So many roads traveled: A career in science and administration

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I have traveled many roads during my career. After spending my first 19 years in Los Angeles, I became somewhat of an academic nomad, studying and/or working in six universities in the United States and three in Sweden. In chronological order, I have a B.A. in Scandinavian languages and literature from UCLA, a Ph.D. in biochemistry from Uppsala University, and an M.S. in toxicology from the Karolinska Institute. I have been in schools of natural science, pharmacy, and medicine and have worked in multiple basic science departments and one clinical department. I have served as a research-track and tenured faculty member, department chair, associate dean, and dean. My research has spanned toxinology, biochemistry, toxicology, and pharmacology. Through all the moves, I have gained much and lost some. For the past 40 years, my interest has been cytochrome P450 structure-function and structure-activity relationships. My lab has focused on CYP2B enzymes using X-ray crystallography, site-directed mutagenesis, deuterium-exchange MS, isothermal titration calorimetry, and computational methods in conjunction with a variety of functional assays. This comprehensive approach has enabled detailed understanding of the structural basis of the remarkable substrate promiscuity of CYP2B enzymes. We also have investigated the mechanisms of CYP3A4 allostery using biophysical and advanced spectroscopic techniques, and discovered a pivotal role of P450-P450 interactions and of multiple-ligand binding. A major goal of this article is to provide lessons that may be useful to scientists in the early and middle stages of their careers and those more senior scientists contemplating an administrative move.

The title of this article derives from my love for the blues and admiration for the guitarist Otis Rush. He was the first to release the song "So Many Roads, So Many Trains." Take the lyrics "So many roads, so many trains to ride, I've got to find my baby before I'll be satisfied." Replace the word "baby" with "purpose," and the course of my career becomes clear.

Childhood

My first memories are of a tract home in North Hollywood in the San Fernando Valley in metropolitan Los Angeles. My mother, Ruth, was a schoolteacher and my father, Saul, a journalist. It was the early 1950s, and life was very much as depicted later in the iconic television shows *Father Knows Best* and *Leave it to Beaver*. Although my parents never pressured me overtly or rewarded me for excelling in school, I understood that much of their happiness depended on my being the top student in every grade. Life outside of school consisted of playing baseball or football in our dead-end street. Parents rarely worried about children outside on their own. The only concern was breaking a car windshield with an errant baseball, but that never happened.

Aside from playing baseball, following the Los Angeles Dodgers was my great joy. In their first year after moving from Brooklyn, the Dodgers were terrible. However, with the recruitment and maturation of new players, they won three World Series Championships between 1959 and 1965. The highlight of my first 10 years was watching Sandy Koufax strike out 18 San Francisco Giants in August 1959. He was my hero after that and to my knowledge is the only Hall of Fame pitcher to retire after a phenomenal last season. I always wanted to end my scientific career like that.

My life changed totally with the acquisition of a chemistry set. It came in a shiny, orange metal case, and the two boys depicted on the front seemed to be having so much fun with chemistry, as did I. Inevitably, however, cookbook experiments of turning water to wine become tedious. Like most boys my age, I wanted to figure out how to blow up something, such as a model airplane I had just glued together. (A disclaimer is in order here: I never exhibited any destructive tendencies later in life.)

Junior high and high school

In order for me to attend better schools, we moved 10 miles to Sherman Oaks. By now my father was a well-respected journalist, and he later became one of the pioneers in television news and a household name to literally millions of Los Angeles residents. My mother went to graduate school in her 40s and obtained a Ph.D. from UCLA and a faculty position at what is now California State University at Northridge. As a lab helper for our junior high school science teacher, Mrs. Munson, I had a small project to determine the normality of an HCl solution by titrating with NaOH in the presence of the pH-sensitive dye phenolphthalein. It sounded so easy, yet I could never get the

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same result twice. Ironically, it was not until almost 60 years later when writing this article that I discovered from the internet that the key is to recognize the right end point, which is a faint pink color that persists for 30 seconds. This episode yielded the first two take-home lessons of this Reflections article. **L1) Know the difference between persistence and stubbornness. L2) Self-reliance and independence are good, but, when really stuck, do not be too proud to ask for help.**

Chemistry was my favorite class in high school, and we all worshiped our teacher, Louie Falb, who never assigned homework on the night when the show *Batman* was on television. I learned a great lesson from my good friend Bob Jones, whom I used to tutor in chemistry. Actually, the tutoring consisted of him calling me up almost every evening and asking me to explain how to solve the homework problems. Eventually, this became annoying, and I started to ask my mother to tell Bob I had gone to the library. Subsequently, Bob had a great revelation that he could solve the problems himself if he read the chapter first. This leads me to the next lesson: **L3) Do not do for people what they can do for themselves.** I have applied this principle in virtually all my personal and professional relationships. Of course, when people really do need help, I gladly provide it.

Although I had grown beyond my chemistry set, I resumed experiments at home with eventual dire results. One time, I ignored all precautions and capped the bottle of an iodine solution that I was heating on the stove to enhance solubility. I was later greeted by the inevitable sound of glass breaking and by a deep purple fog. Fortunately, before our parents came home, my brother Rob and I were able to air out the house and to swab down the walls with household cleaner. However, the next morning we were greeted by the loudest shriek I had ever heard my mother emit. The cleaner had reacted with the iodine rather than removing it, and the kitchen walls and ceiling were a sickly orange color. Repainting by professionals rescued the walls, but even decades later, there were still signs of iodine crystals on the ceiling. The lesson here is: **L4) Lab safety is really important!**

Chemistry student at UCLA

It was natural for me to major in chemistry, and I chose UCLA because I wanted to stay close to my high-school girlfriend. 1967 was a very turbulent time in our country, and life was very confusing. On the one hand, I had the trappings expected of a young man at that time, namely longish hair, beard, and sandals. On the other hand, I was a true chemistry nerd. An example of how the conflict played out in me is that I basically flunked the perpetual love-ins of the day. The music always started several hours late, and generally I would flee back to my chemistry studies, rarely having heard any music. Nonetheless, I did manage to hear more organized concerts by many of the legendary groups of the '60s, such as Jimi Hendrix, the Animals, the Doors, and the Byrds. My brother and I had a small band, and we even earned some money. I remember when we arrived at a university dormitory to play and saw a big banner outside that said "Live Band Tonight." We looked at each other in disbelief. We did fine, but the next lesson is this: **L5) Imposter syndrome is real.**

Finishing the chemistry labs at UCLA on time was a true feat of preparation and organization. Otherwise, I would get trapped behind a long line of students trying to use the lone balance. Before each lab, I wrote down and visualized exactly what to take out of my locker and when and what I needed to do. That way I could be first in each line for common equipment. The obvious lesson here: **L6) Success often requires intense preparation, discipline, and focus.**

Our freshman chemistry professor, Edward Graham, managed to make even the most obscure aspects of chemistry come alive. The highlight of the year started inauspiciously. One day I received a letter inviting me to attend an awards ceremony but without stating why. I felt really shy about going but thought it would be rude if I stayed away, and I arrived at the last second and stood in the doorway. The next thing I remember is that I was named outstanding freshman chemistry student out of around 1,000 students. I was astonished but did not feel like an imposter. At the same ceremony or shortly thereafter, I received a National Science Foundation undergraduate summer fellowship. Naturally, I asked Dr. Graham whether I could work in his laboratory and was so honored when he said yes.

Ironically, the main lesson from my summer experience was the same one I did not quite learn in junior high, namely to ask for the right kind of help. My project in the Graham lab involved the use of a complicated manifold, consisting of glass tubing and stopcocks, which was designed to allow me to trap products of the gas-phase reaction I was studying. Unfortunately, I was always breaking the manifold and never managed to master glassblowing. I had to ask a graduate student to make repairs for me, when I should have asked for a different project. I concluded that I was a wizard in the classroom and in cookbook labs but inept at real research. That feeling definitely impeded my eventual decision to embark on a Ph.D.

My personal frustration during this summer of 1968, my general feeling that the fabric of our society was unraveling, and the fact that all my friends had left LA for college created a great urge to do something different. When I received a letter from UCLA offering me the chance to spend my junior year abroad, I was very excited. For a variety of reasons, I chose Sweden as the destination and never looked back. My second year at UCLA was devoted largely to learning Swedish any way I could (courses, movies, books, and the Swedish Hollywood Club).

Early years in Lund

I arrived in Sweden in August 1969 knowing just enough of the language that I was slightly more proficient than most of my Swedish peers were in English. I loved living in the small university town of Lund, where I could get around by walking or on a bicycle. Because I really wanted to learn Swedish, I started by taking university courses in social sciences. My second semester, I studied organic chemistry for 10 weeks full time. Nonetheless, I fell far behind in credits for a chemistry major at UCLA and did not even consider taking more than four years to graduate. I decided to attend law school upon receipt of my bachelor's degree, for which I could major in any subject. I picked Scandinavian languages and literature and decided to get a head start by staying an extra semester in Lund and studying Old Icelandic, using my very modest inheritance from my

paternal grandfather to support myself. I was in a class with only Swedes who were training to become school teachers of their own language. Although I actually understood the Icelandic, I could not translate it into perfect Swedish and failed the final examination. The lesson here: **L7) No matter how hard you try, there are some things that you will simply not be able to do.** (The professor was kind enough to pass me on an oral makeup exam, probably out of sympathy.)

I returned to UCLA for two quarters and obtained my B.A. However, despite an excellent result on the Law School Aptitude Test, I realized that law school was not my true interest. In a quandary about my future, I decided to return to Scandinavia and was able to secure a Danish work permit through a friend of my parents. Upon arriving in Copenhagen in August 1971, I learned that the job offer had been rescinded. Fortunately, with a Danish work permit, I could apply for a Swedish permit from Denmark. I managed to find a part-time job at Lund University Hospital, working for Dr. Mario Monti, an Italian physician who was pursuing his Ph.D. but had limited lab experience. As the only foreigner and male among ~20 female lab assistants in the department, I was not well-accepted and became the scapegoat for almost anything that went wrong, which leads to this: **L8) Sometimes, for reasons that you can do little about, you will find yourself attacked from all sides.**

In addition to helping Mario with his research on energy metabolism in red blood cells, I had a small project of my own. It had been reported that the glycolytic intermediate 2,3-diphosphoglycerate, which is an important regulator of oxygen release from hemoglobin, could exist in a membrane-bound form (1). No matter how hard I tried, I could not reproduce those results, conjuring up my frustration in junior high with acid base titrations and at UCLA with gas-phase kinetics. I later read another report that a second major research group was unable to reproduce the first group's results (2). The discrepancy was attributed to minor experimental differences in the preparation of cell lysates. From this I learned a lesson that shaped how I managed my research group for almost 40 years: **L9) Two people who obtain different results are inevitably not doing the exact same thing. One needs to watch the other meticulously and continuously, noting even the smallest details that might differ from person to person.** Through application of this principle, we have been able to figure out, for example, why growing protein crystals can seem so idiosyncratic. Insistence on actual observation has solved many difficulties that no amount of discussion would have addressed.

While working for Mario, I took a 10-week biochemistry course in spring 1973 and absolutely fell in love with the discipline. I was enthralled that one could extract proteins from a biological sample and then reconstitute chemical reactions in a test tube. During breaks when other students were drinking coffee, smoking, or using snus, I would go to the biochemistry library and worship the bound volumes of the *Journal of Biological Chemistry*. Reading some of the classical articles was more amazing than any rock concert I had ever attended. I made an oath to myself that someday I would publish an article in the journal. Little did I know that I would be submitting my first *JBC* manuscript only 1.5 years later and would subsequently publish a significant number of articles there and in other top biochemical journals.

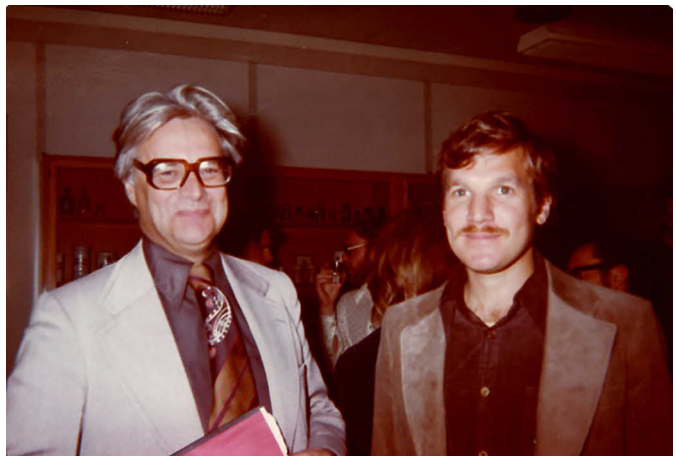

Figure 1. With Jerker Porath after my Ph.D. defense in Uppsala, May 1977.

Uppsala University

In fall 1973, I joined the laboratory of another American, David Eaker, in the Department of Biochemistry at Uppsala University. David had been trained by Lyman Craig at the then-Rockefeller Institute for Medical Research. Uppsala biochemistry had an unparalleled tradition of protein separation science, dating back to the Nobelist Theodor Svedberg (ultracentrifugation) and his student and fellow Nobelist Arne Tiselius (electrophoresis). The department chair was Jerker Porath (Fig. 1), a disciple of Tiselius and co-discoverer of gel filtration. David's group was small, consisting of only two other members, namely the Ph.D. research specialist Evert Karlsson and senior graduate student Jan Fohlman (3). I felt incredibly fortunate to be a part of such a storied biochemistry department.

Still insecure about my technical skills, I found David to be the perfect mentor. He taught me the right way to do virtually everything (see **L9)**, such as solvent distillation, column chromatography, paper electrophoresis, and manual Edman degradation. David's approach to teaching lab skills was to perform the method while I took meticulous notes, which I would then share with him. Once the accuracy of my notes was assured, I would repeat the method myself but now with, for the first time in my life, the expectation that the experiment was going to be successful. From that time forward, I grew into a confident and excellent experimentalist and wished that there were more hours in the day to work.

My first project was to determine the amino acid sequence of notexin, a 119-residue presynaptic neurotoxin from the venom of the Australian tiger snake (4). David's lab and others had determined numerous primary structures of the smaller postsynaptic toxins, the mechanism of action of which was similar to curare. However, no presynaptic toxin had been sequenced, nor was the mechanism of action clear. The expectation was that understanding toxin structure and function in detail would provide novel insights into synaptic transmission. Thanks to the availability of a novel glutamic acid protease provided to us

by Lars Rydén, I was able to cleave notexin into five large peptides. These could then be purified by column electrophoresis, a method developed in Tiselius' laboratory by Jerker Porath and others. Aligning the peptides and completing the sequence of three of them also involved use of trypsin, chymotrypsin, and cyanogen bromide. Nonetheless, with my new self-confidence, I completed the project in 1 year. My proudest moment was performing 21 cycles of manual Edman degradation on a 22-residue peptide, applying the residue directly to the amino acid analyzer, and confirming the C-terminal Glu residue.

Unfortunately, romantic troubles affected my brain, and I started to make careless mistakes. I frequently let David's precious Sephadex columns run dry. The harshest thing he ever said to me was: "This GC on G-25 has to stop." I probably deserved worse but was hurt that I had been chastened rather than asked what was bothering me. So the next lesson is for you mentors: **L10) If a previously capable student starts to make mistakes, ask what is going on.**

I completed two other interesting projects over the next 18 months. One was to show the importance of the phospholipase A2 activity of notexin for the neurotoxicity by chemical modification of a single His-48 residue (5). Because it was difficult to label the protein with 100% efficiency, a key experiment was to separate modified from native protein by ion-exchange chromatography. The second project was to determine the primary structure of another presynaptic toxin closely related to notexin in amino acid composition (6). Now that I knew exactly what I was doing, that sequence took basically 3 months. With these three projects completed and more in the works, I informed David rather boldly that I would be taking a 10-week course in biochemical toxicology at Stockholm University during the second semester of my third year. This was possible because I was supported by an individual stipend from the department, and Swedish universities were very student-oriented.

Stockholm and Karolinska Institute

Stockholm was very different from the student towns of Lund and Uppsala. At first I did not like the big city, but it later became one of my favorite places. At Stockholm University, I first encountered cytochrome P450, which seemed like an odd name for an enzyme. With my interest in toxicology now awakened, I applied to be a member of the inaugural class in a new M.S. program at the Karolinska Institute. I could use the last year of my stipend from Uppsala to start a new program at another university. I was the only non-Swede and only Ph.D. student in the class of 16 students in the toxicology program. (In May 1977, I graduated with my Ph.D. from Uppsala in 4 years, having essentially parlayed 2.5 years of research into four publications and a dissertation.)

At the Karolinska Institute, I met Magnus Ingelman-Sundberg. I also became a roommate with Rune Toftgård, who became a world-renowned cancer researcher. I did my M.S. thesis with Magnus on the purification and characterization of rabbit liver epoxide hydrolase. Once some problems with the assay were solved through advice from another group, the purification went fairly smoothly. The only challenge was to remove the pale yellow color in the penultimate preparation, presumably due to a P450. For this I used a long narrow column. Eight years later, when separating different rat liver P450s from each other, I realized that my successful final purification step for epoxide hydrolase was the result of the P450 denaturing on the column during the prolonged chromatography.

Figure 2. My first meeting in Japan in 1981, standing between Fred Guengerich (*center*) and Ted Kamataki (*far right*).

Magnus realized correctly that purification and characterization of a new enzyme was not sufficient for publication in *JBC.* At the time, he was working on studies of purified P450s in liposomes and suggested that we do the same with epoxide hydrolase. We found some interesting differences in steady-state kinetics between the soluble and membrane-bound preparations but could never really explain the findings. Nonetheless, we submitted two companion manuscripts. Ultimately, mine was relegated to a one-page miniprint supplement to his (7). Magnus was kind enough to let me be first author, and my chagrin subsided. I think the lesson here is for a graduate student: **L11) No matter how smart you think you are, sometimes your advisor knows better than you.**

Vanderbilt University

By fall 1978, and with my Ph.D. and M.S. behind me, it was time to return to the U.S. The ideal place to combine my interests in biochemistry and toxicology was the Center for Environmental Toxicology at Vanderbilt University, which was housed in the Department of Biochemistry. I was thrilled when the center director, Robert (Bob) A. Neal (8), accepted me into his research group. The research environment in the lab and center was very vibrant, and I was truly inspired by the dedication of my lab mates. I had the excellent fortune of getting to know Fred Guengerich (Fig. 2) at the beginning of his own academic career, and I learned so much from Fred and his lab group. I also really liked Tennessee and Nashville, where many of the country and western and bluegrass greats were still performing. I especially recall Bill Monroe, because of his distinctive voice and rapid playing style. I started to practice Tae Kwon Do and compensated for my almost complete lack of physical prerequisites by intense and fierce dedication. (I continued to practice for 11 years, obtaining my black belt along the way. That was the hardest thing I have ever done.)

It is striking how little was known about P450 enzymes when I started at Vanderbilt (Fig. 3). My project was to use mecha-

- Multiplicity: how many P450 enzymes exist in the liver of different mammalian species including humans?
- Substrate specificity: what substrates does each P450 act on?
- Regulation: how is the level of each P450 protein increased or decreased by exogenenous or endogenous factors?
- Human P450s: what role do they play in drug interactions and individual variability in drug metabolism?
- Structure: What are the primary and 3D structures of the P450s and how do the structures relate to function?
- Catalytic mechanism: what are the rate-limiting steps?

Figure 3. Major gaps in knowledge of P450 enzymes in 1978.

nism-based inactivators (MBIs)[2] to label P450 active sites. I plunged totally into the fascinating world of P450 structure and function, which became my life's work. At that time, most P450 MBIs labeled or destroyed the heme moiety of the enzyme, providing key insights into mechanisms of catalysis but no information about active-site residues. There was some evidence from Ted Kamataki (9) (Fig. 2) that parathion might be capable of labeling the protein moiety of P450s. Working with what we now call CYP2B1, I found that parathion did indeed modify the protein moiety but also destroyed the heme group, and the mechanism of inactivation was too complex to help elucidate the nature of the active site. I wrote up a very comprehensive manuscript and submitted it to *JBC* and, to my amazement, received a gold-colored card confirming the acceptance with no revisions (10).

I was fortunate that Chris Chengelis, another postdoctoral fellow in the laboratory, showed me an article suggesting that the antibiotic chloramphenicol might be more suitable than parathion for our purpose. Indeed, key initial findings were that CYP2B1 was inactivated by chloramphenicol with no loss of P450 or heme and that the protein moiety was labeled almost stoichiometrically by a reactive chloramphenicol metabolite (11). In a subsequent sole-author article, I showed that there were two classes of adducts of chloramphenicol and CYP2B1, including *N*-ϵ-chloramphenicol oxamyl lysine. To our knowledge, this was the first clear identification of an adduct of a reactive intermediate of a xenobiotic and a P450 (12).

The time as a postdoctoral fellow at Vanderbilt was wonderful but short by today's standards. After less than 1.5 years, I started to apply for independent faculty positions. At the age of 30, I was feeling too old to still be a trainee, and Bob Neal had accepted a position as president of the Chemical Industry Institute of Toxicology starting around December 1980. I had interviews in several pharmacology departments but none in biochemistry despite my degree and publications in biochemical journals. Clearly, P450 was now viewed as more the province of pharmacology than biochemistry. I learned: **L11) You are not only who you think you are but how you are perceived by the rest of the world.** In any case, the position I was offered was not one I wanted, and the one I wanted I did not obtain.

Back to Sweden again

With a Swedish girlfriend who wanted to return home, I went to work with Jan-Åke Gustafsson at Huddinge Hospital south of Stockholm. He was very generous to me, and I had a modest grant and small lab group of my own. I documented the formation of the lysine adduct of chloramphenicol and CYP2B1 *in vivo* (13) and performed collaborative studies with Eddie Morgan on a female-specific rat liver P450 (14) and with Tapio Haaparanta on an extrahepatic one (15). I think that Tapio and I were among the very first to do Western blotting on a P450. Unfortunately, the time in Sweden was marred by the tragic death of my brother only a few months after I had returned. It became less and less attractive to live halfway around the world from my parents, and I started applying again for faculty positions in the U. S. The major hurdle was not knowing exactly what facet of P450 to pursue. Luck favored me when I confessed my dilemma to Dick Philpot at a meeting in Stockholm, and he suggested "selectivity." I realized immediately that although pharmacologists were very interested in using selective inhibitors to probe P450 function in liver microsomes and *in vivo*, there were no suitable compounds available. Likewise, the biochemists who had identified multiple P450s were generally not really interested in inhibitors. As a biochemist/pharmacologist, I saw a future niche, namely characterizing and enhancing the isoform selectivity of P450 inhibitors. Pursuing that problem was the start of an independent research career that lasted for more than 35 years and led me into so many other aspects of P450 structure-function and structure-activity relationships. To anyone contemplating an academic career in biomedical research I say: **L12) You cannot overestimate the importance of picking the right problem, which must be one in which you are genuinely and intensely interested.**

Assistant and associate professor at the University of Arizona

I joined the Department of Pharmacology and Toxicology in the College of Pharmacy at the University of Arizona in fall 1983. As the first hire of the new department head, Glenn Sipes, I received much personal advice and was in an excellent position to compete for the top graduate students and for training grant slots. I was fortunate to spend a week in Jud Coon's lab in Ann Arbor finalizing a project I had started in Sweden and demonstrating that covalent binding of chloramphenicol to CYP2B1 blocks electron transfer from NADPH-cytochrome P450 reductase (16). In addition to launching the work on inhibitor selectivity, I became involved in a collaborative project with Glenn and a student, Dave Duignan, to purify and characterize the dog liver P450 (now known as CYP2B11) responsible for the unique ability of that species to metabolize and eliminate certain highly chlorinated polychlorinated biphenyls (PCBs) (17). Now the lone biochemist in my own department, I was very grateful that John Law, the head of the

[2] The abbreviations used are: MBI, mechanism-based inactivator; PCB, polychlorinated biphenyl; DXMS, deuterium-exchange MS.

Department of Biochemistry, took an interest in my work. Through him, I reconnected with Jerker Porath, who had started spending winters in Tucson.

After receiving a Faculty Development Award from what is now the PhRMA Foundation, I finally had confidence that my ideas were viable. Later, I obtained an R01 grant and Research Career Development Award from the NIEHS, National Institutes of Health, to pursue the inhibitor studies and a second R01 for the CYP2B11 work. I was fortunate to recruit Jeff Stevens to my lab as an M.S. student to develop methods for determining the selectivity of P450 inhibitors (18). Jeff later did his Ph.D. with me. Our major findings on CYP2B enzymes during this period are summarized succinctly in a review I wrote upon receipt of the Bernard B. Brodie Award in Drug Metabolism (19) and will not be reiterated here. Fortuitously, while purifying CYP2B11, we noted a second phenobarbital-inducible P450 in dog liver, subsequently purified and cloned by Paul Ciaccio and named CYP3A12 (20). That work led to an R01 grant from the NIGMS, National Institutes of Health, on human CYP3A4, which culminated years later in a MERIT Award. The lesson here: **L13) While pursuing your own ideas with passion, keep your eyes open for other opportunities that might come along.** Although I emphasize the funding here, it is important to note how innocent the times were by today's standards. The grants were a means to an end, namely to be able to pursue work that captivated my intellect, and I do not recall ever being asked to report how much grant money I was bringing in.

Much of the research during this time was possible because I was willing to retool my lab to work on recombinant DNA. Within a few years at Arizona, I realized that I would become obsolete if my lab relied solely on P450 preparations from rat liver. I spent one summer immersing myself in every article I could find about cloning P450 cDNAs or genes, and the following fall I took a senior-level biochemistry course in recombinant DNA, including the laboratory. Subsequently, I spent a 1-year sabbatical at UCLA working with Oliver Hankinson and Armand Fulco and was very fortunate that Karen Kedzie, a new postdoc and cloning expert, was willing to manage my lab while I was gone. Karen was the first to show that a single amino acid substitution in a P450 could completely alter susceptibility to mechanism-based inactivation (21). At UCLA, I became sufficiently proficient in basic recombinant DNA techniques to supervise such work upon my return to Arizona. The lesson that emerged: **L14) Stay on top of new technologies by recruiting people who know them, and have enough basic understanding yourself to be a competent mentor.**

An interest in administration

Upon my return to Arizona, I rebuilt my group by recruiting postdoctoral fellows. Grazyna Szklarz showed that a consensus homology model based on bacterial X-ray crystal structures could be used in conjunction with site-directed mutagenesis to pinpoint active-site residues in CYP2B1 (22). Sharon Strobel clarified longstanding confusion about the activities of CYP2B1 and -2B2 (23), and Greg Harlow provided the first evidence of a peripheral binding site in CYP3A4 (24). Yasuna Kobayashi incorporated studies of reaction stoichiometry into our mutagenesis work (25), and a visiting scientist, Julia Hasler, identified key residues in CYP2B11 responsible for the unique activity toward PCBs (26).

We were joined by two research specialists from Shanghai, the sisters You-ai He (27) and You Qun He (28). There were very few Chinese scientists at the university at that time, and You-ai, who came first, had a tough time in the beginning. She rarely spoke, and I did not know how to help besides loaning her some extra money for a few weeks until her paychecks arrived. One day we took You-ai out to lunch, and she almost stepped out in the street in front of a passing car. I pulled her back and said: "Do not get run over; you owe me money." She actually laughed, which told me that she understood both my English and my bad jokes. Once You Qun arrived, the two He's were fine. They stayed with me for almost 15 years, for which I am eternally grateful.

I was promoted to full professor and appointed assistant director of the Center for Toxicology. In that role, I teamed up with my great friend Dan Liebler, a former graduate student of Fred Guengerich's, to prepare an application for a center grant to NIEHS with Glenn Sipes as principal investigator. It was a heady experience to pull large teams of investigators together and plan what research cores and service cores made sense. Dan and I understood each other so well, and we even wrote in a similar fashion. We were elated when the Southwest Environmental Health Science Center was funded on the first try.

With less of my attention devoted to daily running of the lab, I became interested in outside professional activities. I learned a tremendous amount from serving on the NIH Pharmacology Study Section. It was such an honor when the review administrator, Joe Kaiser, asked me to chair the committee. I realized that I could lead a group of scientists far more accomplished than I was. In those days, reviewers would read their entire critiques before giving an initial score. Once appointed chair, I introduced the idea of giving all three scores first and, depending on the level of enthusiasm and consensus, condensing the reading of the critiques. This change was received very well and made the meetings much less tedious.

A golden decade at the University of Texas Medical Branch in Galveston (UTMB)

By 1994, I was determined to build a department and started to apply in earnest for chair positions. Progress was slow until 1997, when multiple factors worked in my favor at UTMB. 1) There were several centers related to pharmacology and toxicology, the directors of which had startup funds but no salary lines for faculty. 2) The dean of medicine, George Bernier, had just come from the University of Pittsburgh, where Pharmacology Chair John Lazo had developed a very successful program in chemical biology. Therefore, my proposal to build such a program at UTMB was received well. 3) The search committee was all center directors and department chairs, who were judging me as a peer, as opposed to faculty members picking their new "boss." I recognize that some will bristle at this approach, as did the faculty in the department, but eventually I won most of them over.

1998 was the perfect time to be a new chair. The NIH started its 5-year plan to double extramural funding, and every assist-

ant professor I recruited over the subsequent few years obtained an R01 grant on the first or second try. I made recruiting my highest priority and a very personal endeavor by spending as much time as possible with each interviewee. I picked them up at the airport and every morning. By the time I drove them back to the airport 2 or 3 days later, I had a very clear idea whether I wanted to recruit the candidate and what it would take to do so. Although the highlight of my own chemistry career was my freshman year at UCLA, I was able to achieve my dream of building a chemical biology program. With help from the Robert A. Welch Foundation and Texas Board of Regents, we established an endowed chair in chemical biology and renovated old anatomy space for chemical synthesis. Our first Welch chair, Scott Gilbertson, remains a friend to this day. Later, I became director of the environmental health sciences center, and our vice-chair and my longtime friend Kathryn Cunningham developed a center in addiction research. At our peak, funding increased 5-fold, and we rose from 61 to 21 in the NIH rankings of pharmacology departments. I was appointed editor of *Drug Metabolism and Disposition*, and, after a few changes in the editorial board, the journal's impact factor started to rise every year. The 5-year review of me and the department went very smoothly. The important lesson: **L15) As a leader, you are only as good as the people around you. Be confident and humble enough to recruit faculty members smarter and more capable than you.**

Six people moved with me from Arizona, with Tammy Domanski (29) taking the lead in organizing the transition and the lone male, Kishore Khan, the heavy lifting (30). Both excelled at UTMB in pinpointing key residues in the CYP3A4 active site. Margit Spatzenegger identified the basis for differential inhibitor selectivity of rabbit CYP2B4 and CYP2B5 (31), and Santosh Kumar engineered CYP2B1 for enhanced activity by directed evolution (32). B. K. Muralidhara (Murali) introduced isothermal titration calorimetry to our group and explored the thermodynamics of multiple-substrate binding in P450eryF, a model for CYP3A4 (33). Our work on CYP2B enzymes entered a new phase once Emily Scott joined our group. Thanks to her incredible talent and indomitable spirit and to the extreme generosity of Eric Johnson and Dave Stout, Emily was able to solve the third mammalian P450 X-ray crystal structure, namely that of a ligand-free form of CYP2B4 (34). That article in *PNAS* and a subsequent publication in *JBC* on the structure of an inhibitor-bound form (35) demonstrated remarkable conformational plasticity of CYP2B4. After Emily left, another talented crystallographer, Yonghong Zhao, joined our group and extended our understanding of enzyme plasticity (36). The work at UTMB enabled Emily's independent academic career, which has been exceptional. I believe that every mentor wants to train one person who eclipses his/her own work, and Emily is definitely that trainee for me.

I also want to acknowledge Dmitri Davydov, who brought his expertise in advanced spectroscopic methods and data analysis and his interest in protein-protein interactions to bear on the problem of CYP3A4 cooperativity. Dmitri later identified a peripheral binding site in CYP3A4 that we had predicted but never established (37), as well as the role of P450-P450 interactions in heterotropic cooperativity (38). Dmitri's personality is as strong as his love for science. The lesson: **L16) Let creative people be themselves to the fullest extent you can handle.**

Figure 4. Second Southwest P450 meeting in 2002. From *left* to *right*, Wayne Backes, Ron Estabrook, Henry Strobel, and Alfred Hildebrandt.

A huge advantage of being in Texas was the proximity to Ron Estabrook (Fig. 4) and Bill Peterson in Dallas, Bettie Sue Masters in San Antonio, and Henry Strobel (Fig. 4) in Houston. Thanks to them, we started a Southwest P450 meeting, which ran from 2001 to 2009. Other leaders in the field, such as Mike Waterman, Steve Sligar, Tom Poulos, John Dawson, and Eric Johnson, attended several meetings. Wayne Backes (Fig. 4) and I learned something important of a nonscientific nature. We had each played singles tennis in high school. During one of the afternoon breaks at the meeting, we understood why people in their 50s should play only doubles and definitely not challenge 20-year-olds to two-man basketball.

Although I did truly have a golden decade at UTMB, two factors prompted me to start to look around for other positions. 1) I was convinced that the concept of the life cycle of a department chair of "hope," "cope," and "mope" was valid. In my eighth year as chair, I was definitely starting to "cope" and did not want to discover what "mope" was like. 2) The perennial tropical storm/hurricane threats from August to October were starting to bother me seriously. I had perfected the art of packing up my office and moving the important papers upstairs in less than an hour with the use of prelabeled rubber bins. We also had our home emergency plan. However, if we were not among the first to evacuate the island, we would be stuck. Every time any storm would enter the Gulf of Mexico, the anxiety level in the department would rise, and I was hardly the person suited to quell it.

Back to pharmacy, this time at UC San Diego

Having previously shown little interest in geography and been willing to go wherever the job took me, I now asked myself

where I would like to be and at what university. The answer was obvious, namely UC San Diego, where I had interviewed 25 years earlier for a position as assistant professor. Fortunately, the relatively new Skaggs School of Pharmacy and Pharmaceutical Sciences was searching for an inaugural associate dean for scientific affairs. Because the school had no departments, one of the most important jobs would be faculty recruitment. That sounded ideal, as did living in one of the most attractive places in the U.S., where I had good friends. I would also be close to my 86-year-old father, who was living on his own after the death of my mother 10 years earlier.

UC San Diego lived up to expectation in all the above respects. We recruited a substantial number of outstanding basic science and clinical faculty members, almost doubling the size of the school. Being close to my father was such a gift, and we got to know each other better than we ever had when I was living at home. I achieved my dream of receiving the Brodie Award from the American Society for Pharmacology and Experimental Therapeutics (ASPET) and was elected ASPET president. Furthermore, a new startup package, outstanding research environment, and relative ease in attracting top-notch talent allowed us to do the best research of my career. Dmitri Davydov and Santosh Kumar had moved with me, and I was fortunate to recruit Ross Wilderman (39), Sean Gay (40), Manish Shah (41), Art Roberts (42), and Keiko Maekawa (40). We worked even more closely with Eric Johnson and Dave Stout, as well as with Qinghai Zhang. The facial amphiphiles he had developed totally transformed our ability to crystallize CYP2B enzymes (43). We finally solved crystal structures of human CYP2B6 (44), and Ross was introduced to deuterium-exchange MS (DXMS) by Virgil Woods (45). An exciting new project with Denise Dearing from Utah on the role of CYP2B enzymes in the desert woodrat (46) in dietary preference for juniper led to my first and only National Science Foundation grant.

By now we had solved ~30 CYP2B structures and had discovered remarkable ligand promiscuity and conformational flexibility largely involving backbone rearrangements of the F-G cassette and I-helix. The DXMS studies suggested that the structural changes observed in crystal structures also occur in solution (Fig. 5). Remarkably, 35 years of research using methods that did not exist in 1978 had provided answers to questions that I could not even contemplate then. I was especially gratified when, together with Paul Hollenberg, my friend of 30 years, and his colleague Haoming Zhang, Sean Gay was able to solve crystal structures of CYP2B4 covalently modified by MBI metabolites (47, 48) (Fig. 6). Each structure took less time than it had taken me at Vanderbilt to identify a chloramphenicol adduct of CYP2B1.

All was not perfect, however. At Arizona and UTMB, I had grown accustomed to being one of the top scientists. At UC San Diego, I was surrounded by National Academy members, Howard Hughes investigators, and Nobel Prize winners, and I felt decidedly average. At UTMB, I had significant institutional influence but virtually none at UC San Diego. I came to the realization that it was too hard for me to be a small fish in a big pond, no matter how beautiful the pond. The lesson: **L17) Before moving to a new university, make sure to understand your own weaknesses and vulnerabilities.** I started to look

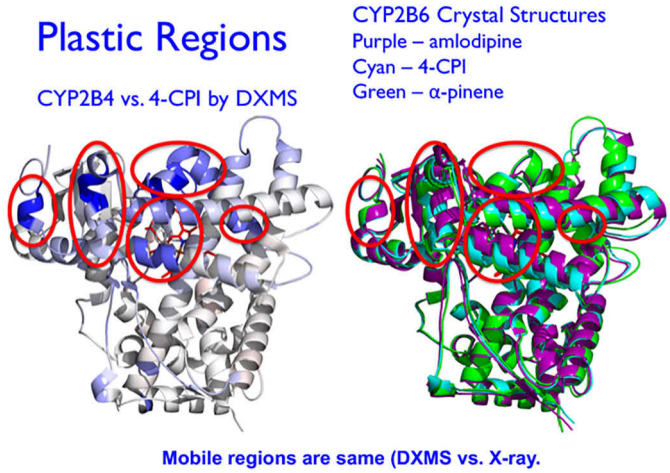

Figure 5. Mobile regions of CYP2B4 in solution (*blue*, *circled*) as identified by DXMS compared with most variable regions identified in CYP2B6 crystal structures (*circled*). *4-CPI*, 4-(4-chlorophenyl)imidazole.

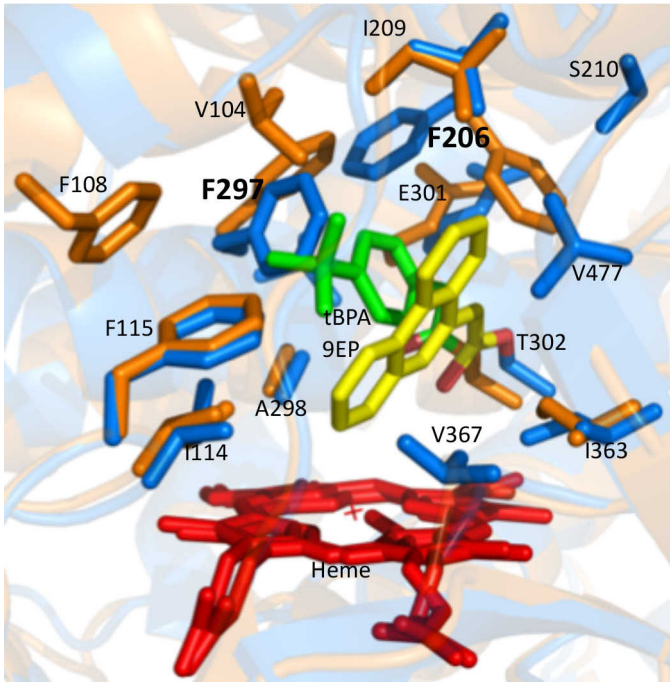

Figure 6. Active sites of covalent complexes of CYP2B4 after modification by reactive products of *t*-butylphenylacetylene (*t*-BPA, *green*) and 9-ethynylphenathrene (*9-EP*, *yellow*). The complex with *t*-butylphenylacetylene is shown in *copper*, and that with 9-ethynylphenathrene in *blue*. Note the very different position of the side chains of Phe-206 (F-helix) and Phe-297 (I-helix).

around for pharmacy dean positions with the full support of our dean at the time, Palmer Taylor.

Dean at the University of Connecticut

The job advertisement for dean of pharmacy at the University of Connecticut (UConn) seemed tailor-made for me. A Ph.D. was the preferred degree for the new dean, who would be reporting to a Ph.D. provost. The school was located on a beautiful rural campus along with the School of Nursing and the traditional main campus schools and colleges. Having lived in Sweden for 11 years, I saw the four seasons as an advantage. Above all, UConn appeared to be in the midst of a major cam-

paign to hire new faculty members in multiple scientific disciplines and increase the overall research enterprise. I arrived in June 2014 full of hope of repeating the "UTMB miracle" and with a detailed plan approved by the provost to do so.

Unfortunately, Connecticut never really recovered from the financial crisis in 2008, and UConn encountered budget cuts every year after my arrival. It was necessary to focus all my effort on maintaining faculty numbers as opposed to building. Nonetheless, there were many successes, including an almost 2-fold annual increase in external funding and a pivotal role for our school, together with colleagues in the School of Medicine and at Yale University, in launching the Program in Innovative Therapeutics for Connecticut's Health. Thanks to wonderful alumni and friends of the school, philanthropy picked up remarkably. Nonetheless, 2014–2019 was nothing like 1998–2003, which leads to my next point: **L18) Timing is everything (or almost everything), and you can do little or nothing about that.** Fortunately, two significant honors were bestowed on me during this time. An Award in Excellence in Pharmacology and Toxicology from the PhRMA Foundation closed the circle on my Faculty Development Award from 1984, and I was selected as an inaugural ASPET fellow in recognition of my research and service to the society. Finally, I am also proud to have been given high marks by the faculty for honesty and keeping promises and for obtaining resources for the school and allocating them fairly. Thus: **L19) You can maintain your core values as a dean, but it is not always easy.**

Ross Wilderman and Manish Shah moved with me to UConn along with a postdoctoral fellow, Jingbao Liu, a chemist who mastered protein biochemistry and structural biology (49). Manish solved additional structures, including woodrat CYP2B35 and -37 (50), and we thought that his observation of halogen-π bonds in numerous CYP2B but not other family 2 enzymes (51) might explain the preference of CYP2B enzymes for halogenated compounds, including the PCBs we had studied in Arizona 30 years before. However, for the first time in decades, a study section just did not accept the concept, and after more experimentation, I became dubious myself. I decided to follow my own lessons **L1, L7**, and **L12** and bow out gracefully, basically handing over the research to Ross to pursue independently. Surprisingly, it has been rather liberating not to worry where the next interesting result will come from or what the reviewers will think of our next manuscript.

Looking back

Over the past 46 years since starting my Ph.D., I have had many joys in science and maintained my love of biochemistry and fascination for enzymes. I am proud that my first publications as a Ph.D. student (4), M.S. student (7), postdoctoral fellow (10), and tenure-track assistant professor (16) were in *JBC*. Thanks to so many terrific trainees, colleagues, and outside collaborators, my research group learned more about P450 structure and function than I would ever have thought possible. After I retired my pipette, my main contributions were a willingness to 1) adopt new methodologies, no matter how daunting or far from my own expertise, and 2) start each grant application 6 months in advance of the deadline and tolerate having my mind consumed by the proposal during that time. I hope that these last two points, along with lessons **L1–18,** will be helpful to the current and next generations of P450 scientists. They have many exciting problems to solve, including using structural information about the enzymes to enhance drug design.

My greatest joy as an administrator has been recruiting faculty, mentoring them as needed, and watching their success. My involvement with ASPET has been so rewarding and allowed me to make friends outside of my own research field. Both research and administration enabled trips to many wonderful parts of the world. My one regret is that I probably moved around too much due to my own impatience. However, from the many moves, I learned the two final lessons of this article. **L20) Figure out what size fish you want to be and pick the pond accordingly; do not ever be a fish out of water. L21) If you move for an opportunity, try to pick a place where you want to be after the opportunity dries up, because it inevitably will.** Those cautions notwithstanding, I am very grateful that I picked the best possible career for myself.

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