Profile of Bob B. Buchanan

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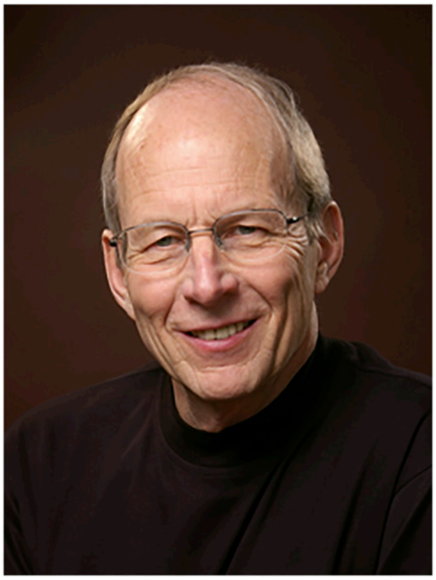
On a clear June day in 1975, Bob Buchanan and two Oslo colleagues pulled up water samples from a lake in Norway, examining the microorganisms growing at various depths. "When we got to 6 meters," Buchanan recalls, "there was a band of *Chlorobium* growing," referring to a genus of green bacteria (now called *Chlorobaculum*) that belongs to a unique class of organisms called photolithotrophs. These bacteria require sunlight for photosynthesis but obtain cellular reductants from sulfur compounds rather than water. The bacteria buck the traditional chemical pathways for energy production and carbon fixation and instead use pathways discovered by Buchanan, a member of the National Academy of Sciences and emeritus professor at the University of California, Berkeley.

From Appalachia to the Laboratory

Buchanan was born in 1937 in Richmond, Virginia. Fearing for the family's safety during World War II, Buchanan's mother took him and his two older sisters to a family farm in Southwest Virginia, near the Appalachian town of Glade Spring, while his father remained in Richmond to work. Farm life shaped Buchanan's early years. He cared for pet lambs as a child and put himself through college by growing beans and milking cows. "By hand!" he says.



Bob B. Buchanan. Image courtesy of Bob B. Buchanan.

Buchanan attended nearby Emory and Henry College, where he took a bacteriology class from zoologist Lee T. Douglas. Under Douglas' mentorship, Buchanan's interest in microorganisms blossomed. "I remember seeing a culture of *Bacillus subtilis*, a bacterium that can grow with or without oxygen, stained with crystal violet under a simple microscope," Buchanan says. "The cells were so brilliant; that's what I wanted to work with." He was fascinated by the concept of life without oxygen.

From Duke to Berkeley

Buchanan moved to Duke University in 1958 for his graduate work. He studied *Actinomyces*, facultative anaerobes known to infect cattle and humans (1). Buchanan characterized a new species collected from an infected human tear duct. The highly infectious isolate, named *Actinomyces propinicus*, differed from known species in cell wall composition and in the formation of propionic acid as a fermentation product. It was later reclassified into the genus *Proprionibacterium*.

While at Duke, Buchanan and his friends in the humanities departments frequented a beer cellar in Chapel Hill, North Carolina, where he explained the anaerobic fermentation processes responsible for their beverages. Another friend at Duke was Melinda Speas, a graduate student in zoology. Their friendship was later rekindled in California and they married in 1965.

After earning his doctorate in 1962, Buchanan secured a postdoctoral fellowship at the University of California, Berkeley, where he would spend the next 55 years. He hoped to work with renowned microbiologist H. A. Barker, but because Barker was on sabbatical leave at the time, Buchanan instead joined the group of Barker's former student, Jesse Rabinowitz. Buchanan and Rabinowitz crystallized the recently discovered ferredoxin protein from *Clostridium* species (2). Watching the protein crystals grow, Buchanan says, was "almost like witnessing the birth of a child." The publication resulting from their work remains his most highly cited paper. Working on ferredoxin set the course for Buchanan's research career. "One can trace the thread from then to today," he says.

Ferredoxin

By the time Buchanan completed his postdoctoral fellowship in 1963, researchers knew that ferredoxin, discovered the year before (3), carried highly energetic electrons and participated in fundamental biological processes (4, 5). At the time, biologists knew of one process by which aerobic organisms produced energy: the Krebs or citric acid cycle. Working with Daniel Arnon, an expert in photosynthesis, and postdoctoral scholar Michael Evans, Buchanan set out to determine whether the energetic electrons carried by ferredoxin could reverse the cycle.

"Textbooks said the cycle was irreversible," Buchanan says, "but it turned out that ferredoxin could do the job." In the citric acid cycle, glucose breaks down into pyruvate, which, in turn, breaks down into acetyl-CoA and carbon dioxide. Buchanan and his colleagues had earlier reversed the reaction, combining acetyl-CoA, carbon dioxide, and reduced

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ferredoxin to generate pyruvate (6). They further reversed another reaction, the breakdown of α-ketoglutarate. With the participation of ferredoxin and three enzymes, the citric acid cycle could be fully reversed (7), fixing carbon dioxide and synthesizing acetyl-CoA and pyruvate. The team recognized the role of such a cycle in anaerobic environments, such as the rumen of cattle or the depths of a lake, where it would enable organisms to synthesize amino acids from organic acids and carbon dioxide. The cycle, now called the Arnon–Buchanan cycle (8), was found in *Chlorobium*, the bacterium that Buchanan would later sample in a Norwegian lake.

Buchanan's cycle immediately encountered controversy, however, challenging the assertion of his Berkeley colleague, Melvin Calvin, that all carbon dioxide was fixed through the Calvin–Benson cycle. Acceptance of the Arnon–Buchanan cycle took about a quarter century (9) and "only then did it appear in textbooks," Buchanan says.

Since that time, the Arnon–Buchanan cycle has been found in various extreme environments, such as deep ocean hydrothermal vents. (10) "This cycle has taken directions I never thought possible," Buchanan says.

Thioredoxin and Chloroplasts

Hoping to find more evidence for carbon fixation via the Arnon–Buchanan cycle, Buchanan then turned his attention to the role of ferredoxin in chloroplasts, the photosynthetic centers of plant cells. Using acetyl-CoA as a reaction substrate with ferredoxin yielded no results. His group then added sugar phosphates as substrates and found that fructose 1,6-bisphosphate increased the uptake of carbon dioxide by chloroplast enzymes in the presence of reduced ferredoxin and ATP. Tracing the chemical path of fructose utilization, Buchanan found that the enzyme fructose 1,6-bisphosphatase was central to this increase and that the enzyme was activated by reduced ferredoxin. (11) "There was no indication that this or any other enzyme could be regulated in this manner," he says.

Part of this discovery was fortuitous. Buchanan had added a small amount of magnesium to the reaction, which turned out to be a cofactor for the fructose 1,6-bisphosphatase enzyme in chloroplasts (12). "Had I used a saturated concentration of magnesium," he says, "we would have missed it." Buchanan's findings were published in 1967. Ten years later, with postdoctoral scholars Peter Schürmann and Ricardo Wolosiuk, Buchanan found that ferredoxin's partner in regulating photosynthetic enzyme activity (13) is a protein called thioredoxin. Ferredoxin reduced by light can reduce thioredoxin via an enzyme Buchanan named ferredoxin–thioredoxin reductase. The reduced thioredoxin activates enzymes of the chloroplast, which are deactivated by a different mechanism in the dark.

Beyond Chloroplasts

Thioredoxin's importance as a redox regulator of enzymes was not limited to chloroplasts. In the early 1990s, Buchanan asked whether thioredoxin participated in plant functions outside the chloroplast. He focused on the process of seed germination. "Seeds and redox had never gotten together before but since I had never really studied plants formally, I could ask simple questions, which may be an advantage," he says.

Buchanan considered the redox state of dried seed proteins. When quiescent, he supposed, seed proteins would be in a stable oxidized state. Exposing seeds to water would reduce the seed proteins, breaking them down to serve as nutrients in germination. In collaboration with graduate student Tom Johnson and visiting scientist Karoly Kobrehel, Buchanan found that reduced thioredoxin served as a redox signal to promote protein solubilization and activate enzymes functional in germination (14).

Thioredoxin has since been found to regulate enzymes throughout biology in bacterial and animal cells alike (15). As a cellular signal, thioredoxin has also found practical application. Overexpression of thioredoxin in barley accelerates germination by about a day (16). "That doesn't sound impressive unless you're in the malt business," Buchanan says. Thioredoxins can also mitigate allergens in wheat (17) and may play a role in cancer and Parkinson's disease research. "In cancer, thioredoxin is the bad guy because it encourages cell division," he says. "In Parkinson's, it's the good guy because you want revitalization."

Most recently, Buchanan and colleague Peggy Lemaux have collaborated with a group at McGill University in Montreal to study the role of redox regulation on the genetic activity of barley. The team identified a gene (*TLP8*) that influenced malting quality (18). The protein produced by the gene binds to β-glucan polysaccharides, which are insoluble, and assists the filtering process in beer production. The researchers found that differential expression of the gene in different barley varieties affected β-glucan binding, as did the redox state of the protein.

Despite his work on plant and grain biochemistry, Buchanan does not keep a garden or brew his own beer. "And I grew up on a farm!" he says. "I'm embarrassed to say that. I really enjoy what I do too much to dilute my time."

In 1999, Buchanan was at home and noticed some difficulty moving his right-side limbs. He went to the hospital and learned he was having a stroke that progressed until he lost almost all mobility on his right side. He says that change in diet and adopting an exercise regimen, which includes regular swimming, are to thank for his recovery. After years of physical therapy, he regained much of his mobility.

Buchanan and his wife have established awards to encourage scientific enthusiasm and excellence. At Wake Forest University, his wife's alma mater, an award honors her father, William E. Speas, who taught physics there for decades. At Emory and Henry, two awards named for Buchanan's ancestors are given to students, typically from Appalachia, studying biology and chemistry. At Berkeley, Buchanan has raised funds to establish endowments for four annual lectures and two graduate fellowships. Close to his original home, Buchanan has also provided an endowed fund for the perpetual upkeep of a family cemetery in Virginia that dates to around 1800. "I feel good about that," he says, "thinking that my ancestors who helped settle the area deserved it."

Buchanan retired from active teaching and research in 2013, but still spends 6 hours a day at the office. Looking back on his career, Buchanan reflects on the factors that brought him success: "Follow your nose, ignore turf, and go with science as it unfolds. If you do that and have a bit of luck, you've got it made."

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