SACRED BOVINES

DECIPHERING HOW CELLS MAKE ENERGY: AN ACID TEST

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How do you determine if something is true? Suppose a prospector offers to sell you some gold. Is it real gold, or a cheap imitation concocted of copper and silver? You could be swindled. How would you know for sure? The answer, of course, is simple: you conduct a *test*. In the case of a gold sample, you apply a bit of nitric acid (what alchemists of old knew as *aqua fortis*). As metallurgists have known for centuries, this acid solution is strong enough to dissolve many metals, but not gold. To 18th-century prospectors, this simple check offered the critical empirical evidence. They called it the *acid test*. Since then, the phrase has become applied more broadly. It now refers to any definitive test for authenticity.

Scientists use tests all the time, too. It's their business to develop reliable claims. Indeed, in conventional depictions of the scientific method – promoted in part by school textbooks – all tests in science seem definitive. Theories yield forth simple questions. Researchers design simple experiments. The results are simple, too. They indicate clearly whether to accept the hypothesis as true or flatly reject it as false. That is, in the popular imagination, every scientific test is an acid test of sorts. This helps foster a reverential deference to anything calling itself scientific. Here, I explore this potent and widespread impression, this month's Sacred Bovine.

Textbooks often turn to history to celebrate how some classic experiment decisively proved a new, possibly controversial theory. One remarkable example from the 1960s helped show how cells process energy. The study demonstrated how membrane gradients, then considered largely a secondary outcome of cell processes, were likely central to generating the cell's standard energy molecule. Coincidentally for the discussion here, the tests involved soaking plant cells in an acid bath. In that sense, they were "acid tests." But from an insider's perspective, they were hardly "acid tests" in the other sense of that phrase, associated with the image of scientific authority. The history on this occasion can thus help inform, and possibly transform, the naive impression into a more authentic view of the nature of science.

○ Jagendorf's Acid-Bath Experiment

In the early 1960s, biologists and chemists were stumped about something as apparently simple as how cells process energy. Everyone knows that we need oxygen to live. And they may know that the oxygen is used by every cell to "burn" the fuel that enters our bodies as food. But it is a controlled burning. And that is a puzzle. The cells manage to capture the energy released – and use it to move muscles that turn the eyes; to send nerve impulses from the eye to the brain; to send more impulses from the brain to the diaphragm, mouth, and tongue; to move the muscles that allow us to vocalize a simple greeting to a friend who has just appeared in sight. How cells process energy is central to life itself.

At the time, the assumption was that chemical energy from food molecules is basically handed off from one high-energy molecule to another. Eventually, the energy would form adenosine triphosphate – more easily remembered as ATP – which acted as the common energy currency, fueling reactions throughout the cell. But one set of high-energy intermediates had eluded researchers. Whoever isolated and identified these last key links in the chain was sure to win a Nobel Prize.

Junior scientist André Jagendorf, at Johns Hopkins University in Baltimore (Figure 1), approached the problem in the early 1960s from the field of plant biochemistry. During photosynthesis, plants make ATP from light (rather than from consumed food). The chloroplasts in the plant cells shared many elements with mitochondria, the object of most ongoing research at the time, but they were much simpler. The simpler system offered an opportunity. "Well," Jagendorf recalled decades later, "I thought that using chloroplasts might make the job of capturing a high

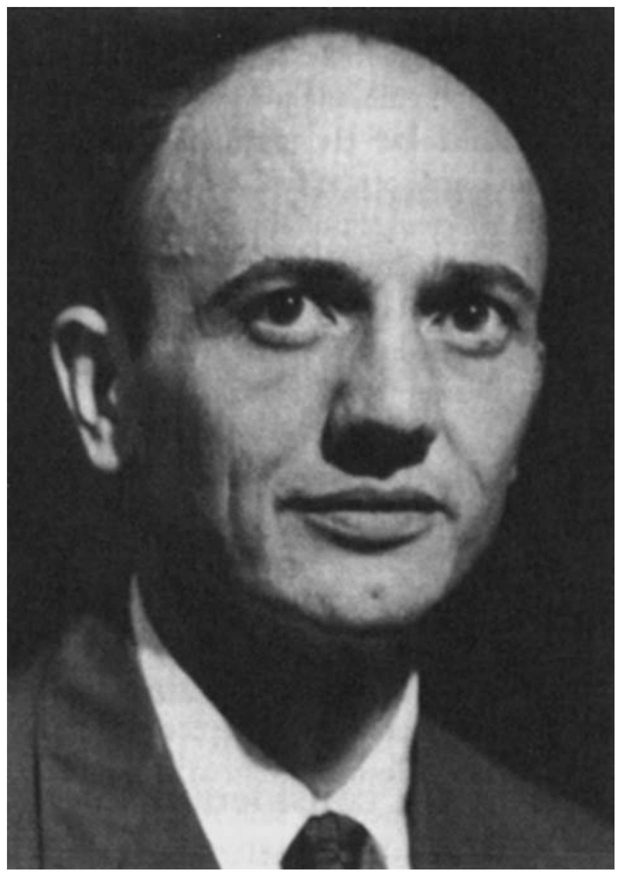

Figure 1. André Jagendorf.

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energy intermediate a little easier, because you could turn off the light so quickly. So I thought I'd jump into the act" (Jagendorf, 1998, p. 221).

At the time, Jagendorf was working with a postdoctoral fellow from England, Geoffrey Hind. Hind's task was to investigate various factors involved in photosynthesis. While analyzing light-scattering, he encountered some unexpected results from a routine control. The pH, or level of acidity, seemed to change. Hind then remembered a 1961 paper by fellow Englishman Peter Mitchell, which speculated that chloroplasts, in producing ATP, might generate a pH imbalance across their membranes. Jagendorf already knew of Mitchell. He had heard him speak at a conference several years earlier. "His words went into one of my ears and out the other," he later recalled, "leaving me feeling annoyed they had allowed such a ridiculous and incomprehensible speaker in" (Jagendorf, 1998, p. 222).

Still, given the odd results, Jagendorf decided to see whether, given short bursts of light, chloroplasts would change the surrounding pH. Was this a relevant experimental variable that had been overlooked? "I stayed in the lab late that same evening, put [chloroplast] thylakoids in a beaker together with PMS, inserted a glass electrode, and watched the needle of the meter rise in the light and fall in the dark" (Jagendorf, 1998, p. 222). Jagendorf was thrilled. "It was an exciting moment to observe this happening using a simple glass electrode" (2002, p. 236). The emotion was exhilarating and memorable. "It was the first time I remember an immediately successful test of a working hypothesis – a most exciting event!" (1998, p. 222).

But in context, the "discovery" was not that exceptional. Hind and Jagendorf published the pH finding amid many other results in the search for the still elusive high-energy intermediate, "X_E". They dutifully cited Mitchell's work, which they characterized as a "rather unusual theory" (Hind & Jagendorf, 1963, p. 607). The *meaning* of the pH effect, while firmly documented, was not yet clear.

In sequel experiments, the lab explored, somewhat blindly, the obscure causal relationships. One lab member later recalled the scene. An "exceptionally able and good natured technician," Marie Smith, spent "hours going around in the coldroom clad in a parka that looked as though it was meant for use in the Arctic and that seemed entirely out of place in Baltimore, especially during the torrid summer months." Graduate student Joe Neumann "used a slide projector in the semi-darkness of a basement lab to illuminate chloroplast preparations and measure pH changes" (McCarty, 1998, p. 229). He tried adding a group of chemicals (called uncouplers) known to disrupt the ATP-forming reactions. Indeed, the pH effect was eliminated. This implied that instead of high-energy intermediate molecules, the light produced a pH gradient. The gradient would then form ATP. That was the essence of Mitchell's "unusual" idea, called the "chemiosmotic hypothesis." Jagendorf noted later:

> At this point, I became rather convinced – although without completely rigorous evidence – that the chemiosmotic explanation was the correct one for the connection between electron flow and ATP synthesis.… I remember more than one biochemical colleague…saying "You have your fingers on the real intermediate, André. What you should do is go in there and fish it out!" To which my response was – "Yes, but maybe it's just a pH gradient, and you can't fish that out." Of course he laughed. (1998, p. 222)

That is, this further set of findings, *combined with the earlier ones*, led Jagendorf to accept Mitchell's still speculative theory. However, he acknowledged that while he was personally persuaded, the evidence was not really complete by scientific standards – and certainly did not persuade others. Were his buoyant emotions biasing his judgment, perhaps?

Jagendorf communicated his findings to Mitchell. A year later, he visited Mitchell's lab in England. There they conceived a new experiment. Yes, light seemed to cause a pH gradient. But could a pH gradient alone, *without any light*, form ATP? That would help demonstrate the prospective series of causes and effects. The strategy – now guided by the emergent theory – was to first keep the chloroplasts in the dark. No light meant no natural source of energy. At the same time, the chloroplasts were incubated in an acid bath, allowing the fluid inside the membrane to reach a low pH. Then, still in the dark, the chloroplasts would be plunged into a much higher pH (basic) solution, creating a sudden pH difference between inside and outside the membrane (Figure 2). Would the pH gradient generate ATP?

The chloroplasts did indeed produce ATP *in the dark*. To ensure that the results were not an accident of circumstances, the experiment was repeated using different ways to measure ATP (firefly luciferase and the uptake of radioactive phosphate). A pH gradient had, surprisingly, produced ATP (Jagendorf & Uribe, 1966).

Ironically, it was the artificial conditions that seemed to indicate what "really" happened in the

Figure 2. Jagendorf's acid-bath experiment, playfully rendered by Dutch biochemist Abraham Tulp.

chloroplasts. Indeed, the experimental design was remarkably clever. By interfering with the natural processes in just the right way, one exposed the role of a previously unknown causal factor. That creativity in experimental design, coupled with the significance of the unprecedented result, made the acid-bath experiment cognitively striking and memorable. Eventually it was described in introductory college biology textbooks, and is still celebrated today.

It was a stunning discovery. The finding was so unexpected. Still, others could easily replicate it and concretely observe it for themselves (and they did, in amazement). Biochemists suddenly had to rethink energetic processes in the cell. Previously, Mitchell's theory had been entertained politely and roundly rejected. Now, it was hard to dismiss. Indeed, years later, biochemists would point back to the acid-bath experiments as key evidence in establishing chemiosmotic theory (Gilbert & Mulkay, 1984a, pp. 108–110; 1984b, p. 29; Robinson, 1986). Accordingly, some present Jagendorf's acid-bath experiment as a historically definitive "acid test" of chemiosmosis.

But Jagendorf's experiment seems conclusive only by abridging the history. Yes, it demonstrated that pH gradients could be relevant. But it did not simultaneously rule out a role for the high-energy intermediates, for which there was already substantial evidence. In that sense, it was not a "test" between two alternative explanations. Indeed, debate between the chemiosmotic and other theories persisted for another decade, at least.

Also, the claims of the experiment were limited. Some saw the setup as *too* artificial. For them, the observations, while "real," did not reflect chloroplasts functioning *naturally*. Many researchers readily conceded that light could produce pH gradients, and that a pH gradient could produce ATP, while also claiming that it all involved side reactions. The central flow of energy, they reasoned, had been rerouted to a secondary process in the cell. The experiment did not test that possibility. That is, the pH gradients could well be peripheral, not essential. Sufficient, one might say, but not necessary. When researchers finally concluded, many years later, that pH gradients were central, they relied on yet further experiments, also artfully designed to exclude alternative interpretations.

After the experiments on chloroplasts, most researchers in the field of plant biochemistry adopted Mitchell's chemiosmotic perspective. However, those studying the corresponding energy system in mitochondria were not so easily persuaded. Jagendorf's assessment that chloroplasts would facilitate research had proved correct. The first parallel experiments in mitochondria were, by contrast, not so clear or dramatic. As noted above, the system was more complex. Experimentalists needed to find more sophisticated strategies to control for the many energy transfers confounding the results. That required many years, too. And more experimental ingenuity.

Jagendorf's experiment was certainly critical historically. But not because it decisively settled a theoretical dispute. Rather, in a sense, it sparked the debate. It demonstrated that pH gradients were causally relevant at a time when no one considered that deeply plausible. That spurred research, rather than resolve it (Robinson, 1984). In addition, the experiment showed just how further research might proceed. It was a material model. It was not merely a theoretical hint or vague prediction. It was a concrete demonstration and exemplar to follow (Kuhn, 1970, pp. 23–42; Allchin, 1992).

The success of the acid-bath experiment reflected some luck, as Jagendorf noted decades later. First, chloroplasts develop measurable membrane gradients much more readily than mitochondria (by regulating subsidiary ion movements). That allowed him to detect the effect much more easily and clearly. Second, Mitchell had made an error in his original 1961 paper about the direction of the gradient in mitochondria. But in chloroplasts, the system is oriented in reverse. Fortuitously, the two errors canceled each other. Finally, Jagendorf used a substrate that minimized extraneous reactions, maximizing the observed effect. The clarity of Jagendorf's results depended on numerous details, a happy convergence of happenstance. For all its elegance, the acid-bath experiment exhibited substantial contingency, complexity, and context-dependence.

○ An Acid Test for How Science Works?

The history of Jagendorf's now renowned acid-bath experiment offers valuable insight into how real science happens. It is much looser than the method so often depicted in textbooks. It is also much more exciting, brimming with chance, uncertainty, opportunity, and creativity. It seems more human, too.

The popular lore dictates that a proper scientific experiment is simple and therefore definitive. Yet consider again the key factors in reaching Jagendorf's monumental finding. It was based on, first, noticing results not formally part of an investigation; then the happenstance of a postdoc's background; personal emotions; hunches about theories in the absence of rigorous proof; correspondence and travel; theoretical context; available materials, such as a slide projector and a parka; multiple cross-checks; local judgment; a series of successive clarifications; disagreement and follow-up experiments; and a few blindly fortunate choices. It was significant, surely, but not as immediately conclusive as common portrayals would lead one to believe. Not every scientific test is an "acid test" – as illustrated, ironically, in Jagendorf's acid-bath experiment on chloroplasts.

One could surely reconstruct a simplified version of Jagendorf's work. For example, one could strip away the context and the human details. One could shoehorn the reasoning into the standard format of hypothesis-deduction-and-test (as has been done in this journal; see Lawson, 2000). But this misrepresents how the great discovery happened. Indeed, an oversimplified account actively misleads students about the nature of science and the basis for scientific authority. Some experiments are ambiguous. They may not be immediately decisive. Knowledge emerges incrementally, through a series of tests. Interpretations may be based on local context, not shared universally. Experiments may open possibilities for new research, not merely justify acceptance of a prospective theory.

One may be tempted to think that the "acid-test" view of science is of no major consequence. Yet it strongly shapes how many persons think about socioscientific issues. For example, critics of evolution or global warming seem to gain traction by appealing to just one negative result or one doubtful-sounding study. They imply that one test is enough to make such major conclusions. The perception of simplicity eclipses the complexity, robustness, and, thus, reliability of scientific theories. Also, when discussing controversial issues, opposing advocates each seem content to cite one research finding and thus to dismiss others as irrelevant (Martin, 1991). These convictions make it much harder to resolve the science in public issues. People expect simple answers, grounded in simple evidence, based on simple tests.

To what degree are these misleading perspectives fostered by simple accounts of science in school? Teachers may certainly know that science is more complex. Yet they can feel compelled by circumstance to simplify things in the classroom. Stripping concepts down to the "gist" can easily seem like a way to respect students who seem burdened by – or at least complain about – things being "too complicated." So, in school settings, the simplified scientific method finds a comfortable home. The ultimate goal of science education in informing social and personal decision making – inevitably complex – can easily get displaced by more proximal concerns. It's not on the test.

However, a "simple" story, full of details and context, may be the first step in remedying the problem of simplicity. Stories are familiar vehicles, easily understood and applied to other cases by analogy. They render the complex on a human scale and in understandable layers of decisions. They can be a new standard for learning about how science works. The vivid, fully elaborated story of Jagendorf's classic experiment may thus be an "acid test" for how one learns a more authentic view of the nature of science.

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