A Professional and Personal Odyssey

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As I began to write this article reflecting on my professional career in biomedical research, many memories of people, activities, situations, and experiences were swirling in my head. Because of the extraordinary support that I have received from my family, they are never far from my thoughts. I believe that sharing a few of these memories will be helpful for those who find themselves in similar situations or periods of professional development. This is not meant to be a comprehensive review of the fields to which I have had the privilege of contributing but a personal stroll down a path in which I have found extraordinary pleasure and from which I have derived a sense of accomplishment.

The Journey Begins

I was born just prior to World War II into a middle class family in the historic town of Lexington, VA. My father was a radio announcer, a nightclub singer (he had a beautiful baritone voice), and the chosen master of ceremonies for most of the local shows in several cities in Southwest Virginia. Because my mother was a full-time parent, he had to hold several jobs to pay the bills. Because he had small children and was working in a vital industry (public information), he was not drafted during the war. Neither he nor my mother was college-educated, although both were well read and surprisingly cosmopolitan to have been raised in southwestern Virginia. Because I was his first-born and my sister came along 2 years later, all of his dreams were wrapped up in what we would accomplish in our lifetimes. One of his oft-repeated admonitions was, “I don’t care what you become as an adult, as long as you do your job well.” However, it never escaped my attention that he wanted us to become professionals. Hence, the fact that we lived next door to a physician, his wife, and two boys had the inevitable effect that we observed that their somewhat higher grade of lifestyle (they owned their home, but we rented our two-bedroom apartment) was achievable.

Both of my parents wanted desperately for their daughters to obtain college educations and they both insisted that we could “be anything we wanted to be.” Unfortunately, I was not born with the artistic talent with which my sister and my brother (who was to arrive 22 years later during my first year in graduate school) were endowed, so my talents had to be sought elsewhere.

In those days, we were known as “tomboys,” and there was little hope for our interests to be more feminine, despite my mother’s attempts, because our only playmates were boys on either side of us. We loved to climb trees, especially the cherry tree between our houses, and to play football and baseball and, best of all, cowboys and Indians. When my parents were vehemently against my getting a football for Christmas, I told my grandmother, and she made sure it was among her gifts for me, much to my mother’s dismay. I loved to collect things, and I particularly loved outdoor activities. By the time I reached high school age, I had read Sir Arthur Conan Doyle’s “The Adventures of Sherlock Holmes”; I admired Holmes’ and Dr. Watson’s analytical skills as sleuths, and perhaps this was an early indication that I would like to solve mysteries of another type. As I matured and entered high school, the only one in Radford, VA, where my father took the position of Program Director for a new radio station, my interests in sports remained, but my teachers
perceived other talents. While taking ballet and tap lessons, when I could work them in around varsity basketball practice, I was able to maintain the highest scholastic average in the school and graduated as valedictorian of my class. In my sophomore year, my chemistry teacher discovered my interest in science and encouraged it. He was a very quiet, "Mr. Peepers"-type man, but he had a twinkle in his eye, and he knew how to draw out his students if they were at all sensitive to his guidance and teaching. In my junior year in high school, I entered the Westinghouse Science Talent Search and placed such that I was given a scholarship to the college of my choice. At that time, I had decided, for some reason unbeknownst to me now, that I wanted to attend William and Mary College in Williamsburg, the oldest land grant college in the United States. Whereas I was readily accepted there, the Dean refused to honor a science scholarship for a woman, so my hopes of attending were dashed. Instead, Roanoke College, a small liberal arts college located in Salem, VA, honored the scholarship and enabled me to complete a double major in biology and chemistry.

As fate would have it, the Chairman of the Classics and Fine Arts Department, Dr. Miles S. Masters, took notice, and when his youngest son, 1st Lt. Robert Masters, arrived home for a leave from active duty as a Marine jet pilot, we met: no matchmaking there! It did not take long for me to realize that "this was the guy," and when he was stationed for active duty in Japan for a year, the letters flowed back and forth while I completed my freshman year and began my sophomore year. My years at Roanoke College seemed to speed by, with labs scheduled 4 days per week leaving very little leisure time, except for playing varsity basketball for a couple of years, participating in biology and chemistry club activities, and editing the school yearbook with a college friend. I graduated as salutatorian of my college class.

The pursuit of a medical degree had been foremost in my mind since I became a teenager, but the economics of my family situation held no hope of this coming to fruition. The next step in my life became very clear when my comparative anatomy professor urged me to consider a career in biomedical research with a major in biochemistry. This was great advice, except that I had no idea what biochemistry was because Roanoke College did not have a biochemistry course in its curriculum. Not being afraid of challenges, I applied with the help and encouragement of my professors and a competitive score on the Graduate Record Examination Aptitude Test to the institutions in the United States best known for their biochemistry programs. At that time, the University of Wisconsin, The Johns Hopkins University, Ohio State University, and Duke University were among the forerunners, and I was accepted into all of the programs with some support from three of them. I entered the Duke University Biochemistry Graduate Program during Dr. Philip Handler’s chairmanship and enjoyed the outstanding teaching of a number of notables, among them, Dr. Handler, Dr. Irwin Fridovich, Dr. Salih Wakil, Dr. Eugene Davidson, and Dr. Henry Kamin. These men (there were no women on the faculty at that time) challenged all of the students as they redesigned the graduate curriculum with our class as guinea pigs. To qualify for Ph.D. candidacy, we had to pass a series of preliminary examinations based on different areas of biochemistry, known only to those who generated the examinations. The students found this guessing game somewhat frustrating, but it did force us to read everything that was coming out in the biochemical literature, as well as to review our notes. I then interviewed with those faculty members whom I believed I could work well with and chose Dr. Henry Kamin as my mentor. This was probably one of the defining decisions of my life insofar as my career was concerned. Dr. Kamin was not only a great teacher and an innovative thinker, but he was truly a Renaissance man who was a lover of history, music, and art; a gourmet cook; and a golf enthusiast. He was known to play golf in faraway places such as Ireland and Australia, but his widow, Dottie, says never very well. He was a master of the English language, and his ability to dictate a manuscript over a Dictaphone directly from my data notebooks, with me at his side discussing the experiments, was unbelievable. The most incredible thing was that these manuscripts were accepted for publication, without further revision, by the Journal of Biological Chemistry on several occasions. His wife, Dottie, a graduate of the University of North Carolina School of Nursing, had become a research associate in pharmacology by this time but remained the “nurse” for numerous faculty members and their families, including me. She was present in the delivery room when our first daughter, Diane, was born in Duke Hospital, trying to keep me calm and cool during the non-air-conditioned July heat.

When I arrived at Duke, Charles Williams had just completed his Ph.D. training under Dr. Kamin and had taken a postdoctoral fellowship position with Prof. Vincent Massey at the University of Sheffield in England. Dr. Williams’ Ph.D. dissertation addressed the cellular localization of TPNH (NADPH)-cytochrome c reductase in the microsomal fraction of pig liver, and Williams and Kamin (1) and Phillips and Langdon (2) published the localization of this activity simultaneously in the Journal of Biological Chem-
In 1950, Horecker (3) had first identified this NADPH-cytochrome c reductase activity in whole liver acetone powder but could not determine its cellular localization from his studies. In 1955 and 1957, respectively, La Du et al. (4) and Gillette et al. (5) reported that the addition of cytochrome c inhibited the TPNH (NADPH)-mediated oxidative dealkylation of monomethyl-4-aminantipyrine and other alkylamines catalyzed by liver microsomal fractions and that this process required oxygen. In the latter article, Gillette et al. (5) were the first to report the generation of hydrogen peroxide in the absence of substrate in these microsomal fractions, an observation that was to have significance much later in the interpretation of data from studies of microsomal oxidases and oxygenases. The discovery of oxygenases, the enzymes that catalyze the incorporation of the atom(s) of molecular oxygen into organic molecules, was made by Hayaishi et al. (6) with pyrocatechase from a pseudomonad and by Mason et al. (7) with a phenolase complex from mushrooms in 1955, but the connection had not yet been made to the NADPH-requiring system in liver microsomes. Following the reports by Klingenberg (8) and Garfinkel (9) in 1958 of a reduced carbon monoxide-binding pigment in mammalian liver microsomes with an absorbance at 450 nm, Omura and Sato (10) published the purification and characterization of a heme-binding protein from rabbit liver microsomes that they determined to be a b-type cytochrome. Although the absorbance maximum of the reduced CO difference spectrum of this purified hemeprotein was at 420 nm, future studies were to show that this was the CO-binding pigment observed by Klingenberg and Garfinkel with an altered absorbance maximum. Thus, the groundwork was laid for a connection to be made between the existence of this hemeprotein and the fixation of O$_2$. This connection was made by Estabrook, Cooper, and Rosenthal (11) and Cooper et al. (12) using the photochemical action spectrum methodology of Warburg. By reversing the CO inhibition of the hydroxylation of 17α-hydroxyprogesterone by adrenal microsomes and the oxygenation of codeine, acetanilide, and testosterone by liver microsomes by light with maximal absorption at 450 nm, they proved that this hemeprotein was the terminal oxidase in these reactions.

Following this period, between 1960 and 1965, I was working on the mechanism of the protease-solubilized microsomal NADPH-specific (NADPH, nicotinamide-adenine dinucleotide phosphate, was called triphosphopyridine nucleotide then) flavoprotein (missing its N-terminal membrane anchor) that reduced cytochrome c and several artificial electron acceptors but whose role in cytochrome P450-mediated reactions remained unproven (13, 14). During this time, I was challenged by Dr. Kamin to purify sufficient quantities (~100 mg from porcine liver, not an easy task) of the reductase to take to Sheffield, England, where I would be able to work with Drs. Quentin H. Gibson and Vincent Massey, gurus in stopped-flow and static spectrophotometry of heme- and flavin-containing proteins, to characterize it kinetically and spectrally. The gauntlet was dropped, and I worked night and day for 6 months to meet this challenge. In the spring of 1962, I left for the University of Sheffield, where Charles Williams was a postdoctoral fellow with Dr. Massey, to perform a raft of experiments that would become the backbone of my dissertation and the subject of two papers in the *Journal of Biological Chemistry* (Fig. 1). As a graduate student and then a postdoctoral fellow, supported by the American Cancer Society and later by the American Heart Association, I was able to show that the enzyme contained 2 mol of flavin/mol of protein and that its catalytic mechanism required one of those flavins to be fully

**FIGURE 1. Bettie Sue Siler Masters at the University of Sheffield in the United Kingdom as a graduate student admiring her favorite flavoprotein, NADPH-cytochrome P450 reductase, then known as TPNH-cytochrome c reductase.** After spending 6 months preparing sufficient quantities of the enzyme, I worked in collaboration with Drs. Quentin H. Gibson and Vincent Massey to obtain stopped-flow kinetics and static spectrophotometric data, respectively.
reduced; the 1-electron-reduced semiquinone form of the enzyme was inactive toward any of the electron acceptors tried (13, 14). I had also contributed to the Williams and Kamin publication (1) with observations that NADPH reduced cytochrome b$_5$ in microsomal preparations but the protease-solubilized, purified enzyme did not. This suggested that the preparation was defective or that a factor was missing. It was shown later that the full-length detergent-solubilized enzyme reduced cytochrome b$_5$. Because my predecessor, Charles Williams, had shown that FAD was a component of his preparations, we were remiss in not re-examining my preparations of reductase, which eliminated the acid precipitation and high ionic strength ammonium sulfate fractionation steps (13) that tend to release noncovalently bound flavins from their apoproteins. Somewhat later, in 1973, Iyanagi and Mason (15) showed definitively that NADPH-cytochrome c reductase, in both the proteolytically solubilized form and the full-length, detergent-solubilized form, contained 1 mole each of FAD and FMN. In 1974, Iyanagi et al. (16) determined from the redox potentials that one of these flavins acted as the entrance flavin and that the other was involved in the exit of electrons, although they did not identify which flavin had the higher potential. Vermilion and Coon (17) showed that FAD accepted electrons from NADPH and then shuttled the electrons one-by-one into the FMN of the reductase, from which the electrons exited to various electron acceptors.

Bob Masters and I were married after our first year in graduate school, and after he earned his MBA at the University of North Carolina, he joined the young company Scientific Products of American Hospital Supply Corp. After a very productive graduate training period, lasting 3 years and 9 months, I decided to remain with Dr. Kamin for additional postdoctoral training and to expand on the studies that I had begun as a student. Our first daughter, Diane, was born barely a month after I was awarded my Ph.D. degree at Duke; my husband and I moved into our first home in Durham that same summer, and a new phase of our lives was to begin.

My American Cancer Society postdoctoral fellowship was to begin immediately, so there was little time to enjoy those first months with our baby daughter. During this period, our second daughter, Deborah, was born, and our days were filled with our family and career commitments as I began an advanced research fellowship with the American Heart Association. My husband was traveling most of the week, and so we engaged full-time help with our toddlers. These were very busy but extremely happy times as we made time for trips to Virginia to visit family and enjoy tent camping by lakes, in the mountains, and at the North Carolina seashore. By the time this training period was to end and my husband, Bob, was ready to move ahead in his company, I had successfully obtained an American Heart Association established investigatorship and a grant-in-aid.

Can the Bird Fly from the Nest?

Let the games begin. It was now time for me to prove to myself and others that I could become an independent scientist and contribute to my research area in a meaningful way. When my husband and I were looking at positions that would be mutually compatible, the choices boiled down to Atlanta or Dallas. During a phone call with Dr. Ronald W. Estabrook, who was to become the new Chairman of Biochemistry at The University of Texas Southwestern Medical School in Dallas, Dr. Kamin mentioned that I had a choice between the two cities. A job offer was made on the spot. Dr. Estabrook had heard me present at a Federation meeting in Atlantic City, and we had met later in Philadelphia at the Johnson Foundation, where I was performing some stopped-flow kinetics studies with Dr. Quentin Gibson, the co-inventor of this technology. My good fortune was to receive the blessing of Dr. Kamin, as I flexed my muscles and tried my wings, and to be given the reductase “problem” to pursue as an independent investigator. Armed with American Heart Association support for salary and a research grant, I arrived in Dallas in the summer of 1968, among a group of four new recruits, to join Dr. Estabrook’s department. I was extremely proud of my first laboratory, all 400 square feet of it, and turned a few heads when I decided to paint the walls above the tile wainscoting a peachy pink. These years were to be highly motivating and productive, extremely busy with a young family, and very exciting as the young Southwestern Medical School gained national prominence due to its visionary leadership, beginning with Drs. Charles C. Sprague and Donald M. Seldin in the late 1960s and their wise decision to build up the basic sciences there.

Evidence accumulated from several laboratories in the late 1960s and early 1970s that the microsomal flavoprotein that I had been studying was the physiological electron donor to cytochrome P450 in liver microsomes (18–21). Utilizing antibodies prepared against the proteolytically solubilized NADPH-cytochrome c reductase, these laboratories demonstrated that inhibition of NADPH-dependent, cytochrome P450-mediated oxygenation of drugs and steroids was obtained. Having in hand the purified preparations I had brought from Duke, I was able to prepare antibodies and to begin our studies on the microsomal metabolism of drugs and ste-
roids immediately (21). Because steroid metabolism was catalyzed by adrenal cortical tissues, we performed immunochemical titration studies on microsomes and mitochondria from the adrenal cortex as well. We decided to prepare antibodies to adrenodoxin, a mitochondrial iron-sulfur protein electron carrier, to compare the effects in the two organs and subcellular fractions. These data showed unequivocally that concomitant inhibition of TPNH (NADPH)-cytochrome c reductase and ethylmorphine demethylation in pig liver microsomes (Fig. 2) was obtained upon titration with anti-reductase γ-globulin. As a bonus to these studies, which confirmed that NADPH-cytochrome c reductase was indeed NADPH-cytochrome P450 oxidoreductase (CYPOR), we were able to show that adrenal microsomes contained a similar activity. Antibodies to the reductase inhibited both liver and adrenal mitochondrial cytochrome c reductase activity but had no effect on NADPH-mediated cytochrome c or P450 reduction by adrenal mitochondria. In addition, because adrenal cortical mitochondria had been shown to catalyze steroid hydroxylations, we determined that the source of electrons was not NADPH-cytochrome P450 oxidoreductase but was dependent, instead, upon the NADPH-mediated pathway in mitochondria involving adrenodoxin. Antibodies to adrenodoxin inhibited only mitochondrial cytochrome P450 reduction. These results demonstrated that the microsomal and mitochondrial P450-mediated pathways involved different electron transport enzymes.

These studies supported the conclusions of Lu, Junk, and Coon (22), who had reconstituted the cytochrome P450-mediated hydroxylation of lauric acid with purified CYPOR, cytochrome P450, and a lipid fraction from rabbit liver microsomes. This was the first successful reconstitution of any cytochrome P450-mediated oxygenation system, and although the substrate was not a drug or a steroid, their beautiful data strongly supported the role of NADPH-cytochrome P450 reductase. In their second publication, however, Lu et al. (23) showed that benzphetamine, aminopyrine, ethylmorphine, and hexobarbital were all metabolized by their reconstitution system. Laurate and benzphetamine were found to be mutually inhibitory, as would be expected if a common “methyl hydroxylase” was involved. The second publication identified the necessary third fraction as a heat-stable, non-protein component that was soluble in organic solvents and behaved like a lipid, which would be expected for optimal activity of membrane-bound components. It is interesting that Lu et al. (23) made the statement that “Although the existence of multiple distinct forms would account for the broad specificity (of the cytochrome P450-mediated reactions), no more than one form of P450 has been identified with certainty by spectral methods.” Whereas this statement was certainly true at that time, to those of us who have labored in this area of research for many years, this has become the ultimate understatement in that thousands of cytochrome P450 genes have now been identified throughout phylogeny, and no fewer than 57 P450 genes exist throughout the human body.

Another microsomal activity that attracted much interest during this time was the enzyme system that degraded...
heme to bilirubin. It was shown by Rudi Schmid’s group (24, 25) that the system required NADPH and molecular O₂, and because of the function of cytochrome P450 in microsomal oxygenation reactions, the prevailing thought was that cytochrome P450 could serve as the terminal oxygenase in the formation of bilirubin. In fact, this group published an article in 1972 that seemed to provide evidence via a photochemical action spectrum that heme oxygenation was catalyzed by cytochrome P450 (26). In 1972, my laboratory collaborated with Schacter and Marver (27) and showed that CYPOR was required as a source of electrons for heme degradation catalyzed by rat and pig liver and spleen microsomes, which gave credence to the idea that cytochrome P450 could serve as a heme oxygenase. This was, however, not the case, and the actual isolation and purification by Mainess et al. (28) of an enzyme that performed this function put this hypothesis to permanent rest. So, an additional physiological electron acceptor was found for CYPOR.

In a review in Methods in Enzymology, I pointed out that the employment of antibodies as diagnostic tools for determining the involvement of enzymes in specific metabolic pathways (29), as well as for tissue and subcellular localization, had proved to be quite useful. For example, it was shown by Thompson and Siiteri (30), using polyclonal antibodies to CYPOR provided by our laboratory, that the aromatization reaction was inhibited, providing further evidence that the three oxygenation steps required for the formation of estrogen from testosterone were catalyzed by a cytochrome P450, later isolated and identified as CYP19A1. Because this reaction was notoriously refractory to CO inhibition, the role of cytochrome P450 in aromatization had been debated in the literature, but this report verified its requirement in the formation of estrogen.

During my 14 years at Southwestern Medical School as I advanced through the academic ranks, I had the privilege of interacting with many outstanding individuals. Foremost among these was Ron Estabrook, the outstanding scientist and newly recruited Chairman of Biochemistry who, as mentioned earlier, offered me my first tenure-track position in academia. Ron was a hands-on scientist who, as mentioned earlier, offered me my first tenure-track position in academia. Ron was a hands-on scientist who spent many hours in the laboratory and loved to show medical and graduate students the mechanics of an experiment to explain how the facts that appear in textbooks are actually obtained. The most memorable was an experiment that he set up using a cuvette containing a mitochondrial fraction mounted with a prism to disperse the wavelengths from a slide projector lamp onto a screen to show how the addition of a reductant produced certain bands that were identified by early investigators as the mitochondrial respiratory pigments. He challenged his faculty constantly, always offering a word of praise before he uttered the word “but” and proceeded to ask a question that would challenge the evidence presented or conclusions drawn. Despite (or because of) this way of motivating the biochemistry faculty, he successfully sent at least half a dozen of his faculty members on to chairmanships at other institutions. He was named to the United States National Academy of Sciences for his studies using the Warburg technique of the photochemical action spectrum (reversing CO inhibition of cytochromes P450 at the optimal wavelength of 450 nm), proving that this pigment was the terminal oxygenase for drug and steroid metabolism by microsomes (11, 12). Ron Estabrook has remained a steadfast supporter, colleague, and friend for over 40 years.

The purification procedure for NADPH-cytochrome P450 reductase was a laborious one, and there were tantalizing reports of biospecific affinity chromatography methods being developed for other enzymes. Dr. Yukio Yasukochi, a visiting professor in my laboratory in Dallas from the University of Tokyo, and I had been attempting to produce our own affinity medium, but our attempts using various coupling procedures for specific ligands for reductase failed to permit release of our enzyme after it was bound or did not bind the enzyme at all. Fortunately, Dr. Klaus Mosbach from Lund, Sweden, was invited as a visiting professor at Southwestern Medical School by Dr. Paul Srere, Professor of Biochemistry. During his visit, he presented a short course on affinity chromatography, and I asked for his help. He made it possible, before his 2’,5’-ADP-Sepharose 4B reached the market through Pharmacia (now GE Healthcare), for us to attempt the purification of CYPOR from rat and pig liver microsomes. Dr. Yasukochi used this chromatographic medium, which took advantage of the specificity of binding of the 2’-phosphate of NADPH, with CYPOR from both sources and produced extremely pure reductase from microsomal preparations (32). Not only has it been the method of choice throughout the field of cytochrome P450 research, but it found its way into the hands of the laboratories of Marletta, Mayer, and Stuehr (33–35), who used it to purify macrophage (inducible) and neuronal nitric-oxide synthases (nNOSs). These enzymes bear ~60% sequence homology in their ~570–640 C-terminal residues, containing both FAD and FMN, to NADPH-cytochrome P450 reductase, as first reported by Bredt et al. (36) for nNOS. Of course, the NOSs are also NADPH-dependent enzymes. To date, this publication in the Journal of Biological Chemistry (32) has been cited over 1300 times and exceeds all other citations of my publica-
tions, illustrating the utility and/or popularity of methods papers.

The excitement over the biospecific affinity chromatography method had spread abroad, and our Japanese colleagues were quite eager to learn how to use it for their experiments. This led to an invitation from the laboratories of Drs. Tsuneo Omura and Ryo Sato to spend a sabbatical as a Japan Society for the Promotion of Science Visiting Professor. So, in 1978, I packed up for the long journey, and among my treasures were several bottles of this new chromatography medium that my Japanese colleagues were eager to try. This 3-month period proved to be one of the most exciting and productive of my career as I worked with students in both the Institute for Protein Research in Osaka and Kyushu University in Fukuoka under the guiding influence of these two great men, who had purified and characterized the first cytochrome P450 (10, 37). With two teenaged daughters at home, I could not extend my stay in Japan, but they were able to visit me with their paternal grandfather and experience this wonderful, exotic country firsthand. My husband, presumably anxious for my return, came to Tokyo, my last stop among many cities and universities, to help me carry all the bags and gifts from my new-found friends back to the United States.

During this time, we had begun to examine the \( \omega \)-hydroxylation of fatty acids, beginning with lauric acid, which had been shown to be a very active substrate in kidney microsomal preparations by our Swedish colleagues. Orrenius and co-workers (38) had reported that kidney microsomes catalyzed the cytochrome P450-mediated oxygenation of fatty acids more efficiently than most other substrates. My first graduate student examined the role of CYPOR in this activity and was able to demonstrate the similarity of the liver and kidney microsomal reductases (39). Shortly thereafter, Dr. Richard Okita joined my laboratory as a postdoctoral fellow, and it was during his fellowship training that he spearheaded the studies of the microsomal cytochrome P450-mediated metabolism of arachidonic acid (40). At this time, we also examined the metabolism of arachidonic acid by purified components from liver and kidney microsomes, produced by Dr. Yasukochi. In collaboration with Drs. Ron Estabrook and Jorge Capdevila, Dick Okita and I demonstrated that purified cytochromes P450 could catalyze the \( \omega \)-hydroxylation of arachidonic acid in reconstitution systems containing CYPOR and dilauroylphosphatidylcholine (41). Later, these metabolites of arachidonic acid proved to be important in the regulation of blood pressure, with \( \omega \)-hydroxyeicosatetraenoic acid (20-hydroxyarachidonic acid) acting as a vasoconstrictor in certain vessels.

In the meantime, my husband, Bob, was working for Scientific Products, owning and running a travel agency, and maintaining his jet pilot skills as a reservist in the United States Marines throughout our 14 years in Dallas. Our daughters completed most of their K-12 education at Greenhill School, which was to prepare them both extremely well for their college educations and subsequent careers and life interests. Bob retired from flying as a Lieutenant Colonel after 27 years, with his last flights being in the F8U Crusader out of the Naval Air Station in Dallas. He claims that he would not trade anything for those years and only regrets there is no civilian flying that can substitute for it!

**Chairing a Biochemistry Department?**

At the age of 38, I was asked to examine a position as chair of a biochemistry department. The offer was tempting, and it appeared to be an exciting opportunity to test my abilities to direct a biochemistry department in its teaching and research programs. Upon careful reflection, I decided that I was too young to tackle such a responsibility at that time and remained at Southwestern for 6 more years before accepting a position as Chair of the Department of Biochemistry at the Medical College of Wisconsin in 1982. Of course, I was frightened at such a move. In addition to the mortgage interest rates having risen to 16%, we were making a drastic change in climate from the mild winters and hot summers in Dallas to the sub-zero, snowy winters and very short summers in Milwaukee. However, the challenge was exciting, and the faculty and I were able to address curricular changes for teaching medical students, recruit new faculty, and build vital resources for the department and the institution. Some of our efforts required some innovative thinking due to limited finances in a private institution. Several of the faculty members recruited during my tenure remain there and have performed extremely well in their respective fields, and of course, some have left for other challenges, including department chairmanships and program and center directors. Also, during the initial period of my 8-year tenure there, I learned of Dr. Helmut Beinert’s imminent retirement from the University of Wisconsin, and knowing that he remained extremely active (he served as a member of the advisory committee to the National Institutes of Health (NIH)-funded Electron Paramagnetic Resonance Center at the Medical College of Wisconsin), I convinced the administration to offer him a Distinguished Scholar-in-Residence position and research space to join our faculty. Dr. Beinert’s presence and mentorship have been invaluable to our students and faculty, and I am grateful for the opportunity to work with him.
ert continued to be extremely successful in winning NIH support for his research, and he brought a colleague, Dr. Mary Claire Kennedy, with him to Milwaukee. The two of them contributed greatly to our research reputation, as well as to our teaching program, throughout my tenure there and for some years after I left. Dr. Beinert died in 2007 at the age of 94, still working at the bench, writing excellent papers and reviews, and presenting plenary talks at meetings.

As a department chair, I felt very strongly that I needed to maintain my own research laboratory at the highest level of activity possible, so I wrote grants along with everyone else. Our studies during this time had focused very much on the inducible cytochrome P450 in lung microsomes that metabolized prostaglandins to their ω-hydroxylated derivatives. At the same time, the Department of Biochemistry recruited Dr. Richard Okita, my former postdoctoral fellow who had initiated our studies on arachidonic acid and prostaglandin metabolism in my lab at Southwestern Medical School. He launched his independent career in the study of 15-hydroxyprostaglandin dehydrogenases (42, 43) and left the Medical College of Wisconsin for Washington State University later, where he became Acting Chair of the Department of Pharmaceutical Sciences. He is now a program official at NIGMS. While we were at the Medical College of Wisconsin, a resourceful postdoctoral fellow, Dr. David E. Williams, demonstrated that pregnancy induced a cytochrome P450 in rabbit lung that catalyzed arachidonic acid and prostaglandin E₂ and F₂α ω-hydroxylation (44). Dr. Williams also discovered a flavoprotein in pulmonary microsomes that was induced by pregnancy and that he characterized as a member of the flavin-containing monooxygenase family (45). These studies were to launch his career at Oregon State University, where he rose through the academic ranks and is now Director of the Marine and Freshwater Biomedical Sciences Center. Although no physiological function has been determined for the cytochrome P450 ω-hydroxylase in lung tissue (now called CYP4A4), a graduate student, A. Scott Muerhoff, was able to show that cytochrome P450 protein, translatable mRNA, and enzymatic activity followed identical patterns during gestation and dropped precipitously toward undetectable levels just prior to parturition (46). He is a successful career scientist at Abbott Laboratories, where he has won recognition and advancement through the Volwiler Society. At the same time, I was recruiting several postdoctoral fellows to continue the work ably conducted by my laboratory group, managed by Marie Leithauser, who also performed some seminal experiments on the isolation and characterization of metabolites of prostaglandin A₁ by whole perfused lungs from untreated and pregnant rabbits using mass spectrometry. These studies showed that pregnancy induced the ω-hydroxylation of prostaglandins in whole lung, through which over 90% of the blood circulates (47). Marie has been highly successful at Bristol-Myers Squibb Co., having been recently promoted to Executive Director. While Scott Muerhoff was completing his Ph.D. requirements, I was learning molecular biological techniques during a mini-sabbatical with Dr. Eric Johnson at The Scripps Research Institute in La Jolla. This 3-month stint at Scripps initiated a collaboration to which members of our laboratory contributed enzymatic studies upon the cloning and expression of three cytochromes P450 from rabbit kidney, the first of the ω-hydroxylases to be cloned (48, 49). During my sabbatical, I received an invitation from Dr. Merle Olson, the Chair of Biochemistry at The University of Texas Health Science Center at San Antonio, to examine the position of the first Robert A. Welch Foundation Endowed Chair at that institution.

**Returning to Direct a Research Laboratory**

When I mentioned the possibility of returning to Texas, my husband, Bob, began to fantasize about the warmer climate and our previous life there. We both loved what we had seen in San Antonio, where we had lifelong friends. After selling our home in Milwaukee County and purchasing one in San Antonio, Bob and Deborah, our younger daughter, packed up to move 5 months ahead of my contractual agreements with both institutions. As the moving truck left our driveway, with the snow flying in February, Bob was heard to say, “If we can get traction, we’re out of here!” And there I was to live with our older daughter, Diane, for the next 5 months in a charming little historic town north of Milwaukee called Cedarburg. It was a wonderful way to end one career and begin another.

Having examined the position in San Antonio, realizing the honor and privilege of holding a Welch Foundation Chair, and knowing the benefits of living in this culturally rich Texas city, I moved my laboratory with five young scientists to San Antonio in 1990 and resumed our experiments relating to arachidonic acid and prostaglandin metabolism, as well as our longstanding research on CYPOR mechanism. To propel our program in molecular biology forward, I sent Dr. Linda Roman, one of the new postdoctoral fellows, to Dr. Johnson’s laboratory at Scripps to follow up on what I had started. She returned with the four clones of the cytochrome P450 4A gene family from rabbit lung and kidney, with which she expressed and purified the wild type and several chimeric constructs of these enzymes from *Escherichia coli*. Our collaboration...
An Exciting Discovery

One of the most exciting happenings after our move to San Antonio occurred in 1991 when I received a phone call from Dr. Beinert alerting me to a publication in *Nature* written by Dr. Solomon Snyder’s group (36), who reported the 36% sequence identity and 58% sequence homology of the 641 C-terminal residues of nNOS to CYPOR. He urged me to step into the field and apply the techniques that we had already used in our studies of CYPOR to examine the enzymology of NOS. After some hesitation about taking on too much, we initiated our studies by convincing a new graduate student, Kirk McMillan, to begin his dissertation work on an enzyme system on which we had never performed an experiment. His decision turned out to be a wise one, and with due diligence, an ability to multitask, and excellent analytical abilities, Kirk showed that nNOS contained heme with a reduced CO difference spectrum identical to that seen with the cytochrome P450 family of hemeproteins (51) using a stably transfected clone in human embryonic kidney 293 cells obtained from Snyder’s laboratory. We probably would not have recognized the possibility of heme as a constituent had our minds not been prepared for such due to our previous experience. Kirk showed that the heme was a $b$-type cytochrome by determining its pyridine hemochromogen character and later determined the cysteine thiolate liganding residue, characteristic of all cytochromes P450, by site-directed mutagenesis (52). These studies led us into a whole new world of flavin- and heme-containing enzymes; until this time, CYPOR was the only known mammalian flavoprotein to contain both FAD and FMN. Contrary to the cytochrome P450-mediated systems, the flavins and heme of the NOSs were bound to the same polypeptide chain, and their constituencies of tetrahydrobiopterin ($H_4$B) and a tetrahedrally coordinated zinc ion set them apart from these multicomponent systems. After an initial rebuff by an NIH review panel that asserted that we could not generate modules of these proteins containing separately heme (oxygenase domain) and flavins (reductase domain), we proceeded to show that we had already succeeded in doing so. Dr. Essam Sheta, a Fulbright Scholar from Egypt, was able to separate these domains by limited proteolysis using immobilized trypsin and to demonstrate that each domain maintained its respective enzymatic properties (53). This success led Kirk McMillan to clone and express the domains independently in *E. coli* expression systems (52), and subsequent studies, including a number of EPR, site-directed mutagenesis, spectroscopic, enzymological, and crystallographic experiments, were to follow using these various constructs.

There was also a need for comparing studies of these domains with a full-length construct of NOS. Dr. Linda Roman responded to this task with her strong background in molecular biology by perfecting a very efficient expression system in which she subcloned the nNOS cDNA into a pCW<sub>ori</sub> vector under the control of a $tac$ promoter and coexpressed this with the chaperonins GroEL and GroES into a protease-negative strain (BL21) of *E. coli* (54). This highly cited procedure resulted in the high level expression of the holoenzyme that was needed for all of the biophysical studies that were to follow.

During this time, Dr. Pavel Martašek joined our laboratory as a visiting scientist from the Czech Republic for a period of 3 years. Dr. Martašek’s interests lay in examining the endothelial isofrom of NOS (eNOS), so he began a series of experiments using eNOS expressed in *E. coli* (55) following a variation of the procedure of Roman et al. (54). These purified preparations of eNOS were then available for the detection of superoxide anion ($O_2^-$) and for spectrophotometric and crystallographic studies. The first of many studies demonstrated the production of $O_2^-$ by EPR spectroscopy (56). The protection (or coupling to NO production) shown in the presence of L-arginine and $H_4$B made this publication a highly cited one, as it presented a paradigm for the control of NOS function *in vivo*. The implications of the disparate actions of fully reduced and oxidized $H_4$B metabolites in the control of eNOS biochemistry were discussed in light of clinical data indicating that $H_4$B levels are important in the regulation of superoxide levels and of endothelial reactivity. Dr. Martašek, with C. S. Raman, a student in the Department of Biochemistry who later became a postdoctoral fellow in Dr. Thomas Poulos’ laboratory at the University of California, Irvine, set up a series of crystallization trials with various constructs of eNOS and finally succeeded with the eNOS oxygenase domain. This enabled determination of the x-ray structure of eNOS.
structure at 1.95 and 1.9 Å of the eNOS heme-containing domain dimer in the absence and presence of H4B, respectively (57). It was this structure, along with the very detailed biochemical studies aided by Dr. Timothy Miller (58, 59) in our laboratory, that led to the identification of a tetrahedrally coordinated zinc ion in the eNOS and nNOS isoforms, a fact that was to be confirmed not only by further studies in our laboratory but by a number of other groups, including another crystallography group (60). The occurrence of zinc was a surprise because the ~3000 Å² of interface between the monomers provide a very tight junction between the monomers of the heme domain dimer, which seemed sufficient to maintain the dimeric structure. Again, although there did not appear to be compelling reasons for the various cofactor substituents in the NOS enzymes, they continued to surprise us and other scientists with their complexity. So, it became our task, and that of other laboratories, to determine what the functions of the various cofactors were, their locations within the structures of NOSs, and how they were involved in the regulation of enzymatic activity. Dr. Raman and colleagues wrote a scholarly review for *The Porphyrin Handbook*, which, even at the present time, is used to guide the questions asked about the NOS isoforms as to how their structural properties determine their functional activities (61).

While the x-ray studies were being pursued, a parallel study was undertaken with Dr. Brian Hoffman’s group using the spectroscopic technique of electron nuclear double resonance. The good fortune of being able to combine heme iron interactions with 15N-labeled substrates and to examine the electron nuclear double resonance spectra of these complexes led to the location of the guanidino nitrogen atom of l-arginine to within 4 Å of the FeN₄ core of the heme (62). This was the first demonstration of the proximity of the reactive guanidino nitrogen of l-arginine to heme iron in any of the NOS isoforms, but its publication was unnecessarily delayed when a reviewer misplaced the manuscript, and the crystallographic structures appeared in the literature first.

**In the Meantime**

Our daughters graduated from their respective undergraduate institutions, the older one from the University of Texas at Austin and the younger from my husband’s and my alma mater, Roanoke College, in the foothills of the beautiful Blue Ridge Mountains in Virginia. Diane pursued graduate studies in anthropology and museology, and Deborah abandoned her business degree for the pursuit of elementary education. Within 3 years of our move to San Antonio, Deborah married a young man who had decided to join my laboratory after graduation from Massachusetts Institute of Technology to discover what he wanted to pursue as his life’s work. That decision was to become a pediatric cardiologist with a subspecialty in echocardiography, and Dr. and Mrs. Michael G. W. Camitta now live in Durham, NC, where Mike is now the Medical Director of the Pediatric Echocardiography Laboratory at Duke University. Diane, who married her artist/sculptor colleague Mark Iwinski, whom she met at the Milwaukee Museum of Art, where they both worked, joined her sister and family in Durham in 2007 from Ithaca, transitioning from the Center for the Study of Economy and Society at Cornell University to the Center for Cognitive Neuroscience at Duke University, where she is a research administrator. Both families seem to be settled in the Durham area, and we are now able to reap the benefits of being the grandparents of two very special grandchildren, one of whom, Christopher Camitta, has already won national recognition as the winner of the first prize for composition in 2008 at the Music Teachers National Association competition at the age of 8; he played his piano composition at the annual meeting in Denver before his proud family and a large audience. Caroline Camitta is a quite energetic, imaginative, and talkative little girl, a voracious reader, and excellent student who aspires to becoming a veterinarian to satisfy her love of animals.

My husband, Bob, retired a few years after we moved to San Antonio and decided to build an airplane, which fortunately, after many years of neglect, will probably never be completed. He maintains our home and our finances and, most importantly, cooks our meals and remains interested in anything related to aviation, including belonging to the Experimental Aircraft Association and attending air shows wherever they may be. He has been the backbone of my existence and an enabler from the beginning of our marriage, which will reach the half-century mark in 2010.

**Lessons from Comparative Biochemistry**

While we were pursuing the structure-function studies of NOS isoforms, our research was continuing on CYPOR. After many years of effort and before the more frequent use of synchrotron facilities, Dr. Jung-Ja P. Kim’s laboratory at the Medical College of Wisconsin succeeded in solving the structure of CYPOR in collaboration with our laboratory (63). This structure now serves as the paradigm for FAD- and FAD-containing mammalian enzymes and has guided further structural studies of the NOS enzymes. Dr. Kim used this structure for the molecular replacement solution of the structure of the FAD/NADPH-binding domain construct of nNOS (64), as did Garcin *et al.* (65) in their solution of the entire reductase domain of nNOS. The structural aspects of NOS holoenzymes remain, how-
ever, a mystery due to the lack of a full-length structure, so the quest for such structures continues.

As we continued to examine the NOS isoforms for their similarities and differences, in collaboration with the laboratories of John Salerno and Steven Gross, we discovered in the constitutive NOS isoforms, nNOS and eNOS, by sequence comparisons with inducible NOS (iNOS) and CYPOR, in their respective FMN-binding domains that seemed to act as regulatory elements (66). These controlling elements seemed to be involved in the activation of the constitutive isoforms upon binding of calmodulin. This was the first such report of intrinsic regulation of NOS and alerted us to examine other sequence differences among the NOSs as possible regulatory elements. Extending this approach, Dr. Linda Roman noticed the C-terminal extensions of all the NOS isoforms beyond the glycine residue homologous to the C-terminal serine in CYPOR, which are 21, 33, and 42 residues longer than CYPOR for iNOS, nNOS, and eNOS, respectively, and decided to engineer truncated constructs of each to determine the effects (67, 68). The exciting result was that, in each case, the removal of these residues resulted in dramatic stimulation of flavoprotein-mediated electron transfer to several electron acceptors, including cytochrome c, in the absence of calmodulin for nNOS and eNOS. In its presence, the activity of the constitutive enzymes reverted to that of their wild-type NOS counterparts in the presence of calmodulin. These experiments indicated that the C termini act as intrinsic regulatory elements that are released upon the binding of calmodulin (69). Although iNOS also contains an extended C terminus, calmodulin is tightly bound upon expression of the C terminus in CYPOR, which are 21, 33, and 42 residues longer than CYPOR for iNOS, nNOS, and eNOS, respectively, and in membrane-bound CYPOR can be achieved by the re-addition of FAD (74) to R459H and V492E mutant enzymes or FMN (C. C. Marohnic, K. M. McCammon, and B. S. S. Masters, unpublished data) to the Y181D mutant. These studies not only demonstrated that the mutations surrounding the FAD-binding site resulted in serious consequences in enzymatic activities, whether measured as electron transport capacity of the resulting flavoproteins alone or upon reconstitution with cytochromes P450, but they suggested that a therapeutic regimen using riboflavin therapy in vivo could reverse the compromised enzymatic activities in these cases. Further studies in animal models need to be performed to determine whether reversal of the phenotype is achievable. In vivo riboflavin therapy in animal models of this nature could be therapeutically beneficial.

Recent studies of the human mutations in CYPOR, which have been demonstrated by Dr. Walter L. Miller’s laboratory at the University of California, San Francisco (72, 73), to be responsible for defects in steroidogenesis resulting in sexual dimorphisms with or without Antley-Bixler syndrome (exhibiting craniofacial and long bone defects), have led our laboratory to demonstrate that both FAD- and FMN-binding defects result from several of these mutations. Drs. Christopher Marohnic and Satya Panda in my laboratory have characterized several of the proteins exhibiting these mutations and demonstrated that rescue of enzymatic function in the purified enzymes and in membrane-bound CYPOR can be achieved by the re-addition of FAD (74) to R459H and V492E mutant enzymes or FMN (C. C. Marohnic, K. M. McCammon, and B. S. S. Masters, unpublished data) to the Y181D mutant. These studies not only demonstrated that the mutations surrounding the FAD-binding site resulted in serious consequences in enzymatic activities, whether measured as electron transport capacity of the resulting flavoproteins alone or upon reconstitution with cytochromes P450, but they suggested that a therapeutic regimen using riboflavin therapy in vivo could reverse the compromised enzymatic activities in these cases. Further studies in animal models need to be performed to determine whether reversal of the phenotype is achievable. In additional studies with Dr. Michel Kranendonk in Lisbon, Portugal, we have been able to demonstrate that both catalytic function and mutagenic activity are affected by such mutations, depending upon the mutagen used (75). The aforementioned and other mutations that may affect interactions of CYPOR with either cytochromes P450 or heme oxygenase-1 are also being examined in collabora-
tion with Dr. Wayne Backes’ group at the Louisiana State University Health Sciences Center.

**Structural Comparisons**

In our continuing collaboration with Dr. Jung-Ja P. Kim at the Medical College of Wisconsin, x-ray structures of several of these mutant enzymes have been obtained. Because of the continuing discovery of new polymorphisms in human CYPOR, it is important to determine the effects of such mutations at the molecular and cellular levels because the degree of severity of CYPOR deficiency could be wide-ranging and could contribute to a variety of problems, including malfunctions in steroid and drug metabolism that may be manifested under stress. Fig. 3 shows the structural relatedness of the precursor molecules flavodoxin and ferredoxin-NADP⁺ reductase, CYPOR, and the nNOS reductase domain, demonstrating visually how similar these molecules are but, at the same time, what modifications have been introduced through evolution to accommodate the new activities that the NOS enzymes were required to perform. Fig. 3A shows the separate structures of flavodoxin from *Desulfovibrio vulgaris* (76) and ferredoxin-NADP⁺ reductase from spinach (77). These structures are shown from the same view as the subsequent structures in Fig. 3 (B–D) of the mammalian proteins NADPH-cytochrome P450 oxidoreductase (CYPOR) and nNOS, the latter having been solved by molecular replacement using the reductase structure without its FMN domain (65). In Fig. 3B, the structure of CYPOR is viewed so that the juxtaposition of the flavins is clearly indicated, with the closest distance between them being 3.5 Å between the C-7 methyl groups of the xylene rings of the isaloxazine ring systems at an angle of 150°. In Fig. 3C, an overlay of the CYPOR structure with the individual flavodoxin and ferredoxin-NADP⁺ reductase structures is shown, demonstrating the conservation of these motifs throughout phylogeny. In Fig. 3D is shown an overlay of the reductase domain structure of nNOS (65) with CYPOR. It is interesting to note that the distance
between the flavins is 4.8 Å in the nNOS reductase structure, still allowing for facile electron transfer, but more distant than in CYPOR. The additional inserts are indicated as AR (autoregulatory insert), BF (β-finger), and CT (C terminus) in the figure. It is these amino acid inserts in the NOS structures that have been shown to function in the intrinsic regulation of catalytic activity upon activation of the constitutive NOS isoforms by the binding of Ca\(^{2+}\)-bound calmodulin.

As we continue the quest for answers to how the three isoforms of NOS are differentially regulated and what structural determinants are important in determining the interactions, both intrinsically and extrinsically, that regulate their activities, our laboratory has sought the most qualified collaborators, who have applied their very specialized approaches to the unraveling of these mysteries. In addition, we are extending our studies of the human CYPOR mutants to understand how they malfunction in vivo with the hope of suggesting interventions where possible. I suppose one could summarize my approach to science as one in which we ask questions that we think are important to answer and then determine the approaches, methodological, technological, or intellectual, to be applied to answer these questions.

Over the years, I served on a number of committees for the American Society for Biochemistry and Molecular Biology (ASBMB), including the Publications, Finance, and Public Affairs Committees, as well as Council, in addition to serving two terms on the editorial board of the Journal of Biological Chemistry. In 2001, I was elected President-Elect of ASBMB and was the second president of the Society, following Dr. Robert D. Wells, to serve 2 years as President. Despite the long 4-year commitment to the presidency, including President-Elect and Past President, I found the continuity it provided to be quite helpful in formulating long-range plans, some of which are still coming to fruition. I had been assigned by Bob Wells to chair the Centennial Planning Committee, and this occupied a great deal of my time, along with a group of dedicated colleagues, who wanted to honor our Society in the most memorable way. With the outstanding ASBMB office administrators, the job was made much easier. Dr. Judith Bond, who was to succeed me as president, would be the hostess of this grand occasion, and her first Council meeting in Hershey, PA, would involve the execution of some of our plans. To my dismay, my term between 2002 and 2004 was interrupted by the necessity for a national search for a replacement for Charles C. Hancock, the Executive Officer of ASBMB for almost 25 years, who decided to retire during my tenure as President. After interviewing a number of outstanding candidates for the position, we selected Barbara Gordon, who had served ASBMB at multiple levels for many years, most recently as Deputy Executive Officer. Her stated goal was to serve the members of the Society and facilitate our plans, which she is continuing to do with a dedicated and talented staff. New approaches were taken to the organization of annual meetings around themes that followed the fields covered by publications in the Journal of Biological Chemistry. The idea was to generate the feeling of a small meeting within the large meeting and to create a community of researchers who would interact with one another throughout the meeting. This has been a successful paradigm, but it was also decided that the annual meeting needed to be supplemented with small meetings focusing on specific fields to promote the development of scientific areas in the Society and to encourage young researchers by giving them a venue for presentations and interactions on a more informal basis. For some small meeting topics, this has proved to be quite successful, and planning for future meetings has been focused on the expansion of this approach with possible interdigitation of the themes between the large annual meeting and the small meetings.

These activities, along with my deep interest in public policy issues, reinforce my dedication to the activities of ASBMB. I have been a member of the Public Affairs Advisory Committee since my presidency because I believe that academicians who understand science and who are willing to interpret it to the public, legislators, and the media must do so because there are numerous issues that depend upon accurate translation of complex scientific principles or technologies on which decisions that have long-term consequences must be made. The recipients of this information will benefit in their everyday lives as they read about scientific breakthroughs, and they will be able to make intelligent decisions when choosing their representatives in our legislative bodies.

In my own state, I was honored to be a charter member and to serve as the first secretary of a new organization named The Academy of Medicine, Engineering and Science of Texas (TAMEST), composed of members of The National Academies and all of the Nobel Laureates of Texas, whose initial mission was to promote young scientists and honor their achievements with prestigious awards in medicine, engineering, and science, as well as technology innovation. The inaugural chairs of TAMEST were Drs. Michael S. Brown and Richard E. Smalley, Nobel Laureates in Physiol-
ogy or Medicine and Chemistry, respectively. My own election to the Institute of Medicine in 1996 qualified me for membership in this august academy. Later, in response to the report “Rising Above the Gathering Storm: Energizing and Employing America for a Brighter Economic Future,” issued by The National Academies in 2007, TAMEST formed the Education Committee, of which I am a member, to prepare a report to identify the opportunities and challenges in science, technology, engineering, and mathematics (STEM) education in K-12 in Texas. This was the first substantive response, chaired by Dr. William R. Brinkley (Baylor) and Dr. Mary Ann Rankin (University of Texas), to this national challenge to the decline in STEM education in the United States, and it has already prompted legislative proposals to address these issues. TAMEST is a unique organization among the various types of state academies.

In 2005, it was one of the most memorable occasions of my life to be awarded the prestigious Doctorem Medicinae Honoris Causae by Charles University in Prague for contributions to the enzymology of NOSs, as well as for the promotion of biomedical research internationally (Fig. 4). It was even more meaningful to share the stage with the Nobel Laureate in Physiology or Medicine, Ferid Murad, M.D., Ph.D., who was being recognized for his seminal contributions to the field of nitric oxide biology as one of its founders and determining the role of NO in activating guanylate cyclase. My immediate family accompanied me to Prague for the ceremony, which was held in a beautiful, restored, and modernized hall, many stones of which were in the original structure from the 1300s, and attended by presidents and provosts from throughout the Czech Republic. The ceremony is being observed under the watchful eye of Charles IV, who founded Charles University in 1348.

In the meantime, I continue to enjoy “practicing” science as an individual but highly collaborative investigator and helping the next generation of young researchers to establish themselves in a world that is infinitely more challenging and complex than the one in which I was trained.

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