CHAPTER 8

Peter Mitchell & How Cells Make ATP

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☐ INTRODUCTION

Science sometimes exudes an aura of the solidity of its facts. In 1963, the study of energy transformations in the cell was far from this image, however. One researcher, at least, could joke about it, observing that "Anyone who is not thoroughly confused just does not understand the situation."

Yet the focus of research—the process whereby cells convert energy from the Krebs cycle into a usable form—was central to the cell. This was where the oxygen we breathe is ultimately used. It was where cyanide, arsenic, and several pesticides can have their deadly effects. It was where cells make most of their **adenosine triphosphate**, or **ATP**—the primary energy-carrying molecule. We use ATP in our muscle cells, for example, to walk, to grasp, to speak, to swallow, to blink, to inhale, to pump blood. Cells depend on the reactions that produce ATP for various other chemical functions as well. Indeed, to the extent that energy is essential to maintaining the organization of living matter, the problem was akin to asking, "What is life?" The reactions were vital. Yet no one knew how cells make ATP.

Biochemists tried to trace the energetic pathways, applying many of the same strategies used by Hans Krebs and his colleagues (Chapter 7), but without success. They had evidence that there were several intermediate steps, and they had set about to isolate and identify the intermediate molecules. Over many years, different chemists announced that they had succeeded, but each claim later turned out to be erroneous. The series of apparent successes, each mistaken, was disheartening. One text writer noted the irony for his student readers: "No worse fate could befall anyone working on [the problem] than to solve it." Failures in science may often recede gracefully into obscurity, but on this occasion another prominent researcher admitted that the chemists' efforts had met with "conspicuous non-success."

Such a field would seem ripe for new ideas. And in fact, it was against this backdrop that Peter Mitchell (Figure 8.1) introduced a novel hypothesis. It was a radical and, in retrospect, triumphant new solution. Mitchell eventually received a Nobel

FIGURE 8.1 Peter Mitchell (left) with his colleague, Jennifer Moyle. *Source:* Courtesy of Glynn Laboratories, Bodmin, England.

Prize for his insights. Yet his ideas were not widely adopted for almost a decade and a half, despite the chemists' continuing frustrations. How did Mitchell solve the vexing puzzle? And why did other chemists not immediately embrace his solution?

A PREHISTORY OF CHEMIOSMOTIC IDEAS

Peter Mitchell's ideas were revolutionary, leading many to rethink chemistry itself in biological contexts. But for Mitchell, the ideas hardly seemed revolutionary at all. They emerged naturally from other ideas in his experience. They had roots extending back many years, to before he even addressed the problem of how cells make ATP. In this case, the history of Mitchell's thinking can help someone understand the ideas themselves.

While a graduate student in biochemistry at Cambridge University in the early 1940s, Peter Mitchell worked with cell membranes. Membranes pose interesting problems because they are barriers that help maintain the vital integrity of cells. Certain molecules enter cells, while others do not. As a result, the fluid inside a membrane is different from the fluid outside. A membrane thus functions as an **osmotic barrier**. Mitchell worked closely with a chemist who asked, how do such barriers work?

Mitchell became fascinated with one aspect of the problem: How are certain materials "actively" transported across the membranes of cells? Some molecules pass through membranes easily. They diffuse passively as though through a filter. Other molecules, by contrast, need energy to actively cross the osmotic barrier. This is espe-

cially true for molecules like nutrients that must concentrate inside the cell. Molecules do not tend to concentrate on their own—quite the opposite: they tend to disperse. Concentrating them (by actively transporting them inside a membrane) requires energy. Mitchell was curious: how is energy involved in moving the molecules?

Scientists often use analogies to think about an unfamiliar problem. Here, you might imagine familiar examples where energy causes movement. For instance, water releases energy as it falls, and the force of its fall can turn a waterwheel. Likewise, a compressed spring can release energy and propel the movements of a toy vehicle. Could something perhaps "fall" or "spring open" on a cellular level in a way that might help move molecules across a membrane?

PROBLEM

Describe a few examples, from your everyday experience, where energy in various forms causes movement. (Consider, for example, batteries, engines, pressurized air or steam, hand pumping, gravity, etc.) Where possible, describe how each example suggests an image for thinking about how molecules might move across a cellular membrane.

In the late 1940s, two largely independent groups of chemists each saw the problem of membrane transport differently. One group of chemists studied membranes. They looked at the physical structure of membranes and how they allowed certain substances to pass through. A second group of chemists studied proteins, enzymes, and the reactions they catalyzed. They recognized the role of energy in Mitchell's problem, but their models of how enzymes worked did not include movement. Mitchell was unusual in thinking about both branches of cellular chemistry at the same time. He thus saw transport as both a physical phenomenon *and* a chemical reaction using energy. The chemical reaction changed reactants on one side of the membrane into products on the other side of the membrane. "Active" transport was a chemical reaction that spanned the membrane.

Later, Mitchell coined a new term, ***chemiosmotic***, to describe these membrane-spanning types of reactions: '*chemi*' because the reactions were chemical reactions, '*osmotic*' because they occurred across an osmotic barrier, or membrane. Mitchell drew on the Greek root, '*osmo*' meaning "to push." The reactions that Mitchell described were not at all related to the physical process of osmosis, though the words share a common root. In fact, Mitchell was upset when textbooks later introduced the term '*chemiosmosis*' a label he felt was grossly misleading. But how could he prevent others from misrepresenting his ideas?

In the early years of his career, Mitchell worked both on his own and in collaboration with Jennifer Moyle, a colleague from Cambridge (Figure 8.1). He experimented on how bacteria transported various compounds across their membranes. At the same time, he reflected theoretically on his problem. Chemical reactions, Mitchell had concluded, occur in three-dimensional space. But how could enzymes catalyze such reactions? Mitchell explored his ideas concretely by building a series of wooden mechanical models of membrane enzymes (Figure 8.2). The model enzymes would first take two reactants from one side of a membrane into a central channel. When they reacted, the enzymes would use energy to change shape. The entering channels would be closed off, and a new exit channel would open on the other side. They

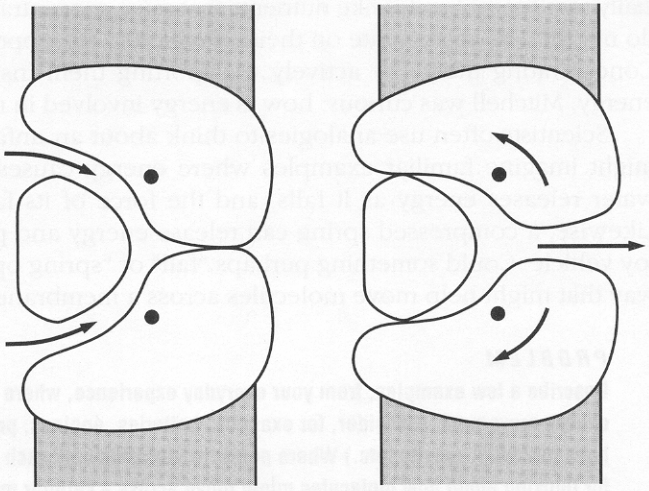

FIGURE 8.2 "Before" and "after" views of a membrane protein, according to Mitchell's early ideas.

would work like one-way turnstiles or the triggered gates found in subways and sports stadiums. Mitchell shared each model with colleagues, asked their opinions, and then revised his models. The models were a way to visualize and develop the notion that transport reactions had direction.

At one point, Mitchell saw the relationship between energy and transport in reverse. Whereas before he had thought only about how energy was used to transport molecules, he now began to think about how the movement of molecules might be used to fuel energy-requiring reactions in the cell. In other words, a molecule traveling from one side of a membrane (where its concentration was high), to the other side (where its concentration was low) would release energy. Could that energy be used to make ATP? To think about the problem, you would reverse the analogies introduced earlier. For example, for a spring to have the *energy* to run a toy, someone has to compress the spring—a form of *movement.* Similarly, *turning* a waterwheel can raise water—*working* against gravity.

PROBLEM

Consider again your earlier analogies linking energy and movement. Describe those cases where the movement could generate energy that could be used or stored. If no case applies, identify another case where movement provides the energy for another process, or where the movement stores energy in a form that can be used later.

In the late 1950s, Mitchell capitalized on a finding from one of his graduate students. She had found that the transport of a particular sugar in bacteria was coupled with the movement of another reactant: a hydrogen ion, or proton. Mitchell recognized that the particles that moved across membranes might be as simple as protons. If the concentration of protons was higher on one side of the membrane, a cell might be able to make ATP when the protons moved.

Mitchell had been working with bacteria, but now he turned his thoughts to cases of ATP production in more complex cells. In eukaryotic cells ATP is produced in the membrane of one organelle, the mitochondrion. ATP is also produced in similar ways in the membranes of chloroplasts in plant cells. Might protons be involved in making ATP there? What would create an imbalance of protons to begin with? Mitchell had ideas about this, as well, and he dispatched a short, speculative article that was published in 1961.

Not long thereafter, Mitchell's professional career took a sharp detour for personal reasons. While teaching at the University of Edinburgh, Mitchell developed gastric ulcers. The institutional environment, he noted later, "did not suit his temperament." At age 43, he was forced to retire from formal academic life. Mitchell moved to the rural region of Cornwall in southern England. There, according to his own testimony, the hand-milking of eight Jersey cows morning and evening for several months did wonders for his ulcers. At the same time, Mitchell nurtured a vision of a small, private research institute where he could work in a less bureaucratic setting. He invited his longtime collaborator, Jennifer Moyle, to contribute her strong experimental skills, and she agreed to join the venture. Mitchell's brother offered the funds. Over the next two years, Mitchell and his family helped renovate a crumbling nineteenth-century mansion into a complex of labs, offices, a library, and a suite of rooms that would be his home. During this three-year pause in research, Mitchell published occasional theoretical papers. But with no lab, his ideas remained relatively dormant.

In 1965, Mitchell was invited to present his chemiosmotic ideas at a conference. Mitchell wanted to present some original data, so he and Moyle hastily assembled some inexpensive and partially homemade equipment. They were able to measure changes in proton concentration (pH) in the solution outside the mitochondria, as predicted by Mitchell's emerging theory. In fact, the results were much better than they expected. Mitchell reported their results at the conference, but few there accepted his ideas. Indeed, a controversy over how cells produce ATP soon flared.

INTERSECTING RESEARCH TRADITIONS

Mitchell had proposed his new ideas in a field that was already well established and had a flourishing tradition of research. After Krebs, chemists were still puzzled about how most of the energy from the citric acid cycle made ATP. The cycle channeled energy into a particular form: high-energy electrons attached to electron-carrier molecules known as NAD and FAD. By the early 1950s, chemists had traced the pathway of energy further. They knew that NAD and FAD transfer their high-energy electrons to a series of molecules in the inner mitochondrial membrane known as the electron transport chain (or the respiratory chain or oxidation chain). In the electron transport chain, the electrons shift to successively lower energy states as they move from molecule to molecule. At the same time, energy is released. The energy that is released produces ATP.

Electrons eventually reach their lowest level when they combine with oxygen and protons, making water. Here is the cellular process associated with our need to breathe. By accepting electrons at the end of the chain, oxygen allows electrons to

flow and to transfer their stored energy to ATP. When oxygen is absent, electrons stop flowing and no ATP is made. In the heart, for example, lack of oxygen means that no ATP is made for the heart muscle cells to contract; as a result, the person has a heart attack. Chemists after Krebs turned to investigate this central question: "How is ATP made as a result of electron transport?"

Chemists were accustomed to thinking in terms of reaction pathways. They reasoned that there must be more intermediate steps. More steps implied more intermediate molecules holding the energy and more enzymes to catalyze each reaction—making what was, for them, a complex process even more complex! Chemists measured the drops in energy levels of electrons to find where enough energy would be released to produce ATP molecules. As Krebs and others before him had done, they set out to find the intermediate molecules.

Mitchell approached the problem of ATP production from an entirely different perspective. The resulting clash highlights, first, just how much the processes Mitchell described differ from other energy reactions in the cell (such as glycolysis and the Krebs cycle). Second, the episode illustrates how scientific knowledge can sometimes undergo revolutionary shifts in perspective.

The electron transport chain was embedded in the mitochondrial membrane and so, Mitchell imagined, the energy system might involve transport across that membrane. In particular, the movement of negatively charged electrons between electron carriers might, through electrical interaction, move positively charged protons across the membrane. In this view, there would be no intermediate molecules as chemists had assumed. Rather, the intermediate energy state would be a different kind of energy: an imbalance of protons on either side of the mitochondrial membrane. The electron transport chain would first create the imbalance. Then the protons would move to restore the balance by traveling through an enzyme, making ATP as suggested in Mitchell's earlier turnstilelike models (Figure 8.2).

For Mitchell, the critical element was how electrons, in cascading down energy levels, could move protons across the membrane. His scheme was based on the idea that as electrons drop energy levels, they also move through space. The electron would start at the first carrier molecule in the electron transport chain, positioned on one side of the membrane. Because of electrical attraction, the negatively charged electron would attract a positively charged proton from the water on that side of the membrane (Figure 8.3(A)). Then the electron would naturally drop energy levels, but because the next electron-carrier molecule was positioned on the other side of the membrane, the electron—with the proton "in tow"—would move across the membrane (Figure 8.3(B)). It would be as if you were riding a pulley down an inclined rope across a river. You would move because gravity pulled you down, but the movement would also take you across the river because the rope would guide how you "fell." If someone grabbed onto you (just as the proton was attracted to the electron), he or she could be carried across the river along with you. The proton, as a "free rider," would now be on the other side of the membrane.

When the electron combined with the electron-carrier molecule on the other side of the membrane, Mitchell postulated, it would no longer be able to hold on to the proton. The proton would then be "dumped" (Figure 8.3(C)). Merely by having

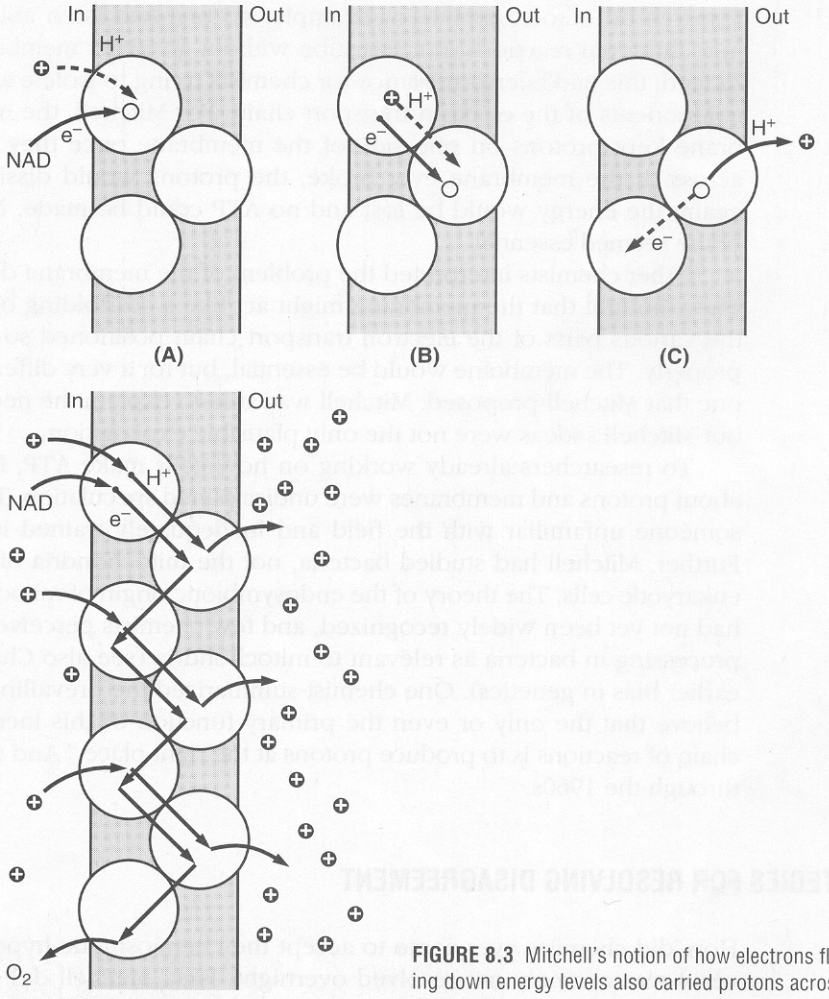

FIGURE 8.3 Mitchell's notion of how electrons flowing down energy levels also carried protons across the membrane.

been attracted to the electron, the proton would have been ferried to the other side of the membrane.

The process would then be repeated as the electron continued to drop energy levels from one electron-carrier molecule to another and onto oxygen. The net effect would be to move many protons from one side of the membrane to the other, thus creating a reservoir or imbalance of protons outside (Figure 8.3(D)). As noted earlier, the high concentration of protons would fuel an enzyme that made ATP as the final step. For Mitchell, the whole process would work because the electron carriers were positioned alternately on opposite sides of the membrane. Here, the chemistry of living things depended on their unique molecular organization.

Mitchell's view of ATP production highlighted several puzzles in the ongoing research on mitochondria. For example, no one had been able to reproduce the mitochondrial reactions in a test tube without an intact membrane being present. Indeed, this had been a nuisance for chemists trying to isolate and study individual components of the electron transport chain. For Mitchell, the mitochondrial membrane kept protons on one side of the membrane once they had been "ferried" across. If the membrane ever broke, the protons would dissipate and even out again; the energy would be lost and no ATP could be made. No wonder a membrane seemed essential.

Other chemists interpreted the problem of the membrane differently, however. They thought that the membrane might act like a scaffolding or skeleton, keeping the various parts of the electron transport chain positioned so they could interact properly. The membrane would be essential, but for a very different reason than the one that Mitchell proposed. Mitchell was able to explain the need for a membrane, but Mitchell's ideas were not the only plausible explanation.

To researchers already working on how cells make ATP, Mitchell's proposals about protons and membranes were undisciplined speculation. They saw Mitchell as someone unfamiliar with the field and inadequately trained in energy reactions. Further, Mitchell had studied bacteria, not the mitochondria of the more complex eukaryotic cells. The theory of the endosymbiotic origin of mitochondria (Chapter 3) had not yet been widely recognized, and few chemists perceived studies of energy processing in bacteria as relevant to mitochondria (see also Chapter 6 for a similar earlier bias in genetics). One chemist summarized the prevailing attitude: "I do not believe that the only or even the primary function of this incredibly complicated chain of reactions is to produce protons at the right place." And so debate continued through the 1960s.

STRATEGIES FOR RESOLVING DISAGREEMENT

How did chemists ever come to accept the chemiosmotic hypothesis? The controversy was certainly not resolved overnight. First, Mitchell did not retreat from his ideas when they were severely criticized. Though Mitchell's disposition was generally gentle and friendly, he was quite tenacious when it came to his ideas. In fact, according to the younger generation of chemists, *all* the leading chemists working during this period had strong egos. There was no doubt, given the magnitude and centrality of the problem, that a Nobel Prize hung in the balance. And the major researchers, including Mitchell, wanted it.

Second, the controversy was resolved in part because the ideas themselves changed. The specific concepts that Mitchell proposed originally and those that chemists came to accept later differed significantly. Mitchell repeatedly revised his theory to address both conceptual problems and new evidence. In his first article, for example, Mitchell had protons moving in the wrong direction! It was a simple mistake, but clearly important for a chemist trying to measure the process. Also, Mitchell had largely guessed about the number of protons needed to make each ATP molecule, and others ridiculed his tentative suggestions as wildly unrealistic. In addition,

the proton imbalance that Mitchell postulated was unprecedented and would likely destroy cells. Mitchell's original notion of the alternating position of electron carriers also did not fit the actual data. He eventually abandoned this concept, replacing it with a far more sophisticated version that other researchers agreed explained the available data very nicely.

Mitchell was both persistent and flexible. He was unwilling to sacrifice his guiding notion that reactions occur in three dimensions, across membranes—and he worked hard to find arrangements that fit the data. Given the limited resources at his private research institute, he had to focus his efforts. In the afternoon, as he sipped tea, he mulled over theoretical problems and identified an experiment to provide critical information. The following morning Moyle would perform the experiment (Mitchell was never known for having good lab skills!). In the afternoon, they would discuss the theoretical consequences of the results. Mitchell would mull some more, sometimes consulting sources in his library, and the two of them would plan where to head next. The close interplay between Mitchell's theoretical adjustments and Moyle's experimental results was essential in revising chemiosmotic interpretations to address everyone's concerns.

Third, Mitchell worked personally with other chemists. Located in a small lab out of the mainstream, Mitchell might easily have become isolated from the scientific community. But he maintained active correspondence with other leading thinkers in the field. He telephoned others when he had specific disagreements with them, presented his point of view, and tried to resolve differences. He also invited others—including his harshest critics—to his lab. Over a one- to three-day visit, they discussed ideas and often conducted short experiments together. These personal visits did not always convince the visiting researchers immediately, but they did help them appreciate the chemiosmotic perspective. And given Mitchell's radical departure from earlier ideas in the field, that sense of understanding was valuable.

AN ARTIFICIAL REALITY

Sometimes, researchers can construct a single crucial experiment to help them decide between two sharply contrasting theories. But here the ideas were too complex. Instead, many tests, each addressing a separate question, were needed to validate Mitchell's ideas. One experiment is especially noteworthy, however, because it exemplifies the kind of dramatic, well-designed experiment that most scientists dream of performing. It also shows how even important results can often be interpreted in different ways.

As Mitchell was completing the renovation of the mansion for his research institute, he received a letter from André Jagendorf at Cornell University. Jagendorf reported that he had encountered some observations in plant cells that confirmed aspects of Mitchell's 1961 hypothesis. He and a graduate student had measured the proton changes due to electron transport in chloroplasts. (These reactions, associated with chlorophyll's use of light energy, closely parallel those in mitochondria.) That was certainly promising. But how could others be sure that the changes were not a coincidence, caused by some unknown factor?

Jagendorf's strategy was to *create* an imbalance of protons artificially and document its effects. First, Jagendorf and a colleague incubated chloroplasts in an acid bath, allowing protons to saturate the solution both inside and outside the chloroplast membrane. They then plunged the chloroplasts into a solution with a lower concentration of protons. This created an imbalance of protons across the membrane. ATP was produced! Because they had conducted the experiment in the dark, they could be confident that the chloroplasts had not used light energy. Rather, the induced imbalance in protons must have made the ATP, mimicking the situation in real chloroplasts, where light was present.

The acid-bath studies were dramatic. The experimenters had induced chloroplasts to perform a natural function under artificial conditions. They had demonstrated that nature behaved in ways that biologists and biochemists had not previously suspected. And they had done so by creating an "unnatural" situation. For many who were accustomed to thinking about intermediate molecules, the experiment was breathtaking.

By today's reckoning, the acid-bath experiments seem like unassailable proof of the chemiosmotic hypothesis (now chemiosmotic "theory"). And indeed, they did confirm one of its controversial predictions. But they did not prove the theory. They did, nonetheless, *demonstrate* an impressive, novel finding. Before the demonstration, the question was whether an imbalance of protons could generate ATP at all. After the experiments, the question was whether ATP could be produced *without* protons. The burden of proof had shifted. Jagendorf's results did not *disprove* the ideas about chemical intermediates (see Question 5 at the end of the chapter). But they did significantly alter the horizon of debate. That was the power of the demonstration. And it was through a series of such demonstrations that chemists began to pursue chemiosmotic ideas, without ever quite explicitly renouncing their earlier ideas.

☐ EPILOGUE

Mitchell received a Nobel Prize in 1978. Later he was portrayed somewhat playfully in a cartoon (Figure 8.4) as the Christopher Columbus of bioenergetics (the field that studies energy in the cell). The caption tells us that "Mitchell sets sail for the Chemiosmotic New World, despite dire warnings that he will be consumed." Note the naysayer on the dock, whose emphatic squiggle, ~, denotes the energy in the hypothesized intermediate molecules. What does this image convey about the process of science and the role of individual genius? (See Question 2.) Mitchell once remarked that "science is not a game like golf, played in solitude." Rather, he observed, it is "a game like tennis in which one sends the ball into the opposing court, and," he added, "expects its return."

Disagreement and controversy can sometimes be construed as symptomatic of weakness in science. If scientists cannot decide between alternative theories, then it may seem as if they do not have strong evidence for either theory. In most cases, however, disputes arise because scientists know too much. In this episode, strong evidence supported each view, yet there seemed no way to reconcile the sometimes conflicting conclusions. Scientists frequently debate theories. This typically means

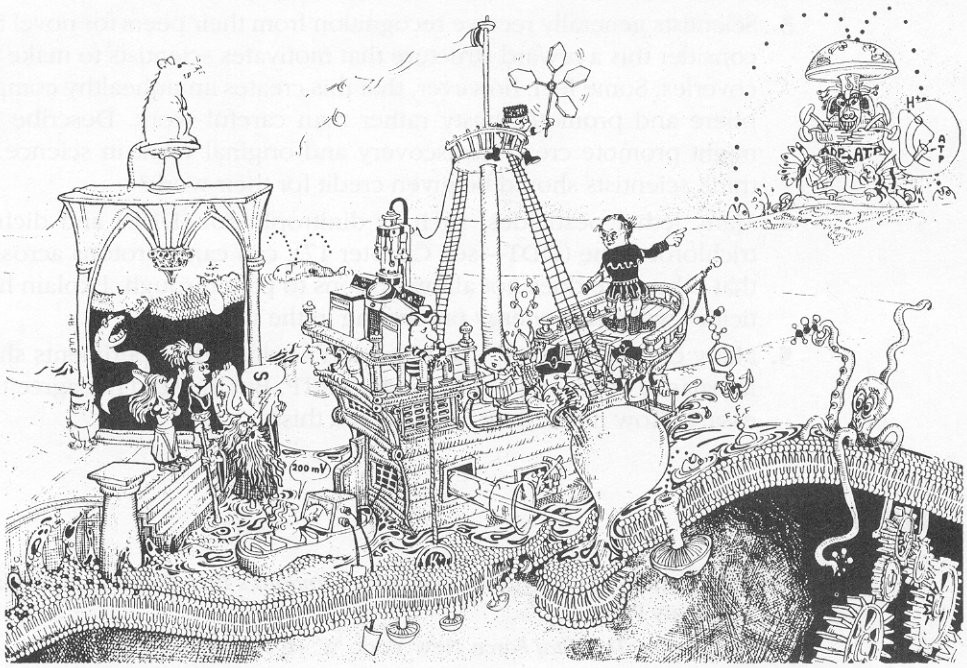

FIGURE 8.4 "Mitchell sets sail for the Chemiosmotic New World, despite dire warnings that he will be consumed." What does this cartoon convey about the nature of scientific discovery? *Source:* Courtesy of Abraham Tulp.

that they are introducing new ideas and struggling with what is not already obvious. Rarely is one side completely "wrong," although they are typically portrayed as such by their opponents! Disagreement, when coupled with constructive dialogue, means that scientists are working towards a fuller, more objective account than either has yet provided. Far from being a weakness, controversy often reflects a healthy, growing science.

QUESTIONS AND ACTIVITIES

1. What does this case show about the following aspects of doing biology?
   — the role of dead ends, blind alleys, and revisions to theory
   — cumulative versus revolutionary growth of knowledge
   — the value of novel findings
   — the collective versus individual nature of scientific inquiry and discovery
   — disagreement
2. Identify several assumptions about the nature of science conveyed in the cartoon image in Figure 8.4. Name at least one other account of a scientific discovery where one or more of these assumptions is evident. Based on this chapter and on other cases in this book, how might you revise the cartoon portrayal? Describe at least two other alternative images or metaphors for science.

3. Scientists generally receive recognition from their peers for novel findings. Many consider this a reward structure that motivates scientists to make important discoveries. Some feel, however, that this creates an unhealthy competitive atmosphere and promotes hasty rather than careful work. Describe how else you might promote creative discovery and original work in science. How do you think scientists should be given credit for their work?
4. Many lethal pesticides, such as dinitrophenol (DNP) and dichlorodiphenyltrichloroethane (DDT—see Chapter 17), can carry protons across a membrane that otherwise does not allow protons to pass through. Explain how these pesticides will affect energy processing in the cell.
5. Many chemists agreed that Jagendorf's acid-bath experiments showed how an imbalance of protons *could* produce ATP. But they did not agree that it was necessary. How might they have argued this?

SUGGESTED READING

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