PSNA NEWS

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PRESIDENT'S LETTER

Invitation to Peoria

In August, our Society will have its annual meeting in the city of Peoria, Illinois. This year's topic is the use of model systems to study secondary metabolism. As in recent years, the organizers chose a hot and important topic for phytochemists. At the beginning of any research project or career questions emerge about the appropriate model system to study a specific problem. In fact, we use several model plants for studies in biochemistry, molecular and cell biology, including *Arabidopsis thaliana, Nicotiana tabacum, Zea mays, Oryza sativa*, and others. The genome of some of these plants has been sequenced and some proteomes are on the way. Choosing a model is not an easy task, particularly in the case of secondary metabolism. During the 1980s and 1990s, several groups developed several models. *Catharanthus roseus*, *Lithospermum erythrorhizon*, *Coptis japonica* and several tropane alkaloid-producing plants, such as *Datura, Atropa*, *Duboisia* were among the most used models. Today many are no longer extensively used. Are there specific criteria one should consider when choosing a model system? Feasibility, of course, is an important one. This is the reason why *Arabidopsis* was adopted as a model. In several cases, choosing the model will depend on who is funding the research. This is not a trivial issue. Today, it is harder than ever to fund and maintain a productive research group. Often, granting agencies expect that the proposal reports *research objectives* as *preliminary results*. Few panels are disposed to fund novel ideas and high-risk projects. Thus, few researchers are able to take this risk. I know several top scientists that are working on *light* projects just to obtain funding and, in parallel, they perform more exciting, *risky* research. As a current member of a grant panel, my major questions are: (a) Is there an important question to answer? (b) Is there a contribution to knowledge? (c) Is the project leader well trained? (d) Does the project leader have a good track record? e) For young scientists, do they have the potential to carry out the proposed research? In reality, there is no single model system to use in the study of living organisms. A genetic approach using Arabidopsis might allow a breakthrough in one area, but a biochemical approach using a lily might make a greater contribution in another. The goal is to bring a diversity of approaches into play to solve fundamental plant problems (see R. Goldberg, Plant Cell, 8: 347, 1996). Plants share their metabolic pathways with many organisms. This is true in secondary metabolism even when the final product is different. Please come to Peoria and share the experience of the model you are working on.

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IN THIS ISSUE

CONTENTS

2003 PSNA Annual Meeting

Secondary Metabolism in Model Systems

August 9 - 13, 2003
Peoria, Illinois

The Phytochemical Society of North America (PSNA) is a nonprofit scientific organization whose membership is open to anyone with an interest in phytochemistry and the role of plant substances in related fields. Annual membership dues are U.S. $40 for regular members and $20 for student members. Annual meetings featuring symposium topics of current interest and contributed papers by conference participants are held throughout the United States, Canada, and Mexico. PSNA meetings provide participants with exposure to the cutting-edge research of prominent international scientists, but are still small enough to offer informality and intimacy that are conducive to the exchange of ideas. This newsletter is circulated to members to keep them informed of upcoming meetings and developments within the society, and to provide a forum for the exchange of information and ideas. If you would like additional information about the PSNA, or if you have material that you would like included in the newsletter, please contact the PSNA Secretary and Newsletter Editor. Annual dues and changes of address should be sent to the PSNA Treasurer. Also check the PSNA website at www.psna-online.org for regular updates.

PHYTOCHEMICAL PIONEERS

Eric Conn - 50 years of plant biochemistry

My career as a biochemist began when I started graduate work at the University of Chicago in September 1946. The subject was not unknown to me as I'd had a course in biochemistry during my last year as a chemistry major at the University of Colorado. I was assigned by the department chair to work with Professor Birgit Vennesland and since her interests were in *carbon dioxide fixation* as mediated by malic enzyme and other such *dark fixation* enzymes, I was quickly introduced to higher plants as experimental material. My first year in her lab was spent attempting to isolate Coenzyme II, or TPN^+, as $NADP^+$ was called in those days, from 50 pounds of hog liver! I followed a procedure published in 1935 by Warburg (the discoverer of TPN^+) in *Biochemische Zeitschrift*. The procedure involved such steps as precipitating the coenzyme as a mercury salt, and then removing the mercury with hydrogen sulfide; further purification as a barium salt, followed by removing the barium with sulfate! Not surprisingly the first attempt failed; the second resulted in about 150 milligrams of TPN^+ that was only 15% pure. Another student and I devised a makeshift *counter current* purification to increase its purity to 77%.

In order to follow the yield of the coenzyme during purification, I had to use the manometric procedure described by Warburg, again from *Biochemische Zeitschrift*, involving glucose-6-phosphte dehydrogenase and *old yellow enzyme*. Both enzymes were obtained from *spent brewer's yeast*, and this necessitated visits to a brewery on Halsted Street behind the Chicago stockyards to collect the yeast we needed. When the coenzyme was sufficiently pure, it could be easily assayed by the characteristic absorption of the reduced form at 340 nm (Vennesland's lab had a new Beckman DU spectrophotometer, commercially available only after the war; its power supply consisted of two 6-volt car batteries.) I mention all these things because this was a time when much biochemical research involved a lot of time and effort just to obtain the reagents needed for the experiments.

The University of Chicago was an exciting place in those years, and the Biochemistry Department had a distinguished faculty that included Vennesland, Konrad Bloch and Albert Lehninger, with Frank Westheimer

and Henry Taube nearby in the Department of Chemistry. The biochemistry graduate students at Chicago considered Vennesland the role model for faculty supervisors, and I was extremely fortunate to have been able to work with her. Her biography can be found on the Web site of the American Society of Plant Biology, where she is listed in section on *Women Pioneers in Plant Biology*. Bloch was deep into his work on cholesterol biosynthesis, for which he later received a Nobel Prize. Lehninger's students were studying the oxidation of pyruvic acid by animal mitochondria, and during that period, discovered that oxidative phosphorylation was coupled to the oxidation of DPNH (i.e. NADH) in animal mitochondria. All of this work was performed in a cold room in the basement of Abbot Hall that contained a tabletop Serval centrifuge that was enclosed inside a metal cage. On one occasion, the head came off that centrifuge while running and nearly demolished the interior of the cold room. Fortunately no one was injured as we were warned not to remain in the cold room when the fuge was operating. Lehninger was an outstanding lecturer and later wrote a classic text in biochemistry. His lecturing skills were just as good as his writing.

My thesis research was not particularly distinguished; it was entitled *Triphosphopyridine Nucleotide Enzymes in Higher Plants*, and mainly concerned the properties of malic enzyme in several species. The thesis also described the enzymatic reduction of oxidized glutathione in wheat germ extracts by TPNH. Since I had the only supply of TPN^+ in the United States, I was probably the first to learn that plant homogenates contain enzymes (nucleotidases) that degrade TPN^+ as well as oxidases that oxidize the reduced form, TPNH. This is not surprising now, but in those days we had little experience with this cofactor. While a crude preparation of Coenzyme I or DPN^+ (i.e. NAD^+) was commercially available from Pabst Brewing, I recall little published work on that cofactor at that time.

Vennesland's laboratory attracted many visitors at that time, and she arranged for them to meet her students. I recall in particular Hans Krebs, Severo Ochoa and his young post-doc Arthur Kornberg, Alexander

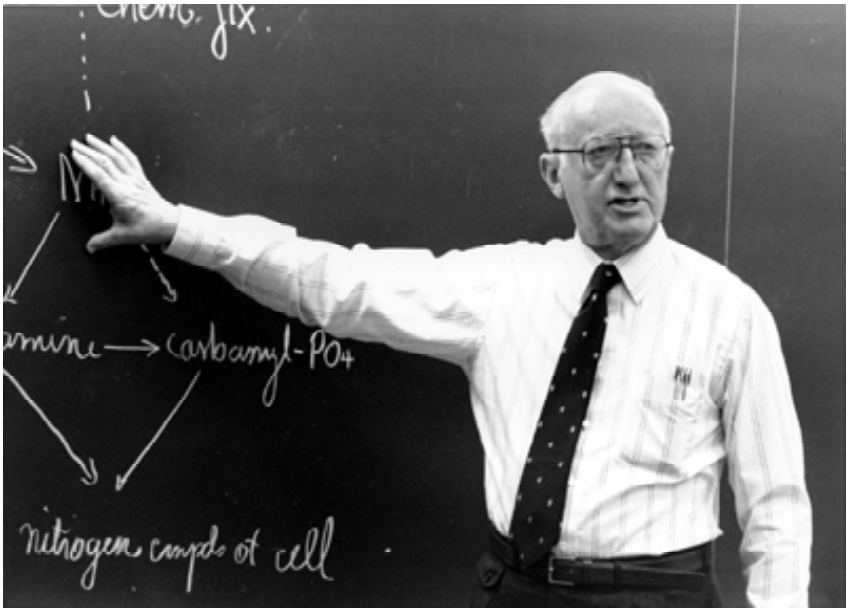
continued on page 6

Todd, Robert Burris and Rutherford Ness (Bob) Robertson. I learned many things from Vennesland, the most important being open, not secretive, about one's research, and being generous in collaboration with others. She stressed that one usually advances a project by collaborating with others who have different but applicable skills and knowledge.

After finishing my Ph. D. I stayed on at Chicago for two years at the urging of Vennesland who wanted me to co-supervise her graduate students while she met other commitments. This allowed me to acquire some valuable experience, and I also taught the introductory biochemistry course in the Biological Sciences Survey, an integrated biology sequence of five courses in the University College. Helen Stafford, who came to Chicago in 1951 as a post-doc to work with Vennesland, also taught botany in that sequence. This gave us valuable teaching experience that was important later when we applied for faculty positions. It also was the start of a long friendship with Helen, another *Woman Pioneer in Plant Biology*.

During those two postdoc years in Vennesland's lab, I was privileged to participate at the beginning of a research problem that has become textbook material. This was the demonstration of the direct enzymatic transfer of hydrogen atoms between substrates and pyridine nucleotides catalyzed by alcohol dehydrogenase (ADH). This was Harvey Fisher's research project initiated under Vennesland. Fisher had to synthesize dideuteroethanol (CH_3CD_2OH) to use as a substrate, and sought Frank Westheimer's advice concerning its synthesis. I provided the crystalline ADH and helped Harvey with the MS measurements. The results were unambiguous (*J. Biol. Chem.* 202, 687 (1953)) and initiated a decade of collaboration between Westheimer and Vennesland that has significantly impacted our knowledge of the stereochemistry of pyridine nucleotide dehydrogenases. Frank Loewus, who came to Chicago just as I was leaving, joined that project and contributed a major share of the experimental design.

I went to the Soils and Plant Nutrition Department in the College of Agriculture at UC Berkeley as an Instructor on September 1, 1992. My biochemical interests were welcomed in that department, made famous for studies on plant nutrition by Dennis Hoagland. However, two years later, an exchange suggested by Paul Stumpf, chair of the small Plant Biochemistry Department in the same College resulted in my moving into that department. I had met Paul when he visited Vennesland in Chicago, and he warmly welcomed me to the Berkeley campus where our relationship developed into a life-long personal friendship. We collaborated in writing the *Outlines of Biochemistry*, and editing the *Biochemistry of Plants* treatise, but never collaborated in research because of our different research interests.



In Berkeley, I initially continued with work involving pyridine nucleotide enzymes and in one project demonstrated that lupine mitochondria could carry out oxidative phosphorylation with the same efficiency (i.e. same P:O ratios) as animal mitochondria. I also had the great good fortune to have, as my first graduate student, Tsune Kosuge, who had a master's degree in plant pathology from Washington State, and sought training in plant biochemistry in order to apply that discipline to plant pathology. Tsune was familiar with the role of coumarin as a precursor of dicoumarol in spoiled sweet clover hay, studied by the legendary K. P. Link at Wisconsin, and proposed investigating the enzymes involved in the biosynthesis of coumarin. Kosuge's thesis research, and papers resulting from it, together with work carried out after he joined the Plant Pathology Department at UC Davis, is probably the first example of a multi-step biosynthetic pathway for a plant natural product described at the enzymatic level. A series of Kosuge's papers documents the evidence that coumarin is formed in sweet clover as follows: phenylalanine → *trans*-cinnamic acid → *ortho*-coumaric acid → *trans-ortho*-coumaroyl-β-glucoside → *cis-ortho*-coumaroyl-β-glucoside → *cis-ortho*-coumaric acid (coumarinic acid) →

coumarin.

The first evidence for PAL, to my knowledge, is Kosuge's thesis research when he observed formation of *trans*-cinnamic acid from phenylalanine in *dialyzed* extracts of sweet clover. These experiments eventually were discussed with Arthur Neish, a distinguished Canadian plant biochemist who, in the 1950s, had already completed an impressive body of work on the biosynthesis of lignin from phenylalanine and tyrosine. In his 1960 review in *Annual Review of Plant Physiology* (11:55 (1960)), Art proposed that *trans*-cinnamic acid might be formed by transamination of phenylalanine, reduction of the keto acid to phenyllactic acid, and dehydration to form cinnamic acid. The first two reactions presumably would require stoichiometric amounts of keto acids and reducing agents (DPNH or TPNH) to accomplish the over-all conversion. Since Tsune's dialyzed extracts catalyzed detectable amounts of cinnamic acid formed from phenylalanine, we believed there had to be another reaction, and proposed a single enzyme catalyzing the deamination, analogous to bacterial aspartase. In 1958 I visited Art at the Prairie Regional Lab (PRL) in Saskatoon and we had a good discussion. I also met Stewart Brown, who did some of the early labeling experiments on coumarins at PRL, and has contributed greatly to the literature in that subject.

One result of this visit was Art coming to Davis in September, 1959 on study leave to look 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 Kosuge, who was located in an adjacent department. Art started looking for TAL in rice, and other grasses, and Jane Koukol, a postdoc from Vennesland, arrived and started working on PAL in sweet clover. Eventually their work on TAL and PAL was published in *Phytochemistry* 1:1 (1961)) and *J. Biol. Chem.* 236: 2692 (1962)) respectively. An amusing problem of nomenclature should be mentioned. We had decided to call these enzymes tyrase and phenylalanase, after the enzyme aspartase. However, the editors at JBC insisted that Jane and I use the newly agreed-upon nomenclature of *phenylalanine ammonia-lyase* for PAL.

I've been queried occasionally about our lack of follow-up on the discovery of PAL as the enzyme gained prominence in plant secondary metabolism. Because it was Tsune's finding initially, I urged that he continue 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 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, my interests turned to the biosynthesis of cyanogenic glycosides and I concentrated my efforts and limited resources studying those compounds. With the help of Takashi Akazawa, a young graduate student from Uritani's lab in Japan, we soon showed that sorghum seedlings should be an ideal tissue to study the biosynthesis of dhurrin, the β-glucoside of *p*-hydroxy-(*S*)-mandelonitrile, the cyanogen in sorghum. While sorghum seed contains only traces of dhurrin, the 3-5 day old etiolated seedlings contain about 5-10% (dry weight) dhurrin. Since tyrosine was the obvious precursor, we fed C^{14} tyrosine to such seedlings overnight and observed a relatively large incorporation (5-10% of the activity fed) into the glycoside.

We reported these results at the Federation Meetings in April 1998, and were surprised, and somewhat chagrined, to hear John Gander, University of Minnesota, describe almost the same experiments in the following paper. Since John had the following summer free, I invited him out to Berkeley where we worked hard to identify intermediates between the amino acid fed and the cyanogen. With that large amount of incorporation, we both thought that it would be a simple matter to find a few spots on chromatograms, identify them and sort out the pathway. Such was not the case, and it eventually took another decade to establish such intermediates. At the end of the summer John returned home and continued to work on the problem for a few months. But, as 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 intact except for loss of the carboxyl carbon into dhurrin, and (b) to postulate possible intermediates, label them with C^{14} and feed them to seedlings. However, progress was delayed by my move to the Davis campus that fall and a delayed sab-

continued on page 8

batical in England in 1960.

I'd applied for and received a 3-yeaar 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 was $120,000, some of which had to accommodate inflation over those 3 decades. About 15 years into those years, when NIH decided that it would no longer fund plant research, someone at NIH deleted the last two words in the grant title. I wanted to complain about that action, but since I had some people on salary in that grant, I took the coward's way out, and accepted the renewal. In 1973 NSF started funding our research usually in 3-year grants.

In the mid 1950s, the University regents approved a major expansion of the university system that included conversion of Davis 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. The prominence of plant sciences at Davis, and the fact that the campus was entering a rapid period of growth was very attractive to us. So, in September 1958, Paul and I moved to Davis to initiate our teaching program. We left our students in Berkeley, since the department's facilities would not be ready until the following summer. That fall I had about 60 students in the introductory course. The following fall, there were over 100. By the end of that decade, we were lecturing to classes approaching 400. I describe this increase because it was primarily responsible for allowing us to hire 8 or 9 additional faculty and offer a well-rounded selection of courses. The students and postdocs moved up from Berkeley in July 1959. Art Neish and his family arrived in August, and he and Jane Koukol initiated work on TAL and PAL.

My sabbatical leave was spent in the Low Temperature Research Station in Cambridge with Tony Swain, hoping 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. I met Tony's boss, the fabled E. C. Bate Smith, 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 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, and this resulted in his spending a year's leave in Davis with us in 1961-1962, and my taking my next sabbatical in his lab.

My wife and I left England in June 1960 to spend the summer vacation on the continent, visiting friends she made while working in Paris during the 4 years 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'd met Hans in Berkeley when he was a post-doc with Melvin Calvin getting experience in the use of radiotracers in metabolism. He often attended seminars in our Department and was aware of Kosuge's enzyme work on coumarin. When he 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've often lectured in our graduate plant biochemistry course on the elegant research done in Hans' Institute, and am sad that he did not live to see the many current applications of molecular biology to his field. I'm also indebted to Hans for advising some of his students to obtain some experience with enzymes in our group. The first person to do so was Klaus Hahlbrock who came to Davis in 1967.

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 post-docs in my lab, and the undergraduates in my courses. Progress was made 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 C^{14}, gave rise to labeled HCN, while the β carbon remained in the *p*-hydroxybenzaldehyde moiety of the cyanogen. Several years later, my graduate student Ernie Uribe, using tyrosine labeled with C^{14} in the α position and N^{15} in the amino nitrogen, showed that the bond between those two atoms was not severed during the synthesis. This important result of course meant that all intermediates in the pathway had to contain nitrogen. However, we still did not know their nature. A later

experiment by Harold Zilg from Freiburg showed that the oxygen atom involved in the glucosyl linkage was derived from molecular O_2. This early result implicated a mixed function oxidase.

Graham Butler made several important contributions during his study leave. Since Gander had shown that dhurrin undergoes metabolic turnover in sorghum seedlings, we wondered whether this involved the release of HCN in the atmosphere. In an off-the–wall experiment, Graham and Shula Blumenthal fed C^{14} HCN to sorghum seedlings in a closed system for several hours and then examined extracts for any labeled products. To our great surprise, a single, heavily labeled compound, quickly identified as asparagine, was observed. Later work by Heinz Floss, Lee Hadwiger and Jackie Miller showed that many (and probably all) plants contain a mitochondrial synthase that catalyzes the replacement of the –SH group of cysteine with –CN to form β-cyanoalanine. While it was easy to understand why cyanogenic species might have this enzyme, it was surprising to find that non-cyanogenic species contained lower amounts of the enzyme. This anomaly was probably solved when Shang-fa Yang and his student Galen Peiser showed that one molecule of HCN is formed in the last step of ethylene biosynthesis when ACC (1-aminocyclopropyl-1-carboxylic acid) is oxidized by ACC oxidase. In their experiments, they could not detect HCN, but showed the C^{14} labeled carbon atom bearing the nitrogen atom in ACC was converted to labeled asparagine in nearly stoichiometric amounts (70% yield).

While in Davis, Graham demonstrated the remarkable efficiency of tops of flax seedlings in converting labeled valine to the cyanogenic glucoside linamarin. The tops could convert 35% of the labeled valine fed in experiments lasting only 7 hours! (Intact seedlings converted at most 5% under similar conditions.) Various experiments were also designed to detect conversion intermediates, but none were found. Experiments with N^{15} showed that the nitrogen atom in valine is retained as valine is converted to linamarin. Since sorghum shoots were also more effective in dhurrin biosynthesis, the two experimental systems were relatively equivalent, and when I was in Graham's group (1965-1966), we worked exclusively on flax.

Butler's student, Brian Tapper, was the first to show that oximes are intermediates in the biosynthesis of cyanogenic glucosides. A labeled compound that had the properties of an oxime glycoside accumulated in flax seedlings fed C^{14}-valine and O-methylthreonine, a metabolic inhibitor of valine. He prepared the oxime of C^{14} labeled isobutyraldehyde, administered it to flax shoots and found that it was converted to linamarin nearly as efficiently as labeled valine; isobutyraldehyde itself was not converted. (*Arch. Biochem. Biophys.* 119:593 (1967). 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.

When Klaus Hahlbrock arrived in Davis, he prepared the nitriles and cyanohydrins corresponding 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 were incorporated, although not as efficiently as the amino acids. The aliphatic cyanohydrin was readily converted to linamarin, but the aromatic cyanohydrin was toxic to leaves. In a joint note with Tapper and Butler (*Arch. Biochem. Biophys.* 125: 1013 (1968)), the plausible biosynthetic pathway cited above was put in writing.

Hahlbrock soon demonstrated the last step in the pathway. He detected a UDPG-ketone cyanohydrin glucosyltransferase in flax seedlings and purified it 120 fold free from β-glucosidase activity. It was 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 purified and characterized the glucosyltransferase in sorghum seedlings.

From 1966 on, my research centered on several different aspects of cyanogenesis, with two important exceptions. The first was work performed 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 using microsomes from such tissue to look for C4H, and was quickly successful (*Arch. Biochem. Biophys.* 122:256 (1967). In an important but seldom cited paper (*J. Biol. Chem.* 246:3870(1971)), David reported numerous properties of the enzyme including its light-reversible inhibition by carbon monoxide (CO), and feed-back inhibition of its activity by the product formed, *p*-coumaric acid. David concluded that his enzyme had all the properties of a P-450 type of cytochrome but that this required studies of the action spectrum for light reversal of the CO-inhibition. My graduate student Rowell Potts subsequently measured the spectrum (*J. Biol. Chem.* 249:5019 (1975)). His spectrum, together with action spectra obtained in Charles West's laboratory in 1969 for two enzymes

continued on page 10

involved in gibberellin biosynthesis are the only such data concerning the many P-450s now described in plants. Although I co-authored the original note in *Archives* with David, I insisted that he be the sole author on the JBC paper, as he had originally proposed the research and had been completely self-sufficient in his work in the lab.

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 because of feeding experiments. Since numerous studies in animal tissues, and some in plants, had shown that microsomal enzyme systems catalyzed C-hydroxylation reactions, I suggested to Ian that he see 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 seedlings in the presence of thiol reagents, and in an early experiment included tyrosine as a control, not expecting it to be acted upon. Astonishingly, he found that tyrosine was oxidized by the particles to form *p*-hydroxybenzaldehyde and HCN. Moreover, this reaction was more rapid than oxidation of *p*-hydroxyphenylacetonitrile. The particles also utilized the aldoxime as a substrate about as well as the amino acid. The properties of this microsomal system were then extensively studied by Ian and Edith Lees, a faculty member on study leave from Sydney (*J. Biol. Chem.* 250:4708 (1975)).

Since the conversion of tyrosine to p-hydroxyphenylacetaldoxime constitutes a 4-electron oxidative decarboxylation, an intermediate in that conversion was likely. Earlier work on glucosinolate biosynthesis in Ted Underhill's group in Saskatoon had indicated *N*-hydroxyaminoacids as intermediates between amino acids and aldoximes in the biosynthesis of glucosinolates. The next step was the synthesis of N-hydroxytyrosine (NHT).

Birger Moller arrived from Copenhagen, just as Ian and Edith were finishing up this work, and decided to work on this problem. He perfected a synthesis of NHT initiated by Ian, and prepared the C^{14}-labeled compound. Its efficacy as a substrate was compared with five other compounds that, on paper, could be intermediates between tyrosine and its aldoxime (e.g. tyramine, *N*-hydroxytyramine, *p*-hydroxyphenylpyruvic acid oxime). Only NHT was metabolized to *p*-hydroxymandelo-nitrile by the particles. Moreover, NHT was produced from [UC^{14}-C]-tyrosine in tracer experiments when unlabeled NHT was added as a trap (*J. Biol. Chem.* 254: 8575 (1979)).

Another major contribution Birger made to the problem while in Davis was to show that the biosynthetic sequence catalyzed by the microsomes is highly channeled (*J. Biol. Chem.* 255:3059 (1980)). His experiments explain why, in the early work done both in Butler's and our labs, we never easily detected any intermediates. Helen Stafford was among the first to propose metabolic channeling in the synthesis of natural products, and she has discussed the criteria that need to be met in her chapter in Volume 7 of *The Biochemistry of Plants*. While this first paper met many of those criteria, recent research from Birger's group in Copenhagen has gone on to show that the three enzymes involved in the biosynthesis form an organized complex. Recently, Meinhart Zenk told me he was skeptical of that early paper until Birger presented his recent work on channeling at a seminar in Munich twenty years later.

Birger returned to Denmark after two years and initially worked at the Carlsberg Institute on aspects of photosynthesis. In 1985, when he was appointed to the chair in Plant Biochemistry at the Royal Agricultural and Veterinary University, he proposed returning to the cyanogenesis field, and I strongly encouraged him to continue with his biosynthetic work. Applying the tools of molecular biology to that and related problems, he and his group have greatly advanced our understanding of the subject. He reviewed their progress at the PSNA meeting in Montreal (*Rec. Advan. Phytochem.* 34:191(2002)), and numerous other papers have appeared since then.

Other problems, usually related to cyanogenesis, attracted the interest of workers in my lab from 1970 until I retired in 1993. Harold Zilg, Kevin Farnden and Mark Rosen, an undergraduate student, studied various stereochemical aspects of cyanogen biosynthesis. Adrian Cutler examined metabolic channeling during biosynthesis in arrow grass and flax. Wendy Swenson and Joe Olechno described new cyanogens from *Acacia sutherlandin* and *Nandina.* Mary Seely, Gary Kuroki and Lang-lai Xu studied the properties of α-hydroxynitrile lyases in several species while Dirk Selmar concentrated on the physiological role of that enzyme. The β-cyanoalanine synthase of lupine was studied by Harland Hendrickson, and Peter Castric and Kevin Farnden discovered a new enzyme that hydrolyzes β-cyanoalanine to asparagine, thereby explaining how the nitrogen in cyanogens is retained by plants rather than being lost as HCN. Compartmentation of cyanogenic glycosides and their catabolic enzymes attracted the interest of Jim Saunders, Jonathan Poulton, Susan Thayer, Eve Wurtele, Mineo Kojima and Marco Frehner. Poulton, Kazuko Oba and Alain Boudet extended such work to enzymes and intermediates of coumarin biosynthesis in sweet clover. Wolfgang Hosel and Ingrid Tober characterized β-glucosidases in sorghum, and Hosel emphasized the

specificity of plant β-glucosidases in a review in *TIBS*.

Another study, not involving cyanogenesis, examined the role of the non-aromatic amino acid, arogenic acid, in the biosynthesis of tyrosine and phenylalanine in sorghum. Earlier studies by Roy Jensen had shown that tyrosine is formed in mung bean, maize and tobacco by transamination of prephenic acid to form arogenic acid (*pre-tyrosine*), followed by oxidative *aromatization* of the 6-membered ring to form tyrosine. Jim Connelly and Dan Siehl showed that sorghum contains prephenic transaminase that utilizes glutamate as amino donor forming arogenate, together with arogenate dehydratase and dehydrogenase required for aromatization of arogenate and formation of phenylalanine and tyrosine. They could not detect the dehydratase and dehydrogenase that aromatize prephenic acid. Bijay Singh and Gary Kuroki examined the regulatory properties of chorismate mutase in several species.

Starting in 1974, my research interests broadened because of collaboration with David Seigler. I told David at the 1972 PSNA meeting of older literature showing that some South African species of acacia contained cyanogens derived from valine and isoleucine, while those in Australian species are derived from phenylalanine. This was the only example at that time of aliphatic and aromatic cyanogens occurring in the same genus. David, who teaches plant taxonomy at Illinois although his Ph. D. is in physical organic chemistry, informed me of 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 UCD arboretum for cyanogenesis, as well as herbarium specimens. Leaf material of any positive species were then obtained, extracted and worked up to identify the cyanogen. This project resulted in several new cyanogenic glycosides being reported.

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. Initially I found two additional cyanogenic species in California, and this led to my last sabbatical in Australia in 1981-1982.

My family and I flatted in Adelaide where I had space in the laboratory of Brian Coombe at the Waite Institute. (I'd met Brian in 1955 when he was getting his Ph. D. in plant physiology at Davis.) The Waite 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 permission to examine both eucalyptus and acacias. Except for Canberra and Perth, it was surprising to see that the other gardens usually had more specimens of northern hemispheric plants than Australian species. However, Canberra's National Botanic Garden is restricted to Australian species and proved especially useful.

While in Perth, I met Bruce Maslin, an expert on the 800-plus Australian species of acacia, many of which are native to Western Australia. Bruce was interested in my project, and I mentioned one species I'd decided was cyanogenic after testing it in several gardens earlier. Because of his taxonomic knowledge, he quickly named several related species and produced herbarium specimens that 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 herbaria require that live specimens be located in the field, tested for cyanogenesis, and if positive, sampled and processed for identification of the cyanogen. This led to several collecting trips with Bruce in subsequent years.

Papers in *Phytochemistry, Kingia,* and the *Western Australian Herbarium Research Notes*, present results of tests on 96% of the described species of the genus. This work showed that cyanogenic species in subgenus *Acacia* contain only aliphatic cyanogens, while cyanogenic species in the two other subgenera *Phyllodineae* and *Aculeiferum* contain aromatic cyanogens. These data support traditional taxonomic evidence that subgenus *Aculeiferum*, distributed mainly in Africa and Asia, is more closely related to the predominantly Australian subgenus *Phyllodineae* than to the pan-tropical subgenus *Acacia*. The work on eucalyptus involved testing about 1400 individual plants representing 348 species, 22 of which were cyanogenic. (*R*)-prunasin, derived from phenylalanine, was identified as the cyanogen in 10 of those species.

I appreciate the invitation to prepare a description of my research career. I've intentionally overemphasized the early part to let younger phytochemists know how different research was then without the techniques, equipment and biochemicals available now. As always, it's a great pleasure to acknowledge the essential contributions of all who have worked with me.

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Editor's note: We are priviledged to have the opportunity to read about the personal and professional events that shaped the lives and careers of eminent scholars, such as Eric Conn. Nominations are still welcome for Phytochemical Pioneers who have made an outstanding impact on their field. Please contact the Editor.