantity equal if the interior concentration of sodium matched the fixed exterior concentration?
d. Subtract the two values found in (c). Divide by the value you found in (b) to get an estimate for the time scale for the sodium to equilibrate after the pumps shut off.
e. Chapter 12 will describe a nerve impulse as an event that passes by one point on the axon in about a millisecond. Compare to the time scale you just found.