



# BIOGRAPHICAL MEMOIRS

## CLINTON E. BALLOU

June 18, 1923–March 8, 2021

Elected to the NAS, 1975

*A Biographical Memoir by Gary R. Gray,  
Patrick Brennan, and Robert Barker*

CLINTON E. BALLOU was a noted biochemist, researcher, mentor, and teacher who was a Professor of Biochemistry at the University of California, Berkeley, for 40 years (1955–1995). Clint, as he was known by all, led a remarkable and impactful life, rising from humble origins in the farm country of Idaho and including service in the U.S. Navy in World War II, before discovering his aptitude for chemistry. He made ground-breaking contributions in several major areas of carbohydrate, polysaccharide, glycolipid, and glycoprotein biochemistry. He devised efficient chemical syntheses for several sugar-phosphates involved in carbohydrate metabolism. He conducted path-finding work on inositol, inositol-polyphosphates, and phosphoinositides. He also made novel discoveries about mycobacterial polymethylpolysaccharides and mycobacterial glycolipids because of their relevance to tuberculosis and delineated the structure and biosynthetic steps required for construction of the phosphomannan component of the yeast (*Saccharomyces cerevisiae*) cell wall. Among other accolades, he received the Claude Hudson Award in Carbohydrate Chemistry from the American Chemical Society (1981). Clint died on March 8, 2021, at age 97 in Alameda, CA.

Clinton Ballou was born on June 18, 1923, in King Hill, Idaho, to William and Mollie (Bernt) Ballou. He attended grade school and the first three years of high school in an eight-room schoolhouse in King Hill and the final year of high school in Boise, Idaho, where he graduated in 1940. After attending Boise Junior College from 1940–1942, he



Figure 1 Portrait of Clinton E. Ballou.

enrolled at Oregon State College where, two years later, he received his bachelor of science degree in chemistry. His research advisor at Oregon State College, William Wagtendonk, suggested that he go to the University of Wisconsin-Madison if he wanted to study biochemistry, and he further suggested that he study with Karl Paul Link. Indeed, Clint enrolled at the University of Wisconsin as soon as his military service in the U.S. Navy was completed in 1946. His work there on alkali-sensitive glycosides earned him master's and Ph.D. degrees in biochemistry.

While in the Link laboratory, Clint met Dorothy Lun Wu, another Link graduate student, and they were married in Link's home in December 1949. After raising daughter





**Figure 2** Clint Ballou and Dorothy Lun Wu at their laboratory retirement. Photo courtesy of Linda Ballou and Philip Ballou.

Linda and son Philip, they resumed working together in the laboratory and authored more than a dozen (17 actually!) papers together. [Figure 2]

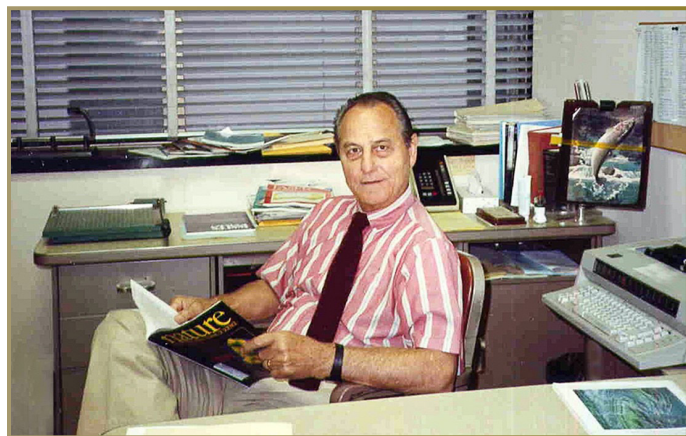
After receiving his Ph.D. in January 1950, Clint was awarded a postdoctoral fellowship from the National Institutes of Health (NIH) to support his work for a year in the laboratory of Edwin L. Hirst in Edinburgh, Scotland. This was followed by a second postdoctoral year with Hermann O. L. Fischer at the University of California, Berkeley. After spending three more years in Fischer's laboratory, he was offered and accepted an appointment as an assistant professor of biochemistry in 1955.

Progressing rapidly through the tenure system, Clint was promoted to associate professor with tenure in 1957 and professor in 1962. [Figure 3]

During his tenure at Berkeley, he and his group made major contributions to many areas of carbohydrate chemistry and biochemistry. His first independent work dealt with mechanistic studies of the glycolytic enzymes enolase with graduate student Finn Wold and phosphoglycerate mutase with graduate student Louis Pizer.<sup>1,2,3</sup> Then, with Louis Pizer, he began an on-again-off-again project on the isolation, characterization, synthesis, and biochemical characterization of phosphorylated inositol glycolipids (phosphoinositides) that would last through much of his career<sup>4,5</sup> as well the glycopospholipids (phosphatidyl inositol mannosides) of mycobacteria.<sup>6-9</sup> The latter work was initiated by postdoctoral fellow Yuan C. Lee but progressed further with the arrival of postdoctoral fellow Patrick Brennan, who had extensive experience working with mycobacteria. Clint is also well known for his work on the isolation and structural characterization

of mycobacterial polymethylpolysaccharides<sup>10-15</sup> and studies of their biosynthesis<sup>16-19</sup> and mode of interaction with fatty acids and fatty acid derivatives.<sup>20-23</sup> The work on polymethylpolysaccharides was again initiated by Lee with the discovery of a polysaccharide containing 6-*O*-methyl-D-glucose in *Mycobacterium phlei*, but structural characterization was accomplished by graduate student Milton H. Saier and continued by postdoctoral fellows Gary R. Gray and William Smith. Gray was also able to isolate another polymethylpolysaccharide from *Mycobacterium phlei*, namely one containing 3-*O*-methyl-D-mannose, and characterize its basic structure. Both methylated polysaccharides were later discovered to help regulate fatty acid biosynthesis in this organism.

Ballou's best-known work might well be the structural characterization and immunochemistry of the mannans of the cell wall envelopes of *Saccharomyces cerevisiae* and other yeasts<sup>24-28</sup> as well as biochemical and genetic studies of the mechanism of their biosynthesis.<sup>29-33</sup> The structural work was initiated by Lee, who discovered that the *Saccharomyces cerevisiae* mannan could be cleaved at its backbone by an acetolysis reaction to yield a mannose-containing disaccharide, trisaccharide, and tetrasaccharide.<sup>34</sup> Structural studies on that yeast as well as others were continued by graduate student William C. Raschke, postdoctoral fellow Tom S. Stewart, and visiting professor Jan Kocourek. Ballou's work with yeasts also included the isolation and characterization of the sexual agglutination factors in certain species<sup>35,36</sup> as well as biochemical and genetic studies of the mechanism of protein glycosylation.<sup>37,38</sup> Much of the work in the latter area was performed in collaboration with his wife Lun, who joined his laboratory after their children were grown.



**Figure 3** Clint in his office at Berkeley. Photo courtesy of Linda Ballou and Philip Ballou.

### PERSONAL REMEMBRANCES BY GARY R. GRAY

It was my good fortune to work in the Ballou lab as an NIH postdoctoral fellow from September 1969 to September 1971 and to continue my research in that lab as a visiting



Figure 4 Clint's Porsche 356 convertible. Photo courtesy of Linda Ballou and Philip Ballou.

assistant professor until September 1972. I have to admit that upon first meeting Clint, he seemed very reserved and unapproachable but, as I got to know him, I came to realize that my first impression could not have been further from the truth. Behind that “stone face” instead was someone who was very thoughtful, helpful, and generous. As I also came to know, he was actually easily approachable, whether for scientific or personal advice. Stated simply, he was a superb mentor!

Both Clint and Lun were extremely generous with their personal time as well. I can recall Clint inviting me to several University of California sporting events and on numerous occasions being invited to dinner, either at their home or at a local restaurant. Clint really enjoyed good food and fine wines, and both he and Lun liked to experiment with gourmet cooking, so such evenings were indeed very memorable! Clint was also a great lover of Porsches, and I was very fortunate on more than one occasion to get a ride in his beautiful yellow Porsche 356 convertible. Those experiences taught me that I needed a Porsche of my own, which I acquired before leaving Berkeley and, in fact, still drive. If only I could have found one like his! [Figure 4]

### PERSONAL REMEMBRANCES BY PATRICK BRENNAN

I was a postdoctoral fellow in Ballou's laboratory as a U.S. Public Health Service Research Fellow for two years from November 1965 to November 1967. I had just completed my Ph.D. studies in the Department of Biochemistry, Trinity College, Dublin, under Frank Winder on the mechanism of action of the front-line tuberculosis drug, Isoniazid, on *Mycobacterium tuberculosis* and had several publications with Frank on the topic. Frank had written a persuasive letter to Clint, who liked the idea of someone in his laboratory with a

knowledge of mycobacterial physiology. As Gary Gray pointed out in his biography of Clint, his education, training, and experience were largely on the chemistry of natural and synthetic products. Primary among these were the synthesis and characterization of phosphorylated inositol derivatives, and Clint had developed a stellar reputation in that area. In that context, Clint and Lun had completed in the early 1960s a one-year sabbatical at the Institut de Chimie des Substances Naturelles (CNRS), in Gif-sur-Yvette, France, in the laboratory of Erna Vilkas and Edgar Lederer. There, he conducted pioneering work on the isolation and structures of the family of phosphatidyl inositol mannosides, which he published in the *Journal of Biological Chemistry*.<sup>39</sup> Incidentally, Gary refers to Clint's enjoyment of “good food and fine wines, and he likes to experiment with gourmet cooking;” Clint made clear that his time at Gif was the genesis of that penchant.

On this return from France, Clint, with postdoctoral fellow Y. C. (Ed) Lee, conducted among the most profound work of his sterling career, the structures of the 6-member phosphatidyl inositol mannosides (PIMs; in fact, glyceryl-phosphoryl-inositol mannosides, because they characterized the deacylated products) of *Mycobacterium tuberculosis*, published in several issues of the *Journal of Biological Chemistry* and *Biochemistry*.<sup>40,41,42</sup> Their profound research was the prelude to mine, a tough act to follow for a still naïve Irishman from the impoverished West of Ireland of the war years. Clint wanted me to initiate work on the biosynthesis of the PIMs, and I was successful in demonstrating that cell-free extracts of *Mycobacterium spp.* could readily catalyze the addition of [<sup>14</sup>C]mannose from GDP-[<sup>14</sup>C]mannose to phosphatidyl inositol (PI) to generate the dominant dimannoside product (PIM<sub>2</sub>). However, I was analyzing the biosynthetic products by thin layer chromatography (TLC) (a technique new to the lab, and Clint enjoyed showing his visitors how I poured my own TLC plates!), and I observed that three lipid products all yielded glyceryl-phosphoryl-dimannoside on deacylation. Clint was ecstatic, as I recall, and said that I had solved a question that he and Ed had long pondered, the intact/physiological nature of these membranous PIMs. It is now well established that each member of the PIM family exists in multi-acylated states. My prestige in Clint's eyes was raised somewhat, and thereafter I was less nervous in his presence. Nicole Kresge, Robert D. Simoni, and Robert L. Hill have written a lovely reflection on the history of Clint and this aspect of his work.<sup>43</sup>

Clinton Ballou was an awesome, daunting, somewhat frightening figure in my eyes and those of fellow foreign post-docs, precipitated, I think, by his impeccable presence, his precision, discipline, and sharp questioning for experimental detail that one had not anticipated. This awe of his magnetism, my preoccupation with him and what he thought of me

and my work were more a product of my own insecurity born of my origins; I had never encountered anyone with that sense of presence, of authority, of command, of elegance (fortified by his elegant Porsche!). But he was a good, kind man, generous in his receptions at home with Lun. (We foreign post-docs claimed that he had a telescope in his home that could peer into his laboratory on the north side of the Biochemistry building and keep an eye on us on weekends and nights!) He had a bench beside mine in his lab and would work there on Saturday mornings, despite being chair of the department, and if UC Berkeley had a home football game that afternoon, he would invite us to join him in the walk up to the Quarry. These were happy days. I met my wife, now of over 52 years, at Berkeley. She was then a graduate student in the Department of Molecular Biology. Fortified by the subsequent letters of support from Clint, I went on to a fulfilling career, all on mycobacterial structure, biosynthesis, and genetics at Trinity College Dublin, University College Dublin, National Jewish Center/University of Colorado Medical School, and now at Colorado State University, thanks to that initial mentoring and guidance from Clinton Ballou. Although I never had any evidence of a religious/spiritual inclination in Clint, I would like to offer him a prayer in the Irish language. As we say in the Irish language, “Ar dheis Dé go raibh a anam dílis” (At the right hand of God his soul was faithful).

### PERSONAL REMEMBRANCES BY ROBERT BARKER

My relationship with Clint was quite different from those who were his scientific colleagues. We were friends. When I went to Berkeley, he was a post-doc with H. O. L. Fischer. He was appointed as an assistant professor about a year before I left. For the last few months of my stay, I worked in his lab finishing up work I had started with Don MacDonald, who had not been tenured. I was an academic orphan. But during most of the time I was in Berkeley, we formed a friendship that lasted a lifetime. His wife Lun and my wife Kaz were good friends. It was the kind of friendship that could lie dormant for years and renew in an instant.

It is in this context that I remember Clint. Together with Finn Wold, Lew Pizer, and Don MacDonald, we had a scotch appreciation “club” that met once a month and settled the world’s and Berkeley’s problems while comparing the selection of the month to Grand Old Par. How that was chosen I have no idea. Clint was always quick to opine. “Ne Plus Ultra” was renamed “Nae So Good.” The club disintegrated when Wold left for Illinois and MacDonald for Germany, but Clint kept all the empties and had them on display on a top shelf in his lab.

One of my recollections that captures Clint’s persona was his response to a request to review a new issue of *Advances in Carbohydrate Chemistry and Biochemistry* around 1957. He



Figure 5 Clint Ballou and Dorothy Lun Wu on Clint’s 80th birthday. Photo courtesy of Linda Ballou and Philip Ballou.

found some errors in one of the articles and said he was considering how to phrase his review. He noted, “The volume is nicely bound and will fill the space between the previous volume and the next perfectly,” but after that the review was to go downhill. Whether he did it or not I don’t know, but the thought was in keeping with his general attitude to matters of no great import.

With Clint I enjoyed many good meals. He liked good food and was pretty adventurous. He liked French food or at least things cooked in butter. He was also a big fan of Foie gras and the wine suitable to take with it. We often shared a meal or two during scientific meetings. One evening we went with Wold and a couple of others to the Parthenon in Chicago. We ordered, got drinks, and waited for the meal. When Clint’s came it was half of a large sheep’s head. He said it was good. Sort of a combination feast and anatomy lesson.

Although we didn’t see each other often in the last of his years, I miss knowing that he is there.

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