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GREAT EXPECTATIONS

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I never expected to find myself in these pages. I have been reading my predecessors in the *Annual Review of Pharmacology and Toxicology* and the *Annual Review of Physiology;* many are inspiring, illustrious, and have high tales to tell. We do have in common that age has taken us unaware. My own advent into medical science was through a series of near-comic accidents and chance events; yet, there is a theme to share with my readers, and that is my title. I have responded to the expectations, which often seemed blind faith, held out to me freely and lovingly by family, friends, teachers, and colleagues from my childhood to this day.

One spring in 1943 I found myself in a strange new place, a Pharmacology Department. It was at Columbia where there was a meeting of people interested in a disease with an even stranger name—filariasis. I had become interested in this although I soon realized that I was mispronouncing it. I cannot recall how I gained entry to the meeting, but I must have invited myself since it would be an exaggeration to say that I was on the fringes of wartime biomedical research. I was a chemist in a small cosmetics company, Wallace Laboratories, much later to gain renown as the maker of Miltown. But then I was toiling enjoyably in the synthesis of thioglycollic (mercaptoacetic) acid to make depilatories, and as a byproduct, we started home permanent waves. I read that antimony thioglycollates had been used in tropical diseases by John J. Abel (a portent) in his one excursion into chemotherapy. It was easy to synthesize a few of these compounds, and my boss, Dr. John Wallace, thought no harm could come of it. It even might give our raffish operation some respectability. Fortunately, canine filariasis (heartworm), although usually confined to the South, had a pocket in New Brunswick, New Jersey where I worked. I arranged with a veterinarian, Dr.

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Morris, to inject monosodium antimony thioglycollate into a few infected dogs. What happiness! The microfilariae disappeared from the blood within a few hours. How nice if one could do this every day. Such an effect was known, but this action seemed fast, effective, and not (another fine new word) toxic. Armed with these data, I came to the meeting in New York where there were not only professors, but also naval commanders, officers of the National Research Council, and representatives from the (real) pharmaceutical industry. I was asked to speak first, which seemed odd at the moment, but this was soon clarified. I gave my report, and was asked to leave. Thus, I was introduced to the amenities of academic medicine, but as I crept away, I did notice a squat, energetic civilian wearing a bow-tie, who eyed me from the back of the room.

The next week this man called, saying that he was Dr. Gilbert Otto, a parasitologist at the Johns Hopkins School of Hygiene and Public Health, and that he would like to drive up to see me. When he came, he revealed his secret; he had a contract from the Office of Naval Research to study the pharmacology of antimony compounds related to filariasis and schistosomiasis (another new word!), but he knew no chemistry. Nor, in wartime, could he find even a barely qualified Ph.D.; he would be willing to settle for me. I told him that I knew less biology than he did chemistry, and the partnership sounded ideal. So I left Wallace; I said that I'd be back after the war. At the same time, I suspended my underground operation of getting a Ph.D. in English at Princeton. I said that I'd be back after the war. But my move to Baltimore closed the one life and began another.

EARLY DAYS AND COLLEGE

I was brought up in Mount Vernon, New York, then a quiet suburb of New York City, which offered country pleasures and good local schools. My father had grown up as a tough kid in the city, a disciple of the local blacksmith, Tommy Burke, and his apprentice, nine-fingered Smitty, who had been prey to one of many accidents which befell city kids who copped free rides on streetcars or the new subways. Indeed, legend had it that my father had been stepped on by a horse. Fortunately, the lesion was localized in the nose which gave him an appearance not unlike the late actor Victor McLaglan. But there was a difference: my father was a scholar! When the blacksmith needed a name for his new horse lotion, my father named it Equisalve—an early advertising triumph! He was also an athlete; somehow he had pulled himself out of Yorkville to DeWitt Clinton High School and Columbia where he played first base. He learned tennis—a game then associated with ladies, England, and Newport—and was occasionally challenged to fights by local bruisers who thought they could take on anyone

walking about with a racket and possible pretensions to the middle class. But he was good with his fists, too. Before he was 20, he had a license to teach school—all subjects from Physical Education to Physics. He scorned educational theories, but advanced to Principal in New York City School System through examinations and his record without ever going to Teachers College. He became a natural sort of authority on delinquent children (without, so far as I know, ever writing anything). In about 1940, Mayor LaGuardia asked my father to organize a school to be called Youth House, so that juveniles who had been accused or convicted of crimes could continue with their educations. My father recruited a faculty, many of whom were also athletes and from poor families. The best way to get to "difficult" children was through athletics and humor. He was kind to these boys, and often very funny, and surely could not have appeared as a jailer. His motto was "laissez faire" at school and certainly at home. He never interfered with anything, and rarely gave advice, and seemed to have no neuroses at all. He could not understand the word "headache," since he had never had one. He loved literature in an idle sort of way, about the way he did his family. He was totally supporting and accepting as long as I "gave him a run for his money." Of course, he did not mean money, and there wasn't much anyway, but the concept was clear. In 1981, he would be called "laid back." My mother was an accepting, loving, and attractive lady who taught me to play tennis. My parents lived rather obscurely, had little social life, and never bothered with an automobile.

In high school, I played tennis, and dabbled unsuccessfully in politics. I had a secret problem: unlike my friends, I did not know what I wanted to be "when I grew up." There seemed to be only three choices: medicine, law, or engineering. Medicine was out since my mother belonged to a sect that forbade it, and my father passively went along with the insanity. I never saw a doctor until I was 18 and in college with a threatened appendix rupture, and he was as frightening as I had dreaded he would be. Law seemed alien; I could not see why my close friends were attracted to it. That left engineering, so I dutifully applied to MIT. In those days there were no terrors in considering the "prestige" schools, or if there were, I was far too naive to perceive them. But when the catalog came, it showed no course in literature, art, or history. What of the poems by Scott and Browning that my father told of? So I withdrew the application. A few weeks later my parents made a rare appearance outside the home at my high school. The history teacher, a Yale man, said to them, "Tommy ought to go to Princeton." I'm still trying to figure that out. And, a curious tour de force was at hand. Someone in the Princeton admission office (of which more later) figured that 85% in New York State Regents was equivalent to College Boards, and so these (very difficult for high school kids, particularly if their minds were chiefly

in sports) were waived. The calculation was off by at least 10 points, but the chance was clear. None of us had heard of the place, but there was a remarkable man in Mount Vernon, a lawyer named Leroy Mills. He was the prime world authority on the science of kicking a football, and being a Princeton graduate, it was his hobby to coach the backfield in this lovely skill. Mr. Mills drove me down, and as I watched the balls float high in the fall air over Palmer Stadium, I entered a new world. The admissions procedure was an interview with a gentle lady in Nassau Hall, the assistant and only staff to a formidable relic from the 19th century with the awesome name of Radcliffe Heermaance. He was both Dean of Admissions and of Freshmen. His Mrs. Williams seemed to wonder what this little 15-year-old was doing away from his mother and not even at Lawrenceville. Perhaps, I was admitted by some maternal compunction. I still intended to become an engineer, but a few weeks into freshman year, I saw that the curriculum was hopelessly restrictive, and I was destined to live in this great atmosphere of liberal arts, and yet outside of it. I told this to the Dean of Engineering in my finest hour of all academic discussions in the next 47 years. I found that if instead I majored in Chemistry, there was full latitude for English, History, Architecture, and yet in those parlous depression years, training for that remote possibility, a job at graduation.

I am too far afield already, and cannot recount the sociological and emotional and intellectual play of undergraduate years. I was relatively indifferent to the great social and economic gulfs separating many of the students from each other. I was too naive or too self-absorbed to be seriously affected by the cruelties that were, and still are, engendered by this situation, but I was part of the first attempt to establish "non-selective" clubs at Princeton. Academically, I was greatly influenced by a remarkable group of young English professors, notably, Donald Stauffer, Willard Thorp, and Carlos Baker. Their scholarship was enormous, the entire great swell and sweep of English literature; usually they had *two* fields of specialization—a "classic" and a "modern"! Somehow this made a deep impression on me, and I tried, perhaps subliminally, to do the same in what was to be a subject as different as could be imagined. In the summer of 1937, a friend's mother treated us to a trip to Europe. My astonished father could afford the passport and a copy of *Tom Jones* brought to the boat. In those far-off days, such a trip was a remarkable adventure, and brought the languages and peoples of France and Germany closer, beginnings which were to continue and become most meaningful in the scientific future. Of Hitler we heard and saw remarkably little; Germany seemed under cover, and we lacked the training and perception to see beneath.

When graduation drew near, I had no prospects. I had failed to get a fellowship in several universities for graduate work in Chemistry, and my

heart was not completely in it. I was not inside the subject the way I was in English, and to a lesser degree, Art and Architecture History. Perhaps, it was the difference in faculties, or that my emotions and perception of the world could not then fit with science. So after my friends were ready for law or medical school, or their Ph.D.'s, or some for business, I was left over. On the evening before graduation in June 1938, a man named Dr. John Wallace came to my room, told me that he was a manufacturing cosmetic chemist with a small laboratory and plant in Jersey City, and that they needed a man to do control work and research. He seemed pleased when he found that I was not going to graduate school, and also that I had not made Phi Beta Kappa. I visited the plant, exchanged a few limericks with his partner, and was hired. The partner, Wilfred Hand, a near-genius, had just invented the antiperspirant, Arrid, and was working on an odorless depilatory and kissproof lipstick. The first fact that I learned was that Arrid Cream contained 21% aluminum sulfate; I had just written in a physical chemistry exam that oil-in-water emulsions were broken by electrolytes. We ordered one of the first pH meters from Beckman, and with great curiosity took the reading with the delicate glass electrode and the lovely little calomel one with the glass sleeve. The pH was 2.7, but in a mysterious way it was buffered by urea (not a buffer) and was non-irritating. It turned out that NH_4^+ was being generated. I checked all batches between stages of the mixing vats and jar filling, but there was also time to begin some chemical syntheses. The chemistry that I had grudgingly learned in college came to life, and I read with excitement the pioneering book of Louis Hammett, *Physical Organic Chemistry.* It seemed that here might be the way out from the cookbook reactions that I was running. I asked the boss if I could go to graduate school at Columbia (I hoped to study with Hammett) part-time, and key it in with my work. The answer, fatefully and surprisingly, was no —I could succeeed in the business without any advanced training. Meanwhile my friends were getting on with their lives, and I was isolated in a loft in Jersey City with no discernible future. What to do? I cast about and struck upon what I loved best—English Literature. I had written some stories in college, but seemed to lack the staying power for real creativity. I was greatly attracted to the scholarship, however, and got admitted to the graduate school at Princeton, quit my job (to the surprise of all), "hung loose," hitchhiking, athletics, reading, studying Medieval Latin (a requirement) for some six months, and returned to Princeton in the fall of 1940.

There followed a memorable year, in which I lived in a single room in the tower of the Graduate College, immersed in Old English, Middle English, Etymology (Linguistics had not yet arrived), and American Literature. Other time was spent studying for what would be a comprehensive exam on all of English Literature. But world events were overtaking us, and

by the next summer, most of our generation was in the services or involved in "war work." Having failed a military medical exam, and being a chemist, I found myself back at the bench, and by a curious chance, at the same company that I had left for graduate school. By night and weekends, I continued to study for that awesome examination, and took it under these unconventional circumstances with a rather disapproving faculty. To my own and their surprise, I passed the written part, but revealed some fatal weaknesses in the process. After all, what could they expect of a chemist? So at the oral, the truth came out: I had not read Wordsworth's *Excursion.* Faculty opinion seemed divided as to whether I should give up the field and do science or try again in a few years. It was just at this time that I met Gilbert Otto, as recounted above. My schizophrenic life in New Jersey was suddenly over; I left uncertain of the future, but never to return.

JOHNS HOPKINS: THE WAR, TROPICAL DISEASE, AND MEDICAL SCHOOL

I went to work in the Department of Parasitology of the School of Hygiene and Public Health, a classical department which had then trained about one third of the parasitologists in the country. The strangeness of the environment made it fun. I had good facilities and fine cooperation from everyone. It slowly dawned on me that I was seeing legendary figures everyday in the basement dining room: E. V. MacCallum, Lowell Reed, W. W. Cort, Kenneth Maxcy, Mansfield Clark, E. K. Marshall; and significant younger men, Emmanuel Schoenbach and David Bodian. The Office of Naval Research contracted with us for a microanalytical method for antimony in blood and tissues; metallorganic compounds of this type had been used in tropical diseases since the turn of the century with no pharmacological base. Those were the days of colorimetric methods which, if done well and luck held, were accurate in the parts per million range. One of the first and most famous was the Bratton-Marshall method for sulfonamides—the same Marshall that I saw at lunch. Methodology seemed to fail with antimony, which was surprising since there was no trouble with other metals, bismuth, arsenic, or mercury. Antimony did form a beautiful red lipophilic dye with Rhodamine B. But the metal seemed to "disappear" during the hot digestion process, and was assumed to be volatilized. However, this was not the case; a reading of Mellor's great tract, *Inorganic Chemistry,* revealed that antimony on oxidation reached a relatively inert tetravalent form. The problem was solved in a few minutes; addition of a reducing agent made the metal miraculously appear as judged by the development of the dye.

Thus, we were ready to study blood levels and excretion patterns in patients receiving antimony compounds for the treatment of schistosomia-

sis, leishmaniasis, and our main target, filariasis. Just at that time, we made another discovery and a surprising one. We screened compounds against the organism that caused filaria in dogs and in cotton rats (where the adult worm was in the pleural space rather than the heart). Antimony had no effect at all on the adult organisms, although it had been used in the human disease, Bancroftian filariasis, for some 30 years! The microfilaria were indeed sensitive to antimony, and since they disappeared rapidly from the peripheral blood when drugs such as tar emetic were injected, there was the comfortable illusion that the disease had been cured. I was ready to drop the antimony project, but Otto gave me an early glimpse of wisdom when he refused, saying that the method might yield valuable theoretical information anyway. In the spring of 1945, the Navy sent us to the Virgin Islands to apply the method to patients still under treatment with antimony. I set up a small laboratory in the hospital at Christiansted. Each morning I climbed the hill protected by my pith helmet, feeling Kiplingesque, and curiously dissociated from the world in that fateful time. The town was still sleeping in the 19th century with the historical and social tradition of having been governed by five countries. The Blacks spoke with a beautiful lilting tongue: "Do not vex me, mon" their hardest rebuke. Reward for some 30 determinations each day was swimming in the tempered azure water off the endless empty beaches. Along with Otto and me, was his great friend and colleague, Harold Brown of Columbia, a parasitologist and tropical disease physician of considerable renown. When dinner conversation lagged, he worked on his project to get me to go to medical school, making Otto (who was not a physician) increasingly uncomfortable. A sign of things to come! The bomb was dropped, the world convulsed, the war ended. We celebrated quietly in the island manner with rum drinks flavored by exotic fruits.

Back in Baltimore, we had another year to work, and an exciting lead that organic arsenicals did kill the adult filarial worm. In what I came to see as the Hopkins manner, unselfish help and "Great Expectations," I was aided immeasurably by a great and kindly man, Harry Eagle, who had a unit for the chemotherapy of trypanosomes and spirochetes and who was a modern Ehrlich in his knowledge of the arsenicals and facilities for synthesis. Our screening, some with his compounds, led to the finding that a p-$CONH_2$ group on phenyl arsenoxide gave some specificity for the filariae. It remained to solubilize the compound by condensing it with my old friend, thioglycollic acid. The compound, which we named arsenamide, received a brief trial in human filaria, but was not pursued because of toxicity and the need for intravenous injection. However, based on our work, the veternarians took it up, and comprehensive studies in Japan showed how it could be used for the prevention of dog heartworm. The drug is still in very wide use.

At lunch in the School of Hygiene, talk was still of wartime medicine. Giant strides had been made, many by these men, in malaria, infectious disease, epidemiology, and the beginnings of the conquest of polio. E. K. Marshall, Professor of Pharmacology, was a leading figure in discussion. His war work was in malaria, following pioneering studies on the sulfonamides, and his earlier work on kidney, in which he had made a major discovery—renal secretion. At 56, he was tall, thin, handsome, rather austere, roughly outspoken, and intractably honest (1). He was at the peak of his intellectual power and influence, and frightened many, but fortunately not me since I had been immunized to these dominant traits by the great Chaucer and Pope scholar at Princeton, Robert K. Root. Marshall did like to hear about my work, and seemed pleased by it.

One day he told me of his plan to train pharmacologists by "taking men from medicine and getting them some chemistry, or taking chemists and seeing them through medical school." Marshall himself had done the latter under the aegis of his predecessor, John J. Abel. Later I was having lunch with Marshall's secretary, a grande dame of Baltimore and also Pharmacology, for she had been Abel's secretary fifteen years before. Miss Wilson said, "Mr. Maren, I think Dr. Marshall is interested in you, and may make it possible for you to go to medical school." I said, "But Miss Wilson I've been out of college eight years, and I'm too old." Said she, "Mr. Maren, you're going to get old anyway." And so, with this dialogue out of Jane Austen, the matter was settled, at least in my mind. Marshall had his plan funded by three drug houses, each contributing $3,000 per year for that many fellowships. So far as I know, it was the first training program in Pharmacology. Over a ten-year period, three of us went to medical school, and ten physicians had the opportunity to study and practice basic science. No Ph.D. degree was involved.

There was the formality of getting admitted, and a brief snag developed when it was discovered that I had never taken a course in Biology. I tried to convince the Dean, Alan Chesney, that this was unnecessary, but that great and kind man (presumably) turned off his hearing device, and gently suggested that I take a summer course in Comparative Anatomy at the Homewood Campus. It turned out to be the hardest course I had ever taken, run by two or three of the most brilliant young biologists in the country. There were surprises for me at every turn. I got a C, and started medical school in the fall of 1946. My mother still "believed" in Christian Science, and I had never been to a doctor. If, as the creed said "sin, disease, and death were unreal," what would happen when I faced that cadaver? Nothing.

Marshall gave me a small and pleasant laboratory office which I had all through medical school. There were three fine Associate Professors:

Kenneth Blanchard, a chemist with universal knowledge of science and its attendant gossip; Thomas Butler, brilliant, incisive, original, becoming one of the best pharmacologists of these generations; and Morris Rosenfeld, a physicist-physician and legacy from Abel. We all had lunch together every day, joined by visitors from all over the world. What we gained and enjoyed from these conversations is incalculable. I was a lab instructor in the Pharmacology course my first year before I had even completed Physiology. I recall not understanding how atropine reversed the hypotensive effect of acetylcholine, and telling the second year students that they must have used the wrong solution. I never did take the pharmacology course, or any other in the subject. The basic sciences were a feast, and quite low keyed under Marion Hines (Neuroanatomy), Philip Bard (Physiology), Mansfield Clark (Physiological Chemistry), and Arnold Rich (Pathology). There seemed ample time for student research. The clinical curriculum offered everything, but economically, and well organized. The major ward clerkships were very rigorous; our examples were interns and residents on 24-hour duty all year. A highlight was the "elective" in dog surgery taken by everyone, an unparalleled opportunity to get the feel of living tissues and palpably understand physiological stress. The heads of specialty departments gave elegant short courses of lectures followed by a week or two in the outpatient clinics. It was a survey of medicine as well suited to the prospective general physician as to the research man. It seemed simple, uncomplicated, and effective. Even the grading system was easy; there were grades, but not given to the students. In light of such experience, what has been accomplished by the curricular convulsions of the past decades?

I finished studies on the distribution and metabolism of the antimony and arsenic compounds, and then began with Marshall on the cinchoninic acids and their relation to pituitary function. He had shown these to be antidiuretic, and gave me the job of finding if they worked through the neurohypophysis or directly on the kidney. He would have been pleased if the latter were the case for such action was (and still is) unknown, and Marshall was ambitious for firsts. Curt Richter, a behavioral biologist in Psychiatry, was one of the few who could perform a clean-cut neurohypophysectomy in the rat (without injuring the anterior lobe), and with his help , I showed that our drugs did act through the neural lobe. Of greater interest was the finding that they also affected the anterior lobe-adrenal system. It was the time of cortisone and ACTH, and Marshall led a clinical experiment at Hopkins using the cinchoninic acids in rheumatoid arthritis. I became interested in the hypothalamus, and another generous and distinguished colleague, David Bodian, supervised the analysis of nuclei in this region in our pituitary ablated rats. We showed for the first time a relation between the anterior lobe and the paraventricular nuclei. In that time was the dawn of neuro-

endocrinology, and I cherish the memory of a visit from Geoffrey Harris, the great English anatomist who had just discovered the portal circulation in the pituitary.

My seven years at Hopkins drew to a close. There was no way to know it then, but 1951 and the few years to follow were watersheds between the ideas and ideals generated in the 19th century, and the very different future in which we now live. Many of the men that I have mentioned were educated before World War I, and both Princeton and Johns Hopkins had a long unchanging period of stability during the first half of our century. The buildings were unchanged, the traditions, curriculum, and even the songs seemed to stay the same. I cannot evaluate such issues, but in a sentence or two, I would guess that the intellectual and ethical benefit was enormous. However, a heavy price was paid in social and possibly financial oppression. Perhaps, this is worth remembering if we wish to recapture the best of those years, some of which has certainly been lost.

I considered clinical training, as I enjoyed medicine very much. The economics seemed impossible for a man with a family and no private income —interns at Hopkins were still paid $25 per month. More important was the question of whether clinical training was worthwhile if I were to continue in basic research. Marshall thought not, and I recall Harry Eagle saying that his year as an Osler intern (that great plum) was the worst of his life. I was not sure then, but feel now, that I did make the correct decision. I had learned enough in medical school to understand basic principles, and to be part of a medical faculty. Besides, I was 33, and better get on with my life. I had a choice between an assistant professorship at $5,000 per year, or several jobs in the pharmaceutical industry, at twice this amount. There was also the novelty and challenge of industry, at a time when not many men with substantial academic training were taking that route. Not so many years back from then the American Society for Pharmacology and Experimental Therapeutics, Inc. did not admit scientists from industry!

THE CHEMOTHERAPY DIVISION OF AMERICAN CYANAMID: DEVELOPMENT OF THE CARBONIC ANHYDRASE INHIBITORS

In the fall of 1951, I joined the Chemotherapy Division of the American Cyanamid Company in Stamford, Connecticut, housed on the top floor of a converted factory. One had to ride an old freight elevator to get there. The rest of the building contained the main research laboratories of this large company devoted to chemistry, physics, plastics, and agriculture. The drugs developed by our division were marketed by Lederle Laboratories, a wholly-

owned subsidiary of Cyanamid. The Chemotherapy Division was remarkable with, as its head, Richard O. Roblin, a talented chemist and true research director. Ten years before, he and his colleagues had discovered sulfadiazine, and from his small chemical team was built his division which now consisted of eight groups: Three were in organic, one in physical chemistry, and these were balanced by the four groups of bacteriology, parasitology, pathology, and pharmacology. The place was strikingly unpretentious; no one had an office except Roblin. Almost no time was spent in administration or meetings; Roblin read the literature all morning and worked in his lab in the afternoon. The eight group leaders were reputable scientists; indeed, a major contribution to theory of drug action had been made here (Bell-Roblin on sulfonamides). They were also pioneers in broad-spectrum and gram-negative chemotherapy (polymixin), and treatment of parasitic diseases in chickens. Roblin believed in a substantial mix of theory and practical development, and he had carte blanche for his programs.

I was hired with the idea of working on the physiology of the hypothalamus and connections with the pituitary. This was related to work that I had started at Hopkins on obesity, using the strain of mice just discovered at the Jackson Laboratory. There also was still some interest in the cinchophen derivatives that Marshall had hoped to use in rheumatoid diseases. But one day that winter, Roblin told me that he had had a call from a gastric physiologist named Henry Janowitz to whom he had given a drug called 6063 which reduced acid output from the stomach. He confused me utterly by talking about enzymes and inhibitors of which I scarcely knew the difference. Finally, he asked if I would work on this drug, and of course, I said yes, being anxious to leave and do some reading. Once again I felt the spirit of "Great Expectations" conveyed by Roblin, unspokenly then and in the years ahead.

I have told the story of the development of the carbonic anhydrase inhibitors elsewhere (2, 3), and now I will try to show some of the strands reaching back into physiology and biochemistry, and how these became woven into my own life. The enzyme was a Cambridge product, discovered by Roughton in 1933, with vital work showing its metallic nature and susceptibility to sulfonamides by Mann and Keilin in 1940. In the following decade, carbonic anhydrase became linked with renal acidification and bicarbonate reabsorption through the work of Davenport, Höber, Pitts, and William Schwartz. The inhibitor used was sulfanilamide, but Roblin, sitting in quiet isolation at Stamford, and drawing on his knowledge of sulfonamide chemistry, realized that possibilities existed for more specific and powerful drugs of this type. There were other strands: The role of the enzyme in gastric and pancreatic secretion, in respiration, and the possibility that inhibition might control epilepsy through alteration in acid-base balance. All of these ideas were in the air on that winter afternoon in 1951,

and by then, Roblin and his associate, Jim Clapp, had synthesized several dozen new compounds, some of which were 1000 times more active against the enzyme than sulfanilamide. He had supplied a few of these to academic physiologists; the best one seemed to be 6063 (later named Diamox, and the generic, acetazolamide—the idea being that this was so difficult to pronounce that only the trade name would be used) on the basis of high activity, stability in vivo, and sharp effect in alkalinizing the urine. Robert Berliner had just published a paper in which he had reported the use of 6063 in a study of K^+ secretion and the action of mercurials. I did not know until much later, however, that Roblin had been advised by some of the leaders in renal medicine not to pursue the project since they thought (correctly, as it later turned out) that such drugs would not be ideal diuretics. Roblin, through broader vision, a taste for gambling, and a chemist's vague sense for physiology, went ahead.

I was assigned the development of this drug with the idea that it might be the first oral diuretic, a treatment of ulcers, epilepsy, and renal stones. Every few days another disease was suggested. How to develop a drug of an entirely new type, and inhibit an enzyme that was thought essential to life, and whose absence had been predicted to lead to "speedy death"? Only in retrospect do these seem formidable, then it was stimulating and great fun. My training with Marshall made the first step reflex; we must have an analytical method for the drug in body fluids. Attempts to develop a chemical method failed, and I fell back on a simple enzymic procedure which is still used. Acute and chronic toxicity work began; to our amazement, the drug was entirely nontoxic; we could not even kill an animal with grams/kilogram by vein! What was going on? Why didn't the diuretic effect and alkaline deficit continue and prove fatal? How about respiration when the red cell enzyme was inhibited? What would be the effect of long-term inhibition on the morphology of the tissues?

The answers emerged slowly over the years, always it seemed with implications for my own life as well as for the underlying physiology. The first question led to a meeting with the now legendary Homer Smith who had just found that seagoing fish did not have a renal response to 6063. He asked me to come to the Mount Desert Island Biological Laboratory (in Salsbury Cove, Maine) to see if there was carbonic anhydrase in fish kidneys. There was not; yet, the fish could absorb any amount of filtered HCO^-_3. At the same time, I was finding in Stamford that acidotic dogs could reabsorb HCO^-_3 when all carbonic anhydrase was blocked. Clearly, there was a second and quantitatively major mechanism for HCO^-_3 reabsorption, and this protected the animal or the patient (as it turned out) from further electrolyte loss after the first day of enzyme inhibition. Thus, we had a solution with theoretical and practical implications, and a triumph of the

comparative method. I have been working at this laboratory every summer since; it has immeasurably enriched my scientific and personal life. E. K. Marshall was still at Salsbury Cove, where he had discovered renal secretion 30 years before. Our friendship deepened as the years passed. There is a curious parallel in his use of the goosefish (no glomeruli) to prove tubular secretion, and mine of the dogfish (no renal carbonic anhydrase) to illustrate ionic HCO^{-}_{3} reabsorption. Both ideas were fought by the renal establishment for at least ten years (1, 2).

The respiratory problem was not solved until much later, as I shall tell. The critical issue of morphological damage was answered in the negative based on a most meticulous chronic toxicity study carried out at Stamford under the direction of my colleague in pathology, Edmund Mayer. From this remarkable man, I seemed to be learning, in those four years, about as much medicine as I had learned at Hopkins. My luck had held; in those spare and obscure laboratories at Stamford, I was still in the presence of masters.

I had no training in renal or electrolyte physiology, and was working in comparative isolation with a few excellent technicians, a fine beagle colony, and a good library. Ten days spent with the young Bill Schwartz and Arnold (Bud) Relman in Boston were of seminal importance. I watched their dog experiments and we traded ideas: chemistry for physiology. By the end of 1953, I emerged and faced the Establishment for the first time at a seminar in Pitt's department at Cornell Medical School. We had given 6063 to dogs and rats for over a year, studying electrolyte balance, drug distribution, effects on growth, and general toxicity. No one had hurried me, but the drug was ready for clinical trials, and the market in the next year. Just then came a totally unforeseen development; Bernard Becker at Johns Hopkins showed that 6063 was effective in glaucoma. Roblin's gamble on the unexpected in science had paid. I have told elsewhere the remarkable story leading to Becker's discovery, based on the ideas and genius of Jonas Friedenwald, and the fine experiments of Everett Kinsey and Per Wistrand (4). Within a few years, acetazolamide became a fundamental part of practice and research in ophthalmology.

This discovery, together with our data showing restricted penetration of acetazolamide to eye and brain, led to development of a second and more diffusable drug, methazolamide, better suited for glaucoma treatment. Attention was also given to other sulfonamides of different physico-chemical types, and correspondingly different affinity for tissues. From these studies, came a third drug, benzolamide, whose affinity for proximal tubule cells made it a specific renal carbonic anhydrase inhibitor. [Attempts to develop benzolamide commercially have failed, and it now has the interesting status of an orphan drug (5).] The Merck, Sharpe and Dohme group under Karl

Beyer, competing with our work on renal carbonic anhydrase inhibition, had the good fortune and technical excellence to find a new series of sulfonamides with a different mechanism, better suited to the treatment of congestive heart disease: the thiazides, and later furosemide and all their congeners, were born.

The Stamford Laboratories offered a great deal. I was able to complete a study on the obese mice, showing that diet restriction caused a preferential loss of protein over fat. Also, we discovered the long-acting antibacterial sulfonamides when it was found that a methoxy group on the N^4 or heterocyclic ring induced avid renal reabsorption. But our Chemotherapy Division had within it the seeds of destruction. First, it was simply too good—perhaps the best pharmaceutical research group in the country. Second, Roblin had unusual power. And third, the drug development units at Lederle were floundering. So, in a stroke combining jealousy, an attack on the "elite," and an attempt to save Lederle, the division was destroyed by the parent company. The various units were to be dispersed at the Lederle Laboratories in Pearl River. (From the vantage point of 25 years, this was a dreadful corporate decision; small *was* beautiful and successful, and big *was* ugly.) But *deus ex machina,* my phone rang; at the other end was George T. Harrell, who said that he was dean of a new medical school at the University of Florida. I recall this conversation with the same embarrassment that I mentioned in connection with Dean Chesney at Hopkins. Only ten years had passed, and I guess I had not mellowed much. I asked Dr. Harrell about himself and his qualifications (which were pretty good) after which he said that he would like to consider me for Chairman of the Pharmacology Department at the school in Gainesville. So that summer (of 1955), I went down and faced the 100 degree heat, walking on the girders of the fifth floor of the building rising from a sinkhole. My department! The deal was settled in a decaying hotel-restaurant at Cedar Key on the Gulf of Mexico, as we ate stone crabs, hearts of palms salad, and as a summer hurricane blew open the doors and out the lights. There were "Great Expectations" in Harrell's voice, and in that wind.

THE UNIVERSITY OF FLORIDA SCHOOL OF MEDICINE: TEACHING OF PHARMACOLOGY

The original faculty of the Unversity of Florida College of Medicine were four basic science chairmen: the others were James G. Wilson (Anatomy), Frank Putnam (Biochemistry), Joshua Edwards (Pathology). In the next year came Emmanuel Suter (Microbiology) and Arthur Otis (Physiology). All, happily, are still alive, and may share with me these brief reminiscences. At first, it was sort of a pick-up game, and we were referred to simply and

conveniently as "the boys," until one day we realized it was a pretty serious business; students were coming in the fall, the building was almost finished, and we were the Executive Committee. We did admissions, curriculum, departmental layouts, and (with the dean) the hiring of the clinical faculty. We were unified by common ideals, a visionary dean, absurdly proud of our little departmental fiefs, but held the school's goals high as well. Classes were small (40–64), there was extensive laboratory work, two free afternoons per week, and in the second year, some 150 hours set aside for independent research for all students. This continued for 13 years, during which nearly 1000 physicians were trained in a system which did encourage independent thinking and inquiry. Changes in the 1970s blew ill winds, and this milieu has vanished without a trace. I mention this with nostalgia, but also in the hope that these notes will encourage my successors in Pharmacology and the other medical sciences to try as hard as possible to reverse the present faceless medical education (in many schools, fortunately not all) in which there are no laboratories, little personal work, limited student-faculty contact, and robot-like examinations. We have turned the clock back a hundred years; the toll is already being counted in the severe decline of M.D.'s in research, an inevitable result of no exposure in the critical years of training. The effects will soon begin to show even more tragically in the decline of clinical skills and good patient care.

I organized the Pharmacology Department, bringing to life ideas that had been germinating for some years. We added "Therapeutics" to the name (*not* "Experimental Therapeutics") to indicate that we had a role in both the theoretical and practical aspects of the subject. The first appointment was a biochemist, Kenneth Leibman, who rapidly and with obvious enjoyment learned physiological as well as chemical pharmacology. Then came Bohdan Nechay, trained in veternary medicine, David Travis, a clinical physiologist with particular knowledge of respiratory and renal medicine, and Aaron Anton, a real Ph.D. pharmacologist. This fine group assembled a course in pharmacology for second-year medical students with heavy emphasis on laboratory work. The planning and teaching in the lab was a vital part of our own training, particularly important since we were all so new at it. (The Dean tried hard to keep our guilty secret: The Pharmacology Chairman was only four years out of medical school.) By 1962, we were ready for a good experiment in education: We would split our course in half, and give the second part in the fourth year. We were still in the "honeymoon" period, and the Executive Committee, including the clinical heads, were willing to give up November of senior year for Pharmacology. And Roger Palmer, a graduate of our first class, fresh from a medical residency at Hopkins, was back, full of idealism for the new program. It worked! Fears that fourth-year students would begrudge time spent in a basic science were

not realized. The split was tried several ways—perhaps the most satisfactory was to emphasize the chemical aspects in the second year and physiological in the fourth. It may make no difference how the topics are arranged: The main advantage was the milieu furnished to the students, and what they brought in the way of attitudes and questions in the two years since we had seen them. This system is still working, and may be unique among American medical schools. I have set down elsewhere some ideas about Pharmacology in medical education (6).

RESEARCH AT FLORIDA: A BACKWARD LOOK, 1981–1958

My work on carbonic anhydrase was done backwards. Although I sought connections between chemical and physiological events, the latter work, as I have recounted, was done first. In Florida, I tried to relate the quantitative data on renal excretion of HCO^-_3 following acetazolamide to catalyzed and uncatalyzed rates of CO_2 hydration, and fractional inhibition of the enzyme. Because the chemical reactions were simple, the same in vitro as in vivo, and the inhibitors completely specific, this did seem possible, and we were able to add a new small dimension to pharmacology. Both the kinetic and the drug data showed, independently, a great "excess" of enzyme so that 99% inhibition was zero on the dose response curve for renal effect. Superficially, it seemed that there was unnecessary enzyme present, but this concealed the principle that a high concentration of enzyme ensures very rapid reactions in a near-equilibrium state, where there is a narrow gradient between substrate and product.

The writing of a comprehensive review on the subject in 1967 (2) showed that we did not understand the role of the enzyme in CSF formation, or in brain fluid. Being already wedded to the Mount Desert Island Biological Laboratory, our initial experiments were done in fish (the small shark, *Squalus acanthias*), a lucky event since an increase in peripheral pCO_2 caused a large and rapid elevation of CSF HCO^-_3, which was blunted when carbonic anhydrase was inhibited. Later experiments with Dr. Betty Vogh confirmed this in the mammal, and showed the linkage of HCO^-_3 formation to Na^+ transport. The excitement of a new and unexpected finding was modified by the difficulty of getting it accepted or even published; it ran counter to prevailing ideas and even seemed unreasonable since bulk CSF is a neutral, not an alkaline, fluid. In relaxed moments, it seemed fun to fall into the great tradition of iconoclasm, and ten years and the work of other groups have now smoothed the concept into the stream of physiological thought.

In about 1970, I decided to finish with carbonic anhydrase. Another wartime malaria program was under way, and I wanted to be a part of it. I was a bit tired of my identification with this enzyme and drug ("Are you still working on Diamox?"), and did people think I was hopelessly stuck? The excursion was fun, and we learned some interesting things about sulfones, but the carbonic anhydrase problem was too central and compelling, so my transgression was brief.

The problem posed by the near-discoverer (Henriques) and discoverer (Roughton) of carbonic anhydrase concerning its quantitative role in respiration had yet to be worked out. It was still not clear how survival was possible in the absence of enzyme activity, or what its role was in exercise. I was fortunate to have a young colleague, Erik Swenson, attack this problem, both as investigator and subject. The answer explained why our high dose animals survived in spite of the dire predictions of the biochemists. In normal respiration, CO_2 is evolved almost entirely from HCO^-_3. When the enzyme is absent, newly established high gradients of CO_2 make its elimination possible by simple molecular diffusion. But this auxiliary non-enzymic route is not adequate in exercise. This remarkable enzyme, with its magically great turnover number, evolved so that HCO^-_3 and metabolic CO_2 are instantly convertible, and that virtually any amount of the gas can be generated from narrow gradients of the ion.

By good fortune, work at the Mount Desert Island Biological Laboratory continues to give hints to solutions of our physiological questions, in addition to those already mentioned. These concern lens, aqueous humor, endolymph, and drug diffusion across the gills. Presently, we are studying the vertebrate phylogeny of carbonic anhydrase. This place is also my window on the world, in science and in spirit.

In the last few years, association with another talented young man, Gautam Sanyal, has led to fundamental studies on the thermodynamics of catalysis, and work on other substrates for carbonic anhydrase and the properties and possible roles of certain of its isoenzymes. So the 30-year Odyssey has taken me back to the homeland of molecular pharmacology: the active enzymic or receptor sites. How different would the story have been if I had started at the beginning? And am I ending here?

BIOPOLITICS: A SMALL DOSE

By instinct or the example of my father, I had no taste for public affairs, or administration, or power, or the judging of others. I found myself a departmental chairman for 22 years, and on NIH study sections for ten years, and served a term as President of the Association for Medical School

Pharmacology (AMSP). I have an uneasy feeling that I "got away with it," but should have been an even more obscure scholar. And I have a residue of warmth from it all—the (too) few fine men I brought into the department, the wide ranging friendships and expansion of view generated by the study section work, the fanning of life to AMSP by organizing "holiday" winter meetings, and precious and supportive exchange of views among men and women in the same profession. Now I have a chance, as a Research Professor, to test my (alleged) affinity for isolation.

I was thrust briefly, and unexpectedly, into Chinese affairs in the time of Mao, when in 1974, I was part of a National Academy of Science sponsored trip to the Mainland to study Herbal Pharmacology. Scientifically we found little, but I returned with two deep convictions: that personal freedom, which I had never before relinquished, was more precious than I had dreamed, and that the potential for accomplishment by the Chinese, once committed, was greater than any we know in the West.

The reader perceives the curious element in this tale: It goes against the grain of good conventional science which is to do one thing, and do it well. What did Mercutio say: " 'Tis not so deep as a well, nor so wide as a barn door, but 'tis enough, 'twill serve." Whether it is my own disposition, or the ubiquitous nature of the problem I was assigned 30 years ago, or a coincidence between them, I do not know, but I have extended myself most unfashionably across these many fields. A modern Dryden might, if I were lucky, notice me as one "Who in the course of one revolving moon/ Was fiddler, statesman, chymist, and buffoon."

I am greatly fortunate to live as part of a community that has no geographic or social boundaries, linked by interests, affection, and even rivalry. My beliefs should be clear from this essay, so I end only in the passionate hope that scholarship and society maintain their equilibrium in the years to come, and that "Great Expectations" are held out to our heirs.

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