

# THE CHEMISTRY SET AND THE ELECTRON MICROSCOPE

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## Beginnings

When asked to talk about yourself, what is the best time to think and reflect on your life? In the morning with a mind cleared of dreams, or during the day when you are doing what you are trained or forced to do at work or by habit? Or better yet, relaxed at the end of the day following dinner under the cloud of fine wine? I believe that my best thoughts come to me when I am on a trout stream with a fly rod in my hand surrounded by all of nature's wonders. It is there that I can reflect on my life unencumbered by daily responsibilities. Fly fishing is not unlike research. At the streamside you formulate a hypothesis depending on stream conditions and the genus, species, and life cycle of the aquatic insect that the fish are likely to be feeding on at that particular moment, and you experiment by choosing and presenting the artificial fly in a way that most closely mimics natural behavior. As with all experiments, sometimes you are successful, but more often than not a second or even third experiment is necessary. When unsuccessful, you analyze where you have erred and try again. Plutarch said, "Research is the act of going up alleys to see if they are blind," and that is why science is so fascinating. But where do I begin and where do I end, where is my alpha and my omega? I am here to describe my journey as an academic, but I have to decide whether my memories are true fact or so colored by the passage of time that they have become part fiction. I will try to be as factual as my memory allows.

I was born in Manhattan during the Depression and grew up in The Bronx. My parents were survivors of the 1914–15 genocide of Armenians by the Ottoman Turks. Those events destroyed both of their families, so I never knew grandparents. My father survived by joining an Armenian brigade of the Imperial Russian army in the First World War. He fought the Turks in the Caucasus and in Anatolia under a legendary Armenian general, Andranik, and came to the United States in 1920 soon after the Bolshevik revolution. American evangelical missionaries had converted my mother's

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family, and my great uncle was the pastor of the local church. In the nineteenth century, mission schools had been established for Armenian children in many towns in what is now Turkey, including my mother's village of Zeitoun. As word of atrocities to the Armenian population began to spread, she and some of her classmates were transported safely under protection of the American missionary staff through Aleppo to schools for orphaned Armenian girls in Beirut, where she completed her secondary education and trained as a nurse. It was there that, at the age of ten, she learned that her entire village had been massacred. Her early training as a nurse undoubtedly later influenced my older sister's choice of nursing for her career, and her education under difficult circumstances instilled in my older siblings and me a respect for the pursuit of learning.

We lived in a working-class neighborhood in The Bronx, where I attended the local public school, P.S. 85. My interest in science probably started around the sixth grade. My teacher, Mrs. Tobler, would send me up to a science room where I was to pick an apparatus to demonstrate to my class a basic physical principle. The one that I liked best was water acting more like a solid than a liquid when under a vacuum or under pressure in a glass water hammer. It was that Christmas that I received an A.C. Gilbert chemistry set. My brother was seven years my senior and had already studied chemistry in high school. With his help I quickly worked through all of the experiments that came with the instructions. It was then that I decided to experiment on my own. It was wartime and I was fascinated with explosives. Black powder is easy to make and I found a recipe somewhere. There was no Google then, so it must have been in the library. It consists of a mixture of charcoal, sulfur, and potassium nitrate. It burned quickly but did not explode, and from that experiment I learned that in order for black powder to explode it must be confined to a space where pressure builds up to accelerate the reaction. For me, chemistry seemed to be everywhere. The radio commercials of the DuPont Company stated, "Better things for better living through chemistry." So I was off to Creston junior high school (P.S. 79), where I was enrolled in an accelerated program to finish two years in a year and a half. My fellow students in that program were as enthusiastic about science as I was. Some years later one of my classmates, Arno Penzias, received the Nobel Prize for his work on the "Big Bang." Our science teacher was Dr. Eisenstein. He stimulated original thought. He gave us the opportunity to get extra credit by presenting a demonstration of chemical synthesis. Synthetic fibers had become even more important during the war because of the scarcity of natural fibers. I decided for my project to demonstrate the synthesis of rayon. Just like black powder, rayon is easy to make. You just have to solubilize cellulose in a caustic solution and then reconstitute it in either a dilute sulfuric or nitric acid solution. I do not remember which of the methods I used — I may have used more than one — but I do remember that a drop of the solubilized cellulose fell on the laboratory bench, dried, and snapped when I put down the beaker in which I had extruded the rayon fiber. It turns out that what I had actually made was nitrocellulose. Explosives again.

## Education

The Bronx High School of Science with its focus on the sciences and mathematics further stimulated my interest in chemistry and physics. Biology was interesting but did not seem to me as quantitative or rigorous as the physical sciences, whose curricula provided stimulation for innovation and invention. In the first semester of our sophomore year, we took a course in mechanical drawing that required us to design a useful tool that we would manufacture in the second semester during a shop course with the pretentious title "Science Techniques Laboratory." I designed and constructed a tilting tripod head that could be set in three axes simultaneously with a single handle. The design and manual skills I learned there proved extremely useful later in my career when I had to design and build my own analytical apparatus to analyze nanoliter volumes of glomerular filtrate obtained in micropuncture experiments of the kidney.

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Since I had accelerated in junior high school, I graduated from high school in the middle of the academic year. As a result my choice of colleges was limited if I wanted to continue my studies without a hiatus. A few schools did admit students at midyear, permitting them to complete freshman year by the following September and to synchronize their subsequent years of university. This was a holdover from the immediate postwar years, intended to accommodate the return of veterans. University College and the College of Engineering of "Uptown" New York University had such a program, so I followed in the footsteps of my older brother, who had graduated from that school. As you may have guessed, I chose chemistry for my major. Mathematics and philosophy were my minors. I relished the interplay of mathematics and physics in chemistry, and my favorite course was physical chemistry. It was there that I had my first exposure to the physical properties of electrolytes in solution and how they are described by the Debye-Hückel-Onsager theory and the Nernst equation. Little did I know at the time that I would later apply those concepts to whether or not there was active transport of electrolytes across the renal tubule. Recently I happened to visit the grave of Lars Onsager in the Grove Street Cemetery, and I think everyone should read his gravestone and compare it to that of fellow physical chemist John Gamble Kirkwood.

A philosophy and logic course was in contrast to the quantitative rationality of the physical sciences, but in reality it was an introduction to the pure reasoning of symbolic logic and the concepts of the "tabula rasa" of Locke and the Kantian extension of reason to morality. It was for me another course in science. The analytic thought process certainly later influenced my formulations of hypotheses. For fun I enjoyed singing and became active in the glee club, the college choir, an *a cappella* group, and a quartet. I even took a course in sight singing and choral conducting. It was my plan to complete college, go to graduate school, and pursue a career in chemistry. Many if not most of my classmates were premeds who took some of the same courses as I did to fulfill requirements for admission to medical school. They did not seem to understand why I was pursuing a curriculum of pure physical science that did not include courses

in biology. My brother was already in medical school, and perhaps that and the persistence of my premed classmates led me in my junior year to an epiphany in my career choice. I threw my hat into the same ring as my classmates and proceeded to take the medical school admission tests and applied to several medical schools. In my senior year I took my first and only biology course to satisfy medical school requirements. It was taught by Professor Horace Wesley Stunkard. He was a parasitologist and parenthetically an Olympic athlete. He introduced us to the complexities of parasites and their choice of hosts and how in their life cycle they lived either symbiotically or acted as a pathogen. He demonstrated that host reaction to an etiological reagent was as (or more) important to the development of disease as the agent itself, a principle that is universally applicable in medicine. I did find biology interesting but still not as rigorous as the physical sciences. Some years later I met Dr. Stunkard again at the Marine Biological Laboratory in Woods Hole. I had a summer laboratory there where I was studying adaptation of kidney and gill function in anadromous fish (trout) migrating from fresh water to salt water and back. I used the opportunity to thank him for introducing me to experimental biology. I think that the dissection of a lobster in the lab section of his course proved pivotal in my appreciation of its culinary value.

### **Medical School**

Just as senior year was starting, I received a letter from the Yale School of Medicine asking me to come to New Haven for an interview. From Grand Central Station I boarded what was then the New York, New Haven and Hartford Railroad on my journey to New Haven. On my arrival at the medical school I was introduced to Dean Thomas Forbes and had a rather perfunctory interview. I was upset by my performance and thought that I had blown my chance for admission. My second interview was with Professor Robert Cooke in the Pediatrics department. In contrast to the formality of the interview with Dean Forbes, Dr. Cooke leaned back in his chair, put his feet up on the desk, and looked over my application and record. He noted that I had not taken any biology courses until the current year and that I had concentrated on the physical sciences and mathematics. He then asked how I thought I might apply that training to medicine. I said that I thought mathematics had a central role in understanding all science. I had studied Fourier analysis of repetitive waveforms in one of my advanced mathematics classes, and I told him that it seemed to me that one repetitive waveform which is used routinely in medicine was the electrocardiogram and there should be ways to reduce the various patterns seen in disease to a series of definable coefficients. I told him that I thought that that type of analysis could eliminate the subjective nature of merely looking at the patterns. My interview with him was truly enjoyable and lasted a little over an hour. He then directed me to my third interview with Assistant Dean Arthur Ebbert. Dr. Ebbert asked me if I had any questions about the Yale School of Medicine or the Yale system of education. I told him that my sister, a nurse, had worked with a graduate of the Yale medical school and that she had told my sister that Yale offered the best medical education and that I should definitely seek admission. That interview was extremely brief and ended by

Dr. Ebbert saying that Dr. Forbes wanted to see me again. I thought that I had been given a second chance and was now fully prepared for a much better second interview. Instead he asked me if I was truly interested in coming to Yale; and when I answered in the affirmative, he said that all I had to do was to send in my deposit.

My classmates and I flourished in the Yale System of Medical Education. We took it at its word as described in the Bulletin of the school at the time:

The plan of study is designed and the formal courses are arranged to assist men and women in their preparation to enter the profession of medicine, and to provide opportunities for the acquisition of the special technical skills essential to teaching and research in the biological and medical sciences. To this same end, an attempt is made to adhere as closely as possible to the graduate type of presentation....The curriculum is designed to provide a minimum of required work and a maximum of opportunity for the development of special interests and talents of individual students.

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While our professors provided structure and scaffolding on which to build our knowledge, we taught ourselves at our own pace and with our own focus. Yale provided the perfect environment for developing our own interests through independent thought and exploration. Our professors also provided a great deal of color to our education. Ed Crelin of Anatomy played the trumpet and led a forties-style dance band. José Manuel Rodriguez Delgado was a neurophysiologist who mapped specific brain function using implanted electrodes. A Spaniard, he implanted electrodes into the brains of bulls so when he fought them in the bull ring he could control their behavior without the fear of being gored. Harry S.N. Greene, chairman of Pathology, defined cancer as autonomous tissue that would grow independently in the anterior chamber of a guinea pig's eye, but dismissed the statistical association of smoking and lung cancer by likening it to the prevalence of baldness in the front row of burlesque houses. Finally, Averill Liebow, chief of Pathology, knew the full name and undergraduate college of every student on the first day of classes and thereby commanded everyone's attention by randomly demanding a specific student to answer a question posed to the entire class.

Pathology changed my opinion about the lack of rigor in the study of biological processes. I saw that careful study of tissues not only described the history of a disease process but also could predict its future outcome. Averill Liebow brought the subject of death to life. He had been taught by M.C. Winternitz, who in turn had been taught by W.H. Welch (a Yale College graduate), who had established the first truly academic Pathology department in America at Johns Hopkins after being educated in the scientific basis of medicine in Europe under pioneers like Waldeyer, Hoppe-Seyler, Virchow, Cohnheim, and Koch. As students we felt as if we had a direct lineage from Virchow's cellular basis of disease to the newly emerging concepts of immunity and carcinogenesis. It was my favorite subject, and in the summer before my clinical years I had the opportunity to do autopsies as a subintern. My first autopsy was on a forty-one-year-old man with end-stage polycystic kidney disease. A photograph of his

kidneys from that autopsy is published in my *Atlas of Renal Pathology*. It was known at that time that this was an autosomal dominant genetic disease, but its natural history and pathogenesis were unknown. We now know the genes that are involved and the specific cellular functions that are disrupted by the mutations. That was the beginning of my interest in pathology and the kidney.

It is in the clinical years that you are exposed to all the various medical specialties and you begin to search for the best fit between your interests and abilities and the choice of a career specialty. Although I had good manual dexterity and could tie surgical knots quickly and securely, I knew that surgery could not satisfy my scientific curiosity. Internal medicine was where pathology and physiology intersected in the understanding of the disease process of individual patients. Our internal medicine professors all had experimental laboratories, and they often brought the results of their studies to the bedside. We now give that process the pretentious name of translational medicine. John P. Peters had introduced quantitative clinical chemistry to the bedside, and Franklin Epstein had trained under him and was my attending on one of my medicine rotations. My interest naturally focused on the chemistry of our body fluids and its regulation, and I chose Frank Epstein as my thesis adviser. I was perplexed, however, by terms like “fixed acid” that were used in the clinical setting. They did not correspond to actual acids but rather to the anions produced by metabolism that could accept a proton and so were actually a base. The kidneys were at that time considered a black box that contributed to excretion of metabolites and acid by poorly understood mechanisms. I saw there were opportunities to apply the principles of physical chemistry to the regulation of body fluids, and my thesis focused on potassium deficiency. In my senior year my classmate Gerry Burrow, who later served as dean here, and I were selected to participate in a case-based seminar led by the chair of Internal Medicine, Paul Beeson, and the chief of Pathology, Averill Liebow. They emphasized that those who were privileged to learn had the obligation to teach others. We studied clinical cases where one of us would act as the clinician and the other the pathologist. From that experience I thought that an academic career in medicine would allow me to combine my interests in physical chemistry and clinical medicine, and I was directed by Paul Beeson to accept an internship in internal medicine at Washington University in St. Louis.

### **Postgraduate Training**

This was long before the institution of the eighty-hour work week for medical residents. As an intern on the ward service, once you admitted a patient that patient was yours twenty-four hours a day seven days a week. We had full responsibility for our patients under supervision of the senior residents and attending faculty. I found that clinical experience rewarding in many ways, both from an educational perspective and from the emotional gratification of seeing patients get well. It also fueled my desire to pursue a career combining clinical medicine with basic research. At graduation from college I had received a commission as a second lieutenant in the infantry of the United States



Army. The army had delayed my activation to allow me to go to medical school, but it now required me to enter active duty as a general medical officer. By this time I had decided that although I enjoyed taking care of patients, a career in pathology would better fit with my ambitions. I knew that one of my medical school mentors, Averill Liebow, had been an officer in the army in the Pacific and had been made a member of the Atomic Bomb Casualty Commission. Thinking that he might in some way be able to influence my military assignment, I called him and asked if he could find a way to have me assigned to a position in pathology. A few weeks passed, and he returned my call to tell me that the army was allowing me to stay in the active reserve while I continued my training as a resident in pathology back at Yale.

So at the end of my medical internship, I returned to Yale as a first-year resident in pathology with a pay cut from \$35 a month to \$25 a month. The chief resident at that time was Frank Carone, with whom I had worked when doing the experiments for my student thesis. Knowing my interest in the kidney, he described a newly emerging experimental technique to investigate kidney function. It consisted of sampling and analyzing fluid obtained by micropuncture of individual renal tubules. There were only three laboratories in this country where this technique was in use, and he asked me if I would be interested in assisting in establishing one here at Yale. This was an exciting idea. No longer would the kidney be a black box when its secrets could be revealed at the microscopic level. We went to visit the Harvard laboratory where they had begun to do micropuncture work on amphibians. Returning to Yale, we proceeded over the next year to scavenge laboratory equipment to try and duplicate what we had seen. What we assembled was quite cumbersome, and although we were able to sample tubular fluid, we did not have the capability of analyzing its contents other than determining the freezing point, which gave us only the total ionic concentration. I decided I needed real laboratory experience, and I applied for and received a Life Insurance Medical Research Fund fellowship for training at one of the more established laboratories.

### **Fellowship**

I chose to go to the laboratory of Karl Ullrich in Göttingen, where Jakob Henle had described the tubular anatomy of the kidney and the entire Physiology Institute was focused on renal physiology. My wife and I, with our three-month-old daughter, set off on a new adventure, and what an adventure it was. One month after arriving in Göttingen, the Berlin Wall went up, and we were just a few kilometers from the East German border. While our families back home were concerned about our safety, daily life there just went on.

The Physiology Institute under Professor Kurt Kramer could not have been a better choice. Dr. Ullrich's laboratory and two other laboratories in the institute were doing micropuncture studies of the kidney. In addition, Dr. Carl Gottschalk, the principal investigator of the most productive of the American micropuncture laboratories, was there on sabbatical leave from the University of North Carolina. The environment was

stimulating, and my project was exactly what I could have hoped for. We set out to determine the transtubular electrochemical potentials of sodium and chloride in renal tubules to see if there was active transport of the ions based on theoretical modeling of material transport across porous membranes. I was back to physical chemistry, but this time I had to produce actual data to see if they fit the model. I developed a novel technique where I could measure membrane potential and tubular ion concentration under steady-state conditions of no net flux, allowing me to apply the theoretical model to the measured electrochemical potential of the individual ions. We called it the “standing drop” technique. Since the measurements of sodium chloride and transepithelial electrical potential were made under steady-state conditions, I was able to use the Nernst equation to calculate the electrochemical potential of sodium and demonstrate that its movement across the tubular epithelium required active transport. Physical chemistry was doing what I had thought it could do: that is, describe biological phenomena. At the Physiology Institute, I shared office space with Karl Heinz Gertz, who was studying the rate of fluid reabsorption with the technique he developed whereby the absorption rate of an isolated drop of tubular fluid could be determined using sequential photographs taken at five-second intervals. This was called “the shrinking drop technique.” In yet another laboratory Klaus Thureau was studying renal circulation. It was in this rich environment that we presented our latest results—both good and bad—for critical review. That is where I learned the vagaries of experimental science and how to overcome them.

### **Back Home**

I returned to Yale as chief resident to complete my training in pathology and was appointed an instructor. I brought with me the two novel micropuncture techniques that I had helped to develop in Germany; and Dr. Epstein, my medical school thesis adviser, helped me establish the first micropuncture renal physiology laboratory at Yale by providing funds to purchase micromanipulators and dissecting microscopes and by assigning a research fellow to work with me. I designed a heated operating table to immobilize the kidney of a rat *in vivo*, and a micro flame photometer to analyze nanoliter volumes of tubular fluid for sodium and potassium and had them constructed by the medical school machine shop. Dr. Yves Warren, who had come from Quebec to do research in Dr. Epstein’s laboratory, and I set out to examine chloride transport in the standing drop with an electrometric method I modified to measure chloride in nanoliter volumes of tubular fluid. We studied the potential contribution of oncotic forces to tubular reabsorption using the shrinking drop technique. With these successes I applied for and received a special research fellowship followed by a career development award from the NIH and was appointed an assistant professor. I could not believe that I was going to earn \$8,000 a year. In addition, the dean asked me to take over the position of resident faculty at Harkness Dormitory, where my wife, my daughters, and I functioned as mini-masters for the next seven years. While my studies were focused on normal tubular physiology, I thought that as a pathologist I should apply the same micropuncture techniques to the



study of an animal model of kidney disease. Two medical students joined my laboratory to do their thesis research, and we investigated the pathophysiology of acute renal failure in two different animal models. They were the first to demonstrate that the decrease in function seen in acute kidney injury was the result of a decrease in glomerular filtration secondary to a feedback regulatory response to decreased tubular reabsorption of sodium chloride and water mediated by the renin angiotensin system.

## **Laboratory and Clinical Research Together**

### ***The Clinic***

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Dr. John Hayslett, who was then a clinical fellow, joined me for research experience in renal micropuncture. In the laboratory we focused on the regulation of sodium reabsorption, but he brought along his clinical responsibilities. Clinical nephrology was in its infancy but was entering a new phase with the introduction of the needle biopsy of the kidney. There were only three or four places in the United States where this technique was being used routinely to diagnose glomerular diseases, and so it was natural for me to want to establish this new diagnostic method here at Yale. Hayslett regularly saw patients in the clinic who could benefit from a renal biopsy, and he mastered the technique of obtaining the tissue that we could study by histology. At the time, renal biopsies were being examined primarily by light microscopy, but this gave limited information about the role of immunity in kidney disease that had been proposed from animal models. Electron microscopy had been used for some years in biology but only in a very limited way in clinical applications. It was being used in addition to optical microscopy in renal biopsy analysis at one or two places where it was shown to contribute significantly to the diagnosis and prognosis in individual patients with glomerular disease. I made the argument that this was critical for proper patient care to my chair, Lewis Thomas, and he provided funds for an electron microscope for clinical use. It was Christmas again. I had a new toy with which I could explore kidney diseases. No explosions this time, but definite curiosity about how to exploit its capabilities both clinically and in the research laboratory. It also gave John Hayslett and me the opportunity to do clinical studies simultaneously with our laboratory studies of renal tubular physiology.

With electron microscopic and immunofluorescence techniques in place, the renal biopsy laboratory was now fully functional. Hayslett ran a clinic for patients with lupus who had renal involvement. Immunosuppressive therapy was just beginning to be used in patients with severe renal disease, and we chose one standard treatment. Prospectively we established a protocol whereby a baseline biopsy was performed before the initiation of treatment and follow-up biopsies at six-month intervals to determine not only the efficacy of treatment but also the clinical and biopsy predictors of long-term outcome. Over the next few years we collected sufficient numbers of patients with complete clinical and biopsy data, all of whom had been treated identically. With the collaboration of Dr. John Esdaile, a clinical epidemiologist, we published the first series of papers that examined in detail the clinical and renal biopsy predictors of response to therapy and long-term outcome. The study also elucidated

how variation of the autoimmune response in individual patients corresponded to the pathogenesis of the different patterns of immune complex deposition seen in biopsies. A few years later Norman Siegel, a pediatric nephrologist, joined the laboratory. We correlated clinical outcomes with biopsy findings in patients with childhood lipid nephrosis and identified the natural history of minimal change disease and focal sclerosis. The renal biopsy laboratory has grown to become a regional and national reference laboratory receiving specimens from as far away as Beirut. The collected biopsy and clinical material has served as the source for the publication of a *Diagnostic Atlas of Renal Pathology*, currently in preparation for its third edition. Now published in both print and electronic format, it has been translated into Chinese and Spanish.

### ***Back to the Laboratory Bench***

These clinical studies were important, but the laboratory was still focused on the basic studies of tubular function. The electron microscope was so useful for clinical evaluation of the kidney that I decided to introduce ultrastructural techniques to investigate ion transport at a cellular level. Epithelial transport studies were the focus of several investigators in the Department of Physiology, where Gerhard Giebisch, who was establishing a program project encompassing the field, and I obtained funding for a core EM facility as part of the project. We used ultrastructural morphometric analysis to examine changes in membrane surface area associated with changes in ion transport. These studies looked at potassium adaptation in kidney tubules with John Hayslett, and in colon epithelium with Henry Binder. The question of the plasticity of intercalated cells of the collecting duct was examined with Gerhard Giebisch. To determine that these cell membrane surface changes reflected changes in specific transport proteins, we developed polyclonal and monoclonal antibodies to NaK ATPase and to colonic K ATPase for use in studies of regulation of ion transport using immunolocalization with confocal and electron microscopy as well as biochemically at the RNA and protein level. With Norman Siegel we demonstrated loss of cell polarity of NaK ATPase in renal tubules following energy depletion in tissue culture of renal epithelium and after ischemia in a rat model of acute kidney injury. These studies led to a series of studies of the cellular and metabolic alterations in energy depletion in renal cell cultures and in vivo ischemic injury in the rat. We identified the role of two different heat shock proteins as chaperones in restoration of cellular integrity. One finding of our metabolic studies was that administration of thyroxine stimulated renal mitochondrial activity, increased ATP synthesis, and ameliorated the effects of renal ischemia on the kidney. The Department of Defense became interested in our observations, and a clinical trial of its potential use in the battlefield was initiated during the brief Gulf War, but unfortunately or rather fortunately there were too few casualties to lead to any conclusions about its military potential. Thyroxine has, however, been incorporated in some solid organ transplant preservation fluids. Another interesting observation was that the neonatal kidney was more resistant to anoxic injury and that this was mediated by regulation of heat shock protein synthesis in the immediate postnatal period by a micro RNA.

There was a constant influence of clinical renal disease on my thinking about renal pathogenesis, and one particular aspect raised some perplexing questions for me. Chronic kidney disease was self-perpetuating even after the initiating cause had been abrogated. Renal fibrosis had a life unto itself. My basic research interests largely ignored the glomerulus even though it was the main focus of renal biopsy diagnosis. Bernd Sterzel had an interest in chronic kidney disease and, knowing of my laboratory's experience in studying cultured kidney cells, approached me to join with him to focus on a new target, the glomerular mesangial cell. Using primary cultures of glomerular mesangial cells both in two-dimensional and three-dimensional cell culture, we studied the regulation of the profibrotic collagen genes and the turnover of their protein products. We identified differential roles for TGF beta, PDGF, and Angiotensin II in regulating collagen genes in culture and an *in vivo* model of glomerulonephritis. To mimic what happens in diabetes, we studied the effect of high glucose and advanced glycosylation end products on the synthesis and degradation of type IV collagen. Collagen synthesis was altered by exposure to high glucose, and these effects were amplified more by periodic exposure to high glucose as compared to constant, elevated glucose levels: the condition that mimics what occurs in patients clinically.

When I closed my laboratory six years ago, I left some projects undone and stopped adding new knowledge to my field, but I did not close my mind to science. There are still alleys I have not explored, and while I cannot test them in the laboratory, I can use my experience to help others with the task. Marcus Aurelius wrote in his *Meditations* that nothing has such power to broaden the mind as the ability to investigate systematically all that comes under observation in life.

The Greek inscription over the entrance to the medical school reads, "That those having torches will pass them on to one another." That inscription reminds us that it is not enough to be a good clinician and a successful investigator; it is necessary to pass your experiences to those who will follow you. I love to teach, and one of my proudest achievements is to have established a Yale College course in MCDB covering human biology. Averill Liebow, whom I consider as my most important mentor, set the example for my career in academic medicine. I have tried to emulate him by striving to be an innovative teacher as well as doing my best in research and in contributing to patient care as a renal pathologist.

None of this could have been accomplished without the help and collaboration of more than seventy-five students and postdoctoral fellows who joined my group and the more than twenty collaborating investigators I have had the privilege of working with over the last fifty years.