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Our Work with Cyanogenic Plants

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Annu. Rev. Plant Biol. 2008. 59:1–19

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

This article's doi: 10.1146/annurev.arplant.59.032607.092924

1543-5008/08/0602-0001$20.00

Key Words

cyanogenesis, biosynthesis, localization, compartmentation

Abstract

The author identifies three individuals who played major roles in the development of his scientific career: his chemistry professor at the University of Colorado, Reuben Gustavson; his Ph.D. supervisor at the University of Chicago, Birgit Vennesland; and his friend and departmental colleague of 55 years at the University of California, Paul Stumpf. He also mentions students, postdoctoral scholars, and professional colleagues he encountered during his career of nearly 50 years as a plant biochemist. Finally, the article describes the author's research on cyanogenic plants. These plants contain hydrogen cyanide in a bound form that is usually released when the plant tissue is macerated. Cyanogenic plants contain cyanogenic glycosides in which the hydroxyl groups of cyanohydrins (α-hydroxynitriles) of aldehydes or ketones are covalently linked to a sugar, usually D-glucose. The biosynthesis, localization, and degradation, by hydrolysis, of these compounds have been examined, especially in sorghum and flax seedlings.

Contents

PREFACE

In a prefatory chapter in this series Ian Sussex (37) commented on how "seemingly chance" events influenced his career as a biologist, and that chance is frequently cited as a major influence on the careers of many scientists. The reader of this article can certainly see where chance events clearly shaped my career, especially in the early years. Patrick J. Hannan (18) authored an interesting book on this subject, in which he attributes a large proportion of such chance events to serendipity. Because the online edition of the Merriam-Webster dictionary defines serendipity as "the gift of finding valuable or agreeable things not sought for," the reader should perhaps conclude that chance, more than serendipity, was responsible for most of the events that resulted in the initiation of my career in science. Later, when my career goals were clarified, serendipity, as defined above, may have occasionally played a role in one or two discoveries.

The invitation to write a prefatory article for the *Annual Review of Plant Biology* probably affords many of the plant scientists so honored their first opportunity to put reminiscences of their careers into writing. I am a possible exception because a few years ago Peter Facchini, then secretary of the Phytochemical Society of North America (PSNA) and editor of its newsletter, invited me to write about my career as a plant biochemist. The PSNA is a small organization of scientists especially interested in plant natural products, and the newsletter is distributed to its 300–400 members. I became a member shortly after the PSNA was formed approximately 45 years ago, and for many years I served as the editor of *Recent Advances in Phytochemistry*, which contains papers presented at symposia held at the annual meetings of the Society.

I enjoyed responding to Peter's invitation and tried to produce an interesting article for the newsletter. Now faced with writing the prefatory chapter for this volume, I find that several things I want to include here previously appeared in the PSNA newsletter (13). Rather than trying to restate or rewrite that material, I have arranged for permission to use different parts of that article and acknowledge the original source of the material.

THE EARLY YEARS

I was born in Berthoud, Colorado on January 6, 1923, the fourth son and last child of William and Mary Anna Conn. My father was the assistant manager of a Farmers' Union grain elevator in that small town north of Denver. During the 1920s and 1930s my father managed and/or owned grain elevators in Nebraska and Kansas; he and my mother had started married life on a Kansas farm in 1910. Because my youngest sibling was seven years older than me, in many ways I lived the life of an only child. By the time I started high school, my siblings had all married and started their families. Although our lives have been very different, I remain close to my brothers and their families.

I grew up in a small town in Kansas in the midst of the Great Depression, where my family experienced the dust bowl years and their effects on the Great Plains. When I was seven, my mother arranged for me to take piano lessons. I mention this because that ability, which was not outstanding, nevertheless

opened many doors to me socially years later. After my parents lost most of their assets except for their home during the Depression, we moved to Fort Morgan, Colorado. That small town of 5,000 had an excellent high school where I did well academically. I had several outstanding high school teachers, one who taught English Literature, and one who taught Advanced Latin (Cicero and Virgil!) to another student and me. Another fine teacher gave an excellent course in American Problems, a course on contemporary American events as they were being influenced by world affairs, especially in Europe in the late thirties. These women (no men here) simply took it for granted that as the class valedictorian I would go to the University of Colorado at Boulder, and encouraged me in that regard. I won an all-tuition scholarship for four years to study there. Although none of my brothers had been able to go to college, my parents encouraged me to do so.

THE BOULDER YEARS

A close friend, who had already spent a year at Boulder, urged me to visit the chemistry department there in the spring of 1940. My friend arranged an interview with a professor of chemistry, Dr. Reuben Gustavson (**Figure 1**), who would eventually have a major influence on my career. Dr. Gustavson suggested that I consider majoring in chemistry, which I declared when I enrolled that fall. Although Dr. Gustavson was the chair of the department, he also was my professor in general chemistry, and there I had my first exposure to an outstanding lecturer. Dr. Gus would enter the lecture room with his notes (a few 3 × 5 cards), and deliver highly organized lectures, filling the chalkboard with structures and equations. Moreover, he soon knew the names of most of the hundred or so students in the course, and after a few days started calling on us by name to answer questions and write and balance chemical equations, all in a nonthreatening way. His performance strongly influenced my determination,

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Figure 1

Dr. Gus was Professor of Chemistry at the University of Colorado when I entered Boulder in 1940. He was an important mentor for me until I entered graduate school at the University of Chicago in 1946. From 1942 to 1959, he held administrative positions at several institutions (Dean and Acting President, University of Colorado; Vice-President and Dean of Faculties, University of Chicago; President, University of Nebraska; Administrator for the Ford Foundation). In 1959 he retired to Arizona where he passed away in 1974.

years later, to perfect my lecturing style and make teaching an important aspect of my academic career.

Courses in analytical and organic chemistry followed; up to this point I had made no overt decision to concentrate on biochemistry. However, Dr. Gus was a steroid chemist—this was before the era of metabolism in biochemistry—and his biochemistry course emphasized sterol structures, chemistry, and their physiological role, if known, in animals. Only a few references to glycolysis and the dicarboxylic acid cycle of Szent-Györgyi were made. Krebs' first detailed review on

the tricarboxylic acid cycle had not yet appeared.

During my last two undergraduate years (1942–1944) I served as a teaching assistant in various chemistry courses. Most of the other undergraduate males had enrolled in Army or Navy training programs, which readily guaranteed their military service during the war. Dr. Gustavson arranged for me to be hired by the Manhattan Project as soon as I graduated, and I caught a train for Oak Ridge. There I worked primarily as an inorganic chemist. My first publication, coauthored of course, was on the half-life of an isotope of nickel produced in the experimental, plutonium-producing uranium pile at Site X-10 (5). As the war wound down, I realized that many of my friends in Oak Ridge were planning to go, or return, to graduate school, and I incorporated that objective into my immediate plans. As I look back over those years now, I realize how much was left to chance, at least in my case.

THE CHICAGO YEARS

I again relied on Dr. Gus for advice when planning for gradaute study. He was now Vice-President and Dean of Faculties at the University of Chicago, and I stopped in Chicago to see him on a trip to Denver in December 1945, when he advised me to apply to the Biochemistry Department there. I applied to Chicago, as well as to Harvard, and when the offer of a teaching assistantship arrived from Chicago (one day before a similar offer from Harvard appeared in the mail), I accepted. At this point I officially launched my career in biochemistry at the University of Chicago.

Figure 2

Birgit Vennesland and Eric Conn in her garden in Berlin-Dahlem, near her laboratory, Forstellung Vennesland, a research unit of the Max-Planck Society.

GRADUATE WORK

Professor Birgit Vennesland (**Figure 2**) was my Ph.D. supervisor, and because she was interested in carbon dioxide fixation as mediated by malic enzyme and other such dark fixation enzymes, I was exposed to higher plants as experimental material. The University of Chicago was an exciting place in those years, and the biochemistry faculty included Konrad Bloch and Albert Lehninger; Frank Westheimer and Henry Taube were nearby in the Department of Chemistry. My entire first year in the Vennesland lab was spent in an attempt to isolate Coenzyme II or triphosphopyridine nucleotide (TPN), as NADP was called in those days, from 50 pounds of hog liver. I was given a procedure authored by Otto Warburg, the discoverer of TPN, that had been brought to the United States by his technician Erwin Hass who had to leave Nazi Germany because he was Jewish. The procedure involved such steps as precipitation of the coenzyme as a mercury salt, and then removal of the mercury with hydrogen sulfide. The first attempt failed; the second resulted in approximately 150 mg of fairly pure TPN. To follow the yield of the coenzyme during purification, I used a Warburg manometer to measure the TPN-dependent rate of glucose-6-phosphate oxidation in the presence of glucose-6-phosphate dehydrogenase (*Zwischenferment)* and "old yellow enzyme." We had to obtain both enzymes from spent brewer's yeast, which necessitated visits to a brewery on Halsted Street behind the Chicago stockyards to get gallons of spent

yeast suspension and haul it back to Abbot Hall in several milk cans. Back at the lab, the yeast was centrifuged down and spread out on lab benches to dry and be processed. All the students in the graduate lab knew where I had been and enjoyed the aroma of the drying yeast. When the coenzyme was sufficiently pure, it could then be assayed by the characteristic absorption of the reduced form at 340 nm. As I recall, our preparation was approximately 70% pure after some additional purification with a makeshift counter-current machine. I mention all this because it was a time when biochemical research involved a lot of effort just to obtain the reagents needed.

At that point, Vennesland's laboratory was the sole source of TPN in the country, and she generously provided a few milligrams to several groups, including Severo Ochoa and his young postdoctoral scholar Arthur Kornberg, who were investigating the role of malic enzyme in animal tissues. Vennesland was certainly aware of the effort that I and another of her students had made in isolating and purifying that TPN, and clearly conserved the supply. I do not recall ever doing another TPN prep there but someone must have done so after I left Chicago, because Vennesland's laboratory was involved in plant enzymology until she left for Germany in 1968. I had the pleasure of coauthoring a biography of Birgit Vennesland that is located in the section on Women Pioneers in Plant Biology on the web site of the American Society of Plant Biologists (ASPB) (**http://www.aspb.org/committees/women/pioneers.cfm#Birgit%20Vennesland**).

POSTDOCTORAL WORK

After I finished my Ph.D., I stayed on at Chicago for two years at the request of Dr. Vennesland, who wanted someone to help her graduate students while she was on sabbatical leave. I participated in several research projects and acquired some expertise in supervising less-experienced students. I also taught introductory biochemistry in

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Figure 3

Helen Stafford joined the Biology Department of Reed College in 1954 and became Emeritus in 1987. At that widely respected undergraduate college, Helen established a highly productive research program in plant biology that was supported by the National Science Foundation.

the Bio-Sci Survey sequence of five courses in the University College. Helen Stafford (**Figure 3**), who had come from David Goddard's lab to work with Vennesland, taught the plant biology quarter in that survey sequence. This course gave Helen and me valuable teaching experience when we moved on to our subsequent careers at Reed College and Berkeley, respectively. Helen established an outstanding research program (funded for many years by NSF) at that excellent undergraduate college, and I authored her biography for the ASPB web site (**http://www.aspb.org/committees/women/pioneers.cfm#Helen%20Stafford**).

One of the projects that I was privileged to participate in during that period went on to become textbook material. This was the thesis research of Harvey Fisher, a graduate student of Vennesland's, who showed that the two hydrogen atoms on the carbon

atom at the 4-position in the dihydropyridine ring of reduced diphosphopyridine nucleotide (DPNH, also known as NADH) are nonequivalent enzymatically, and that alcohol dehydrogenase stereospecifically transfers hydrogen, as a hydride ion, between the reduced substrate (deutero-ethanol) and the oxidized NAD (14). The report of this work was recognized as a classic article by the editors of the *Journal of Biological Chemistry* (35) during its centennial celebration a few years ago. This spring, the American Chemical Society awarded one of its Citations for Chemical Breakthroughs Award program to that paper (39), and installed a plaque in the chemistry department at the University of Chicago. Sadly, Birgit Vennesland, who died in 2001, did not live to see this recognition. Frank Westheimer, who advised Fisher on the chemical syntheses of the deuterated substrates as well as the mechanistic interpretation of the hydride transfer, was able to enjoy the latter acknowledgment a few weeks before his death in 2007.

In addition to providing the highly purified alcohol dehydrogenase used in the first experiments, I also provided emotional support for Harvey Fisher during the early morning hours that he was permitted to use the mass spectrometer in the chemistry department, because chemistry department projects involving mass spectrometry had priority. I recall that Vennesland had instructed us to call her with the results from a particular experiment, no matter what the hour. The results that evening clearly showed that when NADH containing one atom of deuterium (i.e., NADD) is reoxidized by alcohol dehydrogenase in the presence of acetaldehyde, all the deuterium is removed from the reduced coenzyme, which shows that the single deuterium atom in NADD is sterospecifically removed. Our phone call to Vennesland occurred at approximately 3 AM that morning!

Birgit Vennesland was especially thoughtful in introducing her graduate students and postdoctoral scholars to the numerous visitors who came to her laboratory during those years. Usually, these introductions occurred at dinners held in her apartment in University housing on the south side of the Midway. In retrospect I realize that I failed to appreciate the fact that Birgit Vennesland had already achieved an international reputation at that time. In later years, I encountered many of those visitors in my own career. For example, it was at Vennesland's house that I first met Paul Stumpf, as well as the Australian plant physiologist Bob (Rutherford Ness) Robertson, who later hosted me and my family in Adelaide when we were on sabbatical in New Zealand and were visiting Australia. I also recall that Vennesland introduced me to Alan Johns, subsequently director of the Department of Scientific and Industrial Research (DSIR) lab in New Zealand, where I spent my sabbatical in Graham Butler's lab in 1965–66. And of course, I met Severo Ochoa and Arthur Kornberg when they came to discuss the role of malic enzyme in intermediary metabolism in plants and animals.

When Alexander Todd, professor of chemistry at Cambridge, visited the department, I probably made an unfavorable impression—I was asked to oversee the projection of his seminar slides and found that they did not fit the department's projector. I do not recall his specific reaction, and whether it was directed toward me, but I had an opportunity to remind him of that event during my first sabbatical in Cambridge. Our landlady, who rented her ground floor apartment to my wife and me, asked us to join her for sherry one evening, during which she wanted to introduce me to a "biochemical friend" of hers. The friend turned out to be Todd, now Lord Todd, after his recognition for his chemical syntheses of natural products.

Another visitor to Vennesland's laboratory was Daniel Arnon, a member of Dennis Hoagland's famous Department of Soils and Plant Nutrition in Berkeley. Arnon was in Chicago while I was making plans to accept James Bonner's invitation to visit his group at the California Institute of Technology (Caltech) at their expense, ostensibly to

be considered for a postdoctoral position. Before I left on the California trip, Arnon called from California to tell me about a tenure-track opening for a plant biochemist in the Hoagland department, and he invited me to spend a few days there on my way to Pasadena. In those days, I had the time and interest to travel by train and so I routed myself out through Denver, where my parents were living at that time, on to Berkeley, down the coast to Los Angeles and then back through Salt Lake City and Denver to Chicago. I presented seminars in both Berkeley and Pasadena, but I do not recall the material I presented.

The open position at Berkeley was to replace Professor J. P. Bennett, whose research field was dormancy in plants. Arnon and Perry Stout, chair of the department at that time, made it clear that this was an independent position and that I would have to undergo the usual procedures for promotion from instructor through assistant professorship to tenure. That comment was both attractive and challenging. At Caltech, James Bonner's group consisted of numerous people who went on to make their marks in plant physiology and biochemistry, including Bob Bandurski, Arthur Galston, Adele Millard, and Paul Saltman. Barney Axelrod was also at Caltech on leave from the USDA lab in Albany, California. At that time, Anton Lange, as director of the phytotron at Caltech, enriched the plant biology community on that campus. I spent a week in Pasadena because one of my older brothers was living there at the time and I was able to spend evenings with him and his family.

The week in Pasadena was stimulating and very enjoyable, but James emphasized that Caltech had few permanent positions in plant biology and that most of the people in his group would go on to other institutions after they spent several years with him. By the time I got back to Chicago, I had job offers from both institutions; the pay at Berkeley would be $3600 for 12 months, and the pay at Caltech would be $5000. After advice from Vennesland and some thought on my part, I decided to accept the position at Berkeley. I also had to explain my decision to my father by telephone, who probably had never earned more than the lesser salary during his lifetime. I hope he understood, because two weeks later he died from a massive heart attack. Years later when I met James Bonner at meetings, he would jokingly bring up the subject of Caltech paying for my interview at Berkeley.

THE BERKELEY YEARS

Because I was hired to replace a faculty member who had worked on dormancy, some members of the Department of Soils and Plant Nutrition expected me to start working in that area. I lacked any background in plant physiology, so I knew it was unrealistic for me to develop a research program in that area. I took my concern to Perry Stout, the chair of the department. Stout stressed that I had been hired because of my biochemical expertise, and that I was expected to progress by demonstrating my skills in that area, although any biochemical collaboration that might develop with other members of the department would be welcomed. I was assigned space in a basement laboratory in Hoagland Hall where there was a cold room available, as well as an early version of the table-top Serval centrifuge with its cage. Eventually a paper appeared on oxidative phosphorylation by mitochondria from etiolated white lupine seedlings, followed by others on other biochemical activities of plant mitochondria. Arnon was working on photosynthetic phosphorylation in the department, but Bob Whatley was a postdoctoral scholar in his group at that time and there was little need for my biochemical expertise. Because at least two other groups independently observed photophosphorylation around then, I had the opportunity on more than one occasion to stress that Arnon's group had certainly made a similar observation independently.

In 1953, Stout was offered the directorship of the newly funded Kearny Foundation for Soil Science, and he wanted very much to have Connie Delwiche join him in the new

organization. However, Delwiche at that time was located in a new Department of Plant Biochemistry formed by taking Paul Stumpf, H. A. Barker, W. Z. Hassid, and Delwiche out of the Department of Soils and Plant Nutrition and moving them to the Biochemistry and Virus Laboratory on the opposite side of the campus. Now, a chance event occurred that had an enormous influence on my life. That department suggested a transfer of Delwiche into the Kearney foundation and my transfer to their department, so as to not lose a faculty position. Needless to say, when Paul Stumpf (**Figure 4**) proposed this exchange to me, it took me approximately three seconds to agree to the transfer.

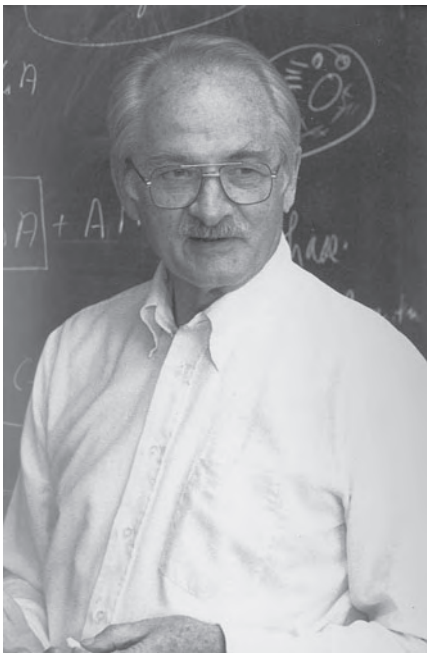

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Figure 4

Paul Karl Stumpf (1919–2007) Paul Stumpf was a distinguished professor of biochemistry at the University of California from 1948 until he became Emeritus in 1988. He founded the field of plant lipid biochemical research and was a mentor to many researchers in the field of oil seed biochemistry. After retirement, he directed the Competitive Grants Program at the U.S. Department of Agriculture from 1988 until 1991 and helped it develop into the National Research Initiative. He was my colleague and good friend for 55 years.

So, starting in the fall of 1954, I found myself a member of the small (four members) but unbelievably rich environment of the Department of Agricultural Biochemistry. Barker and Hassid were very senior scientists—already members of the National Academy of Sciences—and I had presented seminars on their research while still a graduate student at Chicago. Paul had also welcomed me to Berkeley when I first arrived. I was given Delwiche's laboratory and office next door to Paul's lab, and also was assigned to teach a one-semester biochemistry course for nonmajors.

After a few months of settling into this ideal situation, Paul gave me some friendly advice; he was nearer my age and was becoming my closest colleague in the department. Paul was in the process of initiating his lifelong work on lipid metabolism, and found it to be an exhilarating and rewarding experience after his previous work on glycolytic enzymes as a graduate student with David Green. His advice was to "take up a new field and show what you can accomplish."

I was fortunate to have Tsune Kosuge as my first graduate student; he had a master's degree in plant pathology from Washington State University, and wanted to study plant biochemistry to apply that discipline to plant pathology. Tsune came to Berkeley in 1955 hoping to work with Paul Stumpf, but because Paul was going to Bernie Horecker's lab at the National Institutes of Health (NIH) on his first sabbatical leave, Paul graciously suggested that Tsune work with me. Tsune had presented a seminar on the role of coumarin in the formation of dicoumarol in spoiled sweet clover hay, studied by the legendary K. P. Link at the University of Wisconsin, and decided to investigate the enzymes involved in the biosynthesis of coumarin.

Tsune Kosuge's thesis research, and the papers that resulted from it, together with his work carried out after he joined the

Plant Pathology Department at UC Davis, is probably the first example of a multistep biosynthetic pathway for a plant natural product described at the enzymatic level. Tsune's papers document the evidence that coumarin is formed in sweet clover as follows: phenylalanine → *trans*-cinnamic acid → *ortho*-coumaric acid → *trans-o*-coumaroyl-β-glucoside → *cis-o*-coumaroyl-β-glucoside → *cis-o*-coumaric acid (coumarinic acid) → coumarin. (Reference 13, p. 6)

The first evidence for the existence of phenylalanine ammonia lyase (PAL) occurred in Kosuge's thesis research when he observed the conversion of phenylalanine to *trans*-cinnamic acid in dialyzed extracts of sweet clover. These experiments were eventually discussed with Arthur Neish, a distinguished Canadian plant biochemist who had already created an impressive body of work on the biosynthesis of lignin from phenylalanine and tyrosine. In a review (29), Art proposed that *trans*-cinnamic acid might be formed by transamination of phenylalanine, followed by reduction of the keto acid to phenyllactic acid, and dehydration to form cinnamic acid. The first two reactions would presumably require stoichiometric amounts of keto acids and reducing agents (DPNH or TPNH) to accomplish the overall conversion. Because Tsune's dialyzed extracts catalyzed the formation of readily detectable amounts of cinnamic acid from phenylalanine, we believed there had to be another reaction. We proposed a single enzyme that catalyzes deamination, analogous to bacterial aspartase.

In 1958 I visited Art at the Prairie Regional Lab in Saskatoon. (Reference 13, p. 7). We discussed the possibility of a simple one-step deamination of phenylalanine and I wound up inviting Art to spend his upcoming study leave in Davis. I also met Stewart Brown on that trip and we have been good friends and correspondents since those days. Stew was recently recognized for his many contributions to the lignin and coumarin literature (4). The Neishs arrived in Davis in September, 1959, and Art started looking for the deamination of tyrosine (in grasses) and phenylalanine in a wider range of species.

Art brought his supply of labeled compounds as standards, and we provided our new lab facilities for enzyme work, as well as access to Kosuge, who was located in an adjacent department, for moral support. Art started looking for tyrosine ammonia lyase (TAL) in rice and other grasses. Jane Koukol, a postdoctoral scholar from Vennesland's lab, arrived and started working on PAL, initially in sweet clover and then in barley. Eventually their work on TAL (30) and PAL (20) was published in two different journals. We had decided to call these enzymes tyrase and phenylalanase, similar to the enzyme named aspartase. However, the editors at the *Journal of Biological Chemistry* (*JBC*) insisted that Jane and I adhere to the newly agreed-upon nomenclature rules and use the term phenylalanine deaminase for PAL. The term phenylalanine ammonia lyase reflects a later change in the nomenclature of deaminases. (Reference 13, p. 7)

People ask occasionally about my lack of follow-up on the discovery of PAL as the enzyme gained extraordinary prominence in plant secondary metabolism. Because it was Tsune's finding initially, I urged that he continue working with PAL. However, he was keen to wind up his work on coumarin and start looking at problems in plant pathology that might be amenable to solutions using biochemical approaches. He did just that, in such quantity and quality, that he was elected to our National Academy of Sciences in 1988. Tragically, this was shortly before he died prematurely from colon cancer. Later I learned that he was told a few days before his death that both of us were elected that year. As Tsune took leadership on the coumarin problem in Berkeley, my interests turned to the biochemistry of cyanogenic glycosides. (Reference 13, p. 7)

I had read a review of natural products by Geissman & Hinreiner (16) that noted that many natural compounds consist of an aromatic ring and a side chain of three carbon

atoms (i.e., C_6-C_3 phenylpropanes), as well as an aromatic ring and a side chain of a single carbon atom (i.e., C_6-C_1 phenylmethanes), but that C_6-C_2 compounds are far fewer in natural occurrence. Because the cyanogenic glycosides are of the latter type, and also have the triple-bonded nitrile group, I thought these substances might show some interesting enzymology; I therefore concentrated my efforts and limited resources on the study of those compounds.

With the help of Takashi Akazawa, a young graduate student from Ikuzo Uritani's laboratory in Japan, we soon showed that sorghum seedlings should be an ideal tissue in which to study the biosynthesis of dhurrin, the β-glucoside of *p*-hydroxy-(*S*)-mandelonitrile, the cyanogen in sorghum (1). Although sorghum seed contains only traces of dhurrin, the 3- to 5-day-old etiolated seedlings contain approximately 5–10% dhurrin on a dry weight basis. Because tyrosine was the obvious precursor, we fed ^{14}C tyrosine to the seedlings overnight and observed a relatively large incorporation (5–10% of the activity fed) into the glycoside. (Reference 13, p. 7)

We reported these results at the Federation Meetings in April 1948, and were surprised, and somewhat chagrined, to hear John Gander from the University of Minnesota describe almost the same experiments in the following presentation. Because John had the next summer free, I invited him out to Berkeley where we worked hard to identify intermediates between the amino acid fed and the cyanogen. With the large amount of incorporation observed, we both thought that it would be a simple matter to find a few spots on paper chromatograms, identify them, and sort out the pathway. That was not the case, and it eventually took another decade to establish the identities of the intermediates. At the end of the summer John returned home and continued to work on the problem for a few months. But, because success was slow and he had other interests, he stopped working on the problem. At this point, I decided on a dual approach, namely (*a*) to determine whether the tyrosine molecule was incorporated into dhurrin intact except for loss of the carboxyl carbon, and (*b*) to postulate possible intermediates, label them with C^{14}, and feed them to seedlings. However, progress was hindered by my move to the Davis campus in the fall of 1958 and a delayed sabbatical in England in 1960. (Reference 13, p. 7)

I had applied for and received a 3-year U.S. Public Health Service (USPHS) grant in 1957 (for $12,000 per year) entitled "Metabolism of Aromatic Compounds in Plants." It was subsequently renewed in 5-year intervals for a total of 29 years; the final year's amount was $120,000, some of which had to accommodate inflation over those three decades. Approximately 15 years into that period, when the NIH decided that it would no longer fund plant research, someone at the NIH deleted the last two words in the grant title. I wanted to complain about that action, but because I had some people on salary in that grant, I took the coward's way out, and accepted the renewal. In 1973 the National Science Foundation (NSF) also started funding our research, usually in 3-year grants. (Reference 13, p. 8)

In the mid 1950s, the Regents of the University of California approved a major expansion of the university system that included conversion of the Davis campus to a general campus. Paul Stumpf was asked to set up a general biochemistry department in Davis, and I was invited to go with him. We were attracted by the prominence of plant sciences at Davis and the fact that the campus was entering a rapid period of growth. So, in September 1958, Paul and I moved to Davis to initiate our teaching program. We left our students in Berkeley, because the new department's facilities would not be ready until the following summer. (Reference 13, p. 8)

THE DAVIS YEARS

These years started with my marriage to Louise Kachel, a friend from my Chicago days, on October 17, 1959, a few months

before we departed for my sabbatical in England. I spent my postponed sabbatical leave at the Low Temperature Station in Cambridge, England with Tony Swain; I hoped to learn more about plant phenolics. I did a few experiments on a project of Swain's and we published a brief note indicating that gallic acid can also be formed without phenylalanine as an intermediate (8). I met Tony's supervisor, the fabled E. C. Bate-Smith, as well as Jeff Harborne, Arthur Bell, Leslie Fowden, and Trevor Goodwin, among others, and attended an early meeting of the Phytochemical Society. These contacts resulted eventually in visits by Fowden and Goodwin to Davis, where they taught in our graduate course in plant biochemistry when either Paul Stumpf or myself was absent on sabbatical. Another fortuitous event in Tony's lab was a visit from Alan Johns, a New Zealander I had met in Chicago years earlier. I invited Alan to our flat for dinner and learned that evening that Graham Butler was working on cyanogenesis in flax seedlings in Johns' department in Palmerston North. I immediately initiated correspondence with Graham that resulted in his spending a year's leave in Davis with us in 1961–1962, and I eventually took my next sabbatical in his lab. (Reference 13, p. 8)

Louise and I left England in June 1960 to spend our summer vacation on the continent; we visited friends she had made while working in Paris during the four years immediately preceding our marriage in 1959. In Germany I called on Hans Grisebach in Freiburg; this was the first of numerous visits in later years to that beautiful city. I had met Hans in Berkeley when he was a postdoctoral scholar with Melvin Calvin, gaining experience in the use of radioisotopes in the study of metabolism. Hans often attended seminars in our department and was aware of Kosuge's enzyme studies on coumarin. When Hans returned to Freiburg he suggested that we keep in touch. In 1960 he was still a docent in the Chemistry Institute, but soon had his own institute where he trained an entire generation of plant biochemists. I have often lectured in our graduate plant biochemistry course on the elegant research done in Hans' Institute, and I am sorry that he did not live to see the many current applications of molecular biology to his field. I am also indebted to Hans for advising some of his students to obtain experience with enzymes in our group. The first person to do so was Klaus Hahlbrock, who came to Davis in 1967. (Reference 13, p. 8)

PHOTO CREDIT: UC DAVIS

Figure 5

Eric meeting with a student in his office at UC Davis.

Back in Davis, I was eager to continue with my teaching and research. Although I performed my share of service on university committees, my primary commitment was to the students and postdoctoral scholars in my lab, and the undergraduates in my courses (**Figure 5**). We made progress on the cyanogen problem; Jane Koukol showed that the carbon bond between the α and β carbons in the side chain of tyrosine was not severed during the conversion of the amino acid to the cyanogen. She also showed that the α carbon atom of tyrosine, labeled with ^{14}C, gives rise to labeled HCN, whereas the β carbon remains in the *p*-hydroxybenzaldehyde moiety of the cyanogen (21). Several years later, my graduate student Ernest Uribe, using tyrosines labeled with ^{14}C in the α position and ^{15}N in the amino nitrogen, showed that the bond between those two atoms is not severed during the synthesis of dhurrin (38). This important result meant that all intermediates in

the pathway must contain nitrogen. However, we still did not know the nature of the intermediates. A later experiment by Harold Zilg from Freiburg showed that the glucosyl linkage oxygen atom is derived from molecular O_2 (40). This early result implicated a mixed function oxidase in this pathway. (Reference 13, pp. 8–9)

Graham Butler made several important contributions during his study leave in Davis. Because Gander had shown that dhurrin undergoes metabolic turnover in sorghum seedlings, we wondered whether this process involved the release of HCN in the atmosphere. In an off-the-wall experiment, Graham and Shula Blumenthal fed ^{14}C-labeled HCN to sorghum seedlings in a closed system for several hours and then examined plant extracts for any labeled products. To our great surprise, we observed a single, heavily labeled compound, which we quickly identified as asparagine (3). (Reference 13, p. 9) This experiment probably fits the definition of a serendipitous result. We hoped to find the incorporation of radioactivity into the cyanogenic glycoside dhurrin, and instead observed the intense labeling of asparagine.

Later work by Heinz Floss and Lee Hadwiger (15) and by Jackie Miller (26) showed that many, and probably all, plants contain a mitochondrial β-cyanoalanine synthase that catalyzes the replacement of the –SH group of cysteine with –CN to form β-cyanoalanine. Although we understood why cyanogenic species might have this enzyme, we were surprised to find that noncyanogenic species also contained the enzyme, albeit in lower amounts. Shang-fa Yang and his students (31) eventually solved this anomaly; they showed that one molecule of HCN is formed in the last step of ethylene biosynthesis when 1-aminocyclopropyl-1-carboxylic acid (ACC) is oxidized by ACC oxidase. They could not detect HCN in their experiments, but they showed that the ^{14}C-labeled carbon atom bearing the nitrogen atom in ACC is converted to labeled asparagine in roughly stoichiometric amounts (70% yield). (Reference 13, p. 9) Thus, any plant tissue that produces ethylene also produces an equivalent amount of HCN.

While in Davis, Graham demonstrated the remarkable efficiency of rootless shoots of flax seedlings in the conversion of labeled valine to the cyanogenic glucoside linamarin. The shoots could convert 35% of the labeled valine fed in experiments that lasted only seven hours! Intact seedlings converted, at most, 5% under similar conditions. Various experiments were also designed to detect intermediates in the conversion, but no intermediates were found. Experiments with ^{15}N showed that the nitrogen atom in valine is retained when valine is converted to linamarin. Because sorghum shoots are also much more effective in dhurrin biosynthesis than sorghum seedlings, the two experimental systems are relatively equivalent. When I was in Graham's group (1965–1966), we worked exclusively on flax. (Reference 13, p. 9)

Butler's student, Brian Tapper, was the first to show that oximes are intermediates in the biosynthesis of cyanogenic glucosides. A labeled compound with the properties of an oxime glycoside accumulates in flax seedlings fed ^{14}C-valine and O-methylthreonine, a metabolic inhibitor of valine. Brian prepared the oxime of ^{14}C-labeled isobutyraldehyde, administered it to flax shoots, and found that it is converted to linamarin nearly as efficiently as labeled valine; isobutyraldehyde is not converted. Because oximes can be dehydrated to form nitriles, and the latter might be oxygenated to form cyanohydrins, Brian's results suggested this biosynthetic sequence: amino acid → → aldoxime → nitrile → α-hydroxynitrile (i.e., cyanohydrin) → cyanogenic glycoside, all of which contain one nitrogen atom. (Reference 13, p. 9)

When Klaus Hahlbrock arrived in Davis, he prepared the nitriles and cyanohydrins that correspond to possible intermediates in the biosynthesis of linamarin and prunasin, the cyanogen derived from phenylalanine. When fed to appropriate tissues (flax shoots and petioles of cherry laurel leaves, respectively)

Klaus found that the nitriles are incorporated, although not as efficiently as the amino acids. The aliphatic cyanohydrin is readily converted to linamarin, but the aromatic cyanohydrin is toxic to the leaves. In a joint note with Tapper and Butler that described these findings (17), we put the plausible biosynthetic pathway stated above in writing for the first time. (Reference 13, p. 9)

Klaus soon demonstrated the last step in the pathway. He detected a uridine diphosphoglucose (UDPG)-ketone cyanohydrin glucosyltransferase in flax seedlings and purified it 120-fold, free from β-glucosidase activity. The enzyme is equally active on the cyanohydrins of acetone and butanone, forming linamarin and lotaustralin, respectively. In subsequent work, he concluded that the flax enzyme is responsible for formation of both cyanogenic glucosides in flax, a fact confirmed years later using cloned enzyme. Later Peter Reay (33) purified and characterized the glucosyltransferase in sorghum seedlings. (Reference 13, p. 9)

From 1966 on, research in my group centered on several different aspects of cyanogenesis, with two important exceptions. The first exception was work on cinnamic-4-hydroxylase (C4H) by David Russell. In his thesis research with Arthur Galston, David had studied the increase in kaemperferol derivatives mediated by phytochrome in pea seedlings. He proposed the use of microsomes from such tissue to look for C4H, and was quickly successful. In an important but seldom cited paper (34), David reported numerous properties of the enzyme, including its light-reversible inhibition by carbon monoxide (CO) and the feedback inhibition of its activity by the product, *p*-coumaric acid. David concluded that his enzyme had "all the properties of a P-450 type of cytochrome" and that this required studies of the action spectrum for light reversal of the CO inhibition. My graduate student Rowell Potts subsequently measured the spectrum (32). His spectrum, together with action spectra obtained in Charles West's lab in 1969 for two enzymes involved in gibberellin biosynthesis, were the only data of this kind concerning the many P-450s now demonstrated to exist in plants, before their expression in heterologous hosts became relatively common. Although I coauthored an earlier note in *Archives* with David, I insisted that he be the sole author on the *JBC* paper (34), because he had originally proposed the research and had been completely self-sufficient in his work in the lab. (Reference 13, pp. 9–10)

The other study that did not involve cyanogenesis concerned the role of the nonaromatic amino acid, arogenic acid, in the biosynthesis of tyrosine and phenylalanine in sorghum. Roy Jensen's earlier studies (19), showed that tyrosine is formed in mung bean, maize, and tobacco by transamination of prephenic acid to produce arogenic acid (pretyrosine), followed by oxidative aromatization of the six-membered ring to form tyrosine. Because of the large flow of carbon atoms into the cyanogenic glycoside dhurrin in sorghum, as well as the formation of many phenylpropanoid compounds in all plants, it seemed important to learn more about the final steps of phenylalanine and tyrosine biosynthesis in sorghum. Results obtained by Dan Siehl, Jim Connelley, and Bijay Singh established that the final steps of phenylalanine and tyrosine biosynthesis in sorghum clearly utilize the arogenic acid alternative described by Jensen and coworkers.

A major advance in the cyanogen biosynthetic work occurred when Ian McFarlane arrived from Michael Slaytor's lab in Sydney. By that time, aldoximes, nitriles, and α-hydroxynitriles were considered likely intermediates in cyanogen biosynthesis because of the results from feeding experiments. Because at that time numerous studies in animal tissues, and some in plants, had shown that microsomal enzyme systems catalyze C-hydroxylation reactions, I suggested to Ian that he determine if sorghum microsomes could catalyze the oxidation of *p*-hydroxyphenylacetonitrile to *p*-hydroxymandelonitrile in the presence of NADPH and oxygen. He isolated microsomes from

etiolated sorghum seedlings in the presence of thiol reagents, and in an early experiment he included tyrosine as a control, not expecting it to be acted upon. Astonishingly, Ian found that tyrosine was oxidized by the particles to form p-hydroxybenzaldehyde and HCN. Moreover, this reaction was more rapid than the hydroxylation of *p*-hydroxyphenylacetonitrile. The sorghum particles also utilized the aldoxime as a substrate about as well as they utilized the amino acid. The properties of this microsomal system were then extensively studied by Ian and Edith Lees; Edith was a faculty member on study leave from the University of Sydney (25). (Reference 13, p. 10)

Because the conversion of tyrosine to p-hydroxyphenylacetaldoxime constitutes a four-electron oxidative decarboxylation, it was likely that an intermediate existed in that conversion. Earlier work on glucosinolate biosynthesis in Ted Underhill's group in Saskatoon indicated N-hydroxyamino acids as intermediates between amino acids and aldoximes in the biosynthesis of glucosinolates. The next step was the synthesis of N-hydroxytyrosine (NHT). (Reference 13, p. 10)

Birger Moller arrived from Copenhagen, just as Ian and Edith were finishing up their work, and decided to work on this problem. He perfected a synthesis of NHT that Ian had initiated, and prepared the ^{14}C-labeled compound. He compared the efficacy of NHT as a substrate with five other compounds that, on paper, could be intermediates between tyrosine and its aldoxime. However, only NHT was metabolized to p-hydroxymandelonitrile by the particles. Moreover, labeled NHT was produced from [U-^{14}C]-tyrosine in tracer experiments when unlabeled NHT was added as a trap (27). Birger made another major contribution to the problem while in Davis; he showed that the biosynthetic sequence catalyzed by the microsomes is highly channeled (28). His experiments explain why, in the early work done in both Butler's and our lab, we never readily detected any intermediates. (Reference 13, p. 10)

Helen Stafford (36) was the first to propose metabolic channeling in the synthesis of natural products. Whereas Birger's paper met many of the criteria listed in Helen's review for a channeled system, research from his group in Copenhagen has gone on to show that the three enzymes involved in the biosynthesis form an organized complex or metabolon.

Work continued on cyanogenesis in plants until I retired in 1993, and many excellent students, postdoctoral scholars, and visiting scientists contributed to that effort. Over the years I have discussed their work in different reviews (9–13) and the interested reader can consult those papers. This research would not have been possible without the essential contributions of all the highly talented people whose work is described in those reviews.

I take great pleasure in the fact that Birger Moller has greatly developed the field of cyanogenesis in plants after his appointment to the Chair of Plant Biochemistry in the Royal Veterinary and Agricultural University in Copenhagen in 1985. He proposed returning to the cyanogenesis field at that time, and I strongly encouraged him to do so. Applying the tools of molecular biology to cyanogenesis and related problems, he and his group have greatly advanced our understanding of these compounds. The progress in Moller's group was recently reviewed (2).

CYANOGENESIS IN TWO LARGE PLANT FAMILIES, ACACIAS AND EUCALYPTUS

Starting in 1974, my research interests broadened significantly owing to a collaboration with David Seigler in the Plant Biology Department at the University of Illinois. At the 1972 PSNA meeting, we discussed the older literature that reported that some South African species of acacia contain cyanogens derived from valine and isoleucine, whereas cyanogens in Australian species are derived from phenylalanine. At that time this was the only example of aliphatic and aromatic cyanogens occurring in the same genus.

David, who teaches plant taxonomy at the University of Illinois, educated me on the taxonomic complexity of the genus, and we decided to check out the earlier work. After confirming those studies, I started testing acacias in the UC Davis arboretum for cyanogenesis, as well as testing herbarium specimens of the species. Leaf material of any positive species was then obtained, extracted, and worked up to identify the cyanogen. This project resulted in the identification of several new cyanogenic glycosides (**Figure 6**). (Reference 13, p. 11)

While testing acacias in California gardens, I also started looking at eucalyptus. Because there was only one documented report of cyanogenesis in that large genus—prunasin in *E. cladocalyx*—I reasoned there should be some additional cyanogenic species of *Eucalyptus*. Initially I found two additional cyanogenic species in California, and this finding led to my last sabbatical in Australia in 1981–1982. (Reference 13, p. 11) My family and I had an apartment in Adelaide, where I had space in Brian Coombe's laboratory at the Waite Institute. I had met Brian in 1955 when he was getting his Ph.D. in plant physiology at Davis.

The Waite Institute has over 300 species of eucalyptus in their botanic garden and that kept me occupied for several weeks. We then started visiting botanic gardens in Adelaide, Brisbane, Canberra, Melbourne, Hobart, Sydney, and Perth, where I arranged for permission to test the eucalyptus and acacia species in those gardens for cyanogenesis. Except for the gardens in Canberra and Perth, I was surprised to see that the other gardens usually had more specimens of northern hemispheric plants than Australian plants. However, Canberra's National Botanic Garden is restricted to Australian species and proved especially useful. (Reference 13, p. 11)

While in Perth, I met Bruce Maslin (**Figure 7**), an authority on the 1000-plus Australian species of acacia, approximately half of which are native to Western Australia. Bruce was interested in my project, and I mentioned one species I had decided was

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Figure 6

Eric Conn admiring Acacia blossoms in the Louise and Eric Conn Acacia Grove in the UC Davis Arboretum.

Figure 7

Bruce Maslin works at the Western Australia Herbarium in South Perth. He is an internationally recognized authority on the systematics (taxonomy) of acacias and continues to find and describe new species of the genus Acacia, especially in Western Australia. Bruce played a major role in establishing an internet site called the WorldWideWattle.com that contains much information regarding a group of related genera (*Acacia*, *Senegalia*, and *Vachellia*) that are spread around the world. (Wattle is Aussie-speak for acacia.)

cyanogenic after the results of earlier tests in several gardens. Because of his taxonomic knowledge of the genus, he quickly named several related species and produced herbarium specimens, which we tested. During that first afternoon, we identified more cyanogenic species than I had managed to find during several months of work in gardens! Such results from herbarium specimens require that live species be located in the field, tested for cyanogenesis, and if positive, sampled and processed for identification of the cyanogen. Our results led to several collecting trips with Bruce in subsequent years. (Reference 13, p. 11)

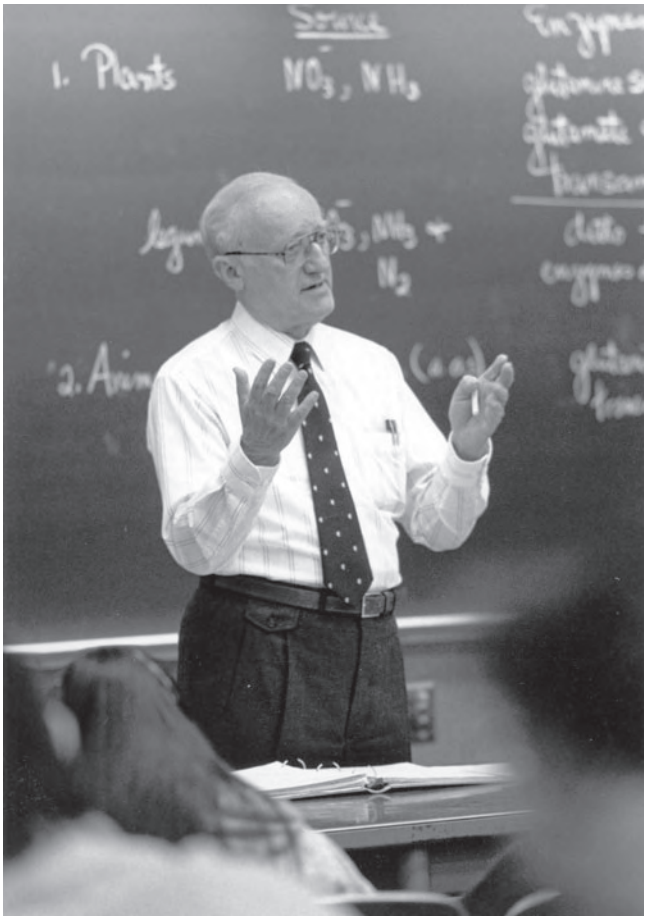

PHOTO CREDIT: UC DAVIS

Figure 8

Eric Conn lecturing to the undergraduate course in General Biochemistry at UC Davis. Courtesy of the University of California at Davis.

Papers in *Phytochemistry* (7, 23), *Kingia* (22), and *Western Australian Herbarium Research Notes* (6) present the results of tests on 96% of the described species of the genus *Acacia*. This work showed that cyanogenic species in the pantropical subgenus *Acacia*, found mainly outside Australia, contain only aliphatic cyanogens, whereas cyanogenic species in the two other subgenera, *Phyllodineae* and *Aculeiferum*, contain aromatic cyanogens. These data support traditional taxonomic evidence that the pantropical subgenus formerly known as *Aculeiferum* (species of this group are now called *Senegalia*, *Mariosousa*, or *Acaciella*) is more closely related to the predominantly Australian subgenus *Phyllodineae* (to which the name *Acacia* is now restricted) than to the pantropical subgenus *Acacia*, now called the genus *Vachellia* (Reference 13, p. 11). Maslin discussed the recent changes in classification of acacias in Reference 24.

The work on eucalyptus involved testing approximately 1400 individual plants and herbarium specimens, which represents 348 species, 22 of which were cyanogenic. (*R*)-prunasin, derived from phenylalanine, was identified as the cyanogen in 10 of those species.

TEACHING

Because Paul Stumpf and I had taught the one-semester biochemistry survey course in Berkeley, we designed a more comprehensive but still introductory course and offered it on the Davis campus. Approximately 60 students enrolled in the course in the fall of 1958. The following fall, there were over 100 students. By the end of the 1960s, I was lecturing to classes approaching 400 students. The course became a requirement for numerous undergraduate majors at Davis, and also attracted graduate students who needed biochemistry in their research. We were surprised that this course attracted more students on a campus of four or five thousand than a similar course at UC Berkeley, with its enrollment of 20,000. We attributed this interest to the large amount

of activity in various biological sciences at Davis, primarily in the College of Agriculture. The increase in course size over the years was the primary reason that allowed us to hire eight or nine additional faculty and eventually offer a well-rounded selection of biochemistry courses (**Figure 8**).

That course also led to the writing of our introductory textbook, *Outlines of Biochemistry*. The first edition appeared in 1963 and went though five editions before we "laid it down." *Outlines of Biochemistry* was the first brief biochemistry text that emphasized the principles of intermediary metabolism and did not attempt to be encyclopedic. However, as plant biochemists we made sure to include chapters on photosynthesis and nitrogen fixation that were notoriously lacking in texts written for medical schools. When I returned from my sabbatical in England, Paul and I offered a graduate course in plant biochemistry in which approximately 75–100 students enrolled for many years.

I mentioned above my admiration of Dr. Gus, my first chemistry professor at Boulder, and how I resolved to model my teaching style after his. I apparently was successful in doing so, for I received the Davis Academic Senate teaching award in 1972, the second year it was offered. Later, when the campus established the UC Davis Prize for Undergraduate Teaching and Scholarly Research accompanied by $25,000, I was the recipient in the third year it was offered.

PHOTO CREDIT: UC DAVIS

Figure 8

Eric and Louise Conn in the garden of their home in Davis.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Little of this research would have been accomplished without the contributions of the graduate students, postdoctoral scholars, and visiting scientists who collaborated with me over the years. I consider myself extremely fortunate to have been able to attract talented individuals who became both valued scientific colleagues and also treasured friends. I also acknowledge grants from the NSF for 16 years and from the USPHS for 29 years. I also wish to acknowledge the support of my dear wife Louise during the 43 years of our marriage (**Figure 9**). She contributed immeasurably by helping the postdoctoral scholars and scientists and their families settle into life in Davis on their arrival, entertaining them in our home on numerous occasions, and especially on Thanksgiving Day. Louise believed Thanksgiving was a typical American holiday and wanted to share the experience with visitors from overseas. My wife was not a scientist, but her social skills were superb. Sadly, Louise died in March 2002, leaving a great void for my two sons and me.

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