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Pushing Forward the Future Tense: Perspectives of a Scientist

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Abstract

This review is a somewhat chronological tale of my scientific life, emphasizing the why of the questions we asked in the lab and lessons learned that may be of value to nascent scientists. The reader will come to realize that the flow of my life has been driven by a combined life of the mind and life of the soul, intertwining like the strands of DNA.

CONTEXT: MY LIFE IS A GRATITUDE LIST

When I was invited to write this autobiographical review, I made a mental list of all to whom I am grateful in my life. That list is long. When trainees ask, "When did you realize you wanted to be a 'scientist'?," I must confess to not having had a plan. At each stage of my life, I have been blessed by being at the right place at the right time with many advocates. My father was my first cheerleader and encouraged me to do whatever I wanted to do with my life—a courageous point of view for someone without the privilege of a college education and who had been deeply harmed mentally by World War II. I went to college with no idea of what I would pursue, and I perhaps drifted toward the sciences because my brother, five years older, had pursued engineering. However, to be honest, even my graduate training and the earliest years of my postdoctoral research were, in my mind, a holding pattern until we had children; I had too few role models who managed both a career and family life.

THE COLLEGE OF WOOSTER: A STARTING POINT

My first opportunity to behave as a scientist began at the College of Wooster in Wooster, Ohio. Wooster sets aside the fourth year principally for faculty-mentored independent study—a requirement for all students. My mentor was an analytical chemist, Theodore Roosevelt Williams, an African American among a principally white faculty and student body during the era of the Civil Rights movement and the coincident distrust evoked by the Vietnam War. Based on his life experience, precedent for him was not a compelling reason to do, or not do, anything. Consequently, when I showed no interest in departmentally suggested independent study projects, he encouraged me to pursue my interest in a more medically relevant question. Though clinical research expertise did not exist at the College, it could be found at Duke University, where my fiancée, Tom Limbird, was studying medicine. Ah, you see the linkage here, don't you? Ultimately my studies, which included terrorizing fellow Wooster students with blood draws before and after climbing lots of stairs, were detailed in my Independent Study document, "Role of CPK Isoenzymes in the Diagnosis of Myocardial Infarction," comentored by Galen Wagner at Duke. This topic presaged my PhD dissertation three years later. Most importantly, however, Wooster was the perfect place for me to spend my formative years. As a first-generation college attendee, I was perpetually fearful and always watching others before jumping in. Wooster offered enthusiastic teachers who were as committed to our personal growth as they were to our learning.

PHD TRAINING: INTERRUPTED

I entered graduate school in the fall of 1970. With a trivial graduate school stipend to live on, the only outlet for angst was taking courses. I completed the didactic requirements of the PhD program in Biochemistry at the University of North Carolina (UNC) at Chapel Hill in two semesters. However, I did not perceive that same joy in the courses I took there as I had at Wooster, nor did I identify faculty whose research truly excited me in my graduate studies. I justified leaving graduate school by reasoning that if the path to a PhD is not interesting or exciting, perhaps I need to reconsider whether this is the goal I want to pursue.

Having resigned from graduate school after only two semesters, I was fortunate that Galen Wagner, who had coadvised my independent study at Wooster, navigated my continued ability to work on creatine phosphokinase (CPK) isoenzyme detection for clinical application as a research assistant in Duke's Pediatric Division of Metabolic Disease with Dr. Charles Roe. In collaboration with cardiac surgeons and cardiologists, we demonstrated the value of detecting the MB isozyme

of CPK, which in humans is only expressed in myocardial tissue, for diagnosis of cardiac infarction, particularly in patients whose total CPK and lactic dehydrogenase (LDH) isoenzyme levels are confounded by concomitant surgery (1–5).

Thanks to the advocacy of my former graduate student colleagues and Dr. Robert Bell, Director of Biochemistry Graduate Studies at UNC Chapel Hill, my CPK isoenzyme research at Duke served as my UNC PhD degree, awarded in 1973. Though I was not first author on any of the CPK isoenzyme research at Duke, my efforts had been noticed by Dr. Andrew Wallace, Chief of Cardiology, who found resources for me to join the laboratory of a new faculty member in Medicine, Robert J. Lefkowitz, MD. This opportunity aligned with my desire to move from direct clinical research to a more detailed molecular understanding of cardiac disease; resources for my postdoc were available due to a program fostered by James Wyngaarden, then Chair of Medicine, to fund reciprocal training of basic scientists in clinical problem-solving and clinicians in basic science approaches.

POSTDOCTORAL RESEARCH AND THE "LEFKO LAB" MEMORIES

As Bob's first postdoc, I helped set up the lab while benefitting from his side-by-side teaching of methods for creating myocardial particulate preparations, building cardboard-box incubators to perfuse proteolytically dissociated myocardial cell suspensions, and refining laboratory assays. Though I lacked relevant laboratory expertise for the early projects I undertook, this was not a factor for Bob, or Bobby Jo, as I nicknamed him. Bob was just delighted that I was married to a Duke surgical resident and so would be available to work nights and weekends. He was right!

My early work with Bob explored how guanylyl cyclase was regulated by acetylcholine, a question not central to Bob's interest or expertise. It was about this time that my husband, Tom, and I learned of our infertility—a huge blow. I began to think, if this is all I am going to do for my life, I am going to have to take the direction of my projects more seriously. I summarized the guanylyl cyclase studies in unremarkable publications (6, 7) and switched directions to central themes in Bob's lab. About this time, the lab had found a way to specifically identify the β-adrenergic receptor with radiolabeled antagonists (8, 9), which made possible the pursuit of several parallel lines of investigation. Bob encouraged me to examine reconstitution of receptor-mediated activation of adenylyl cyclase after detergent solubilization of the receptor. After banging my head against the wall for several months, I reached out to the authors of the preliminary reports of successful reconstitution only to learn that they, like me, could not repeat those findings (see the sidebar titled Lessons Learned).

Further consideration of reconstitution studies made me realize that there were underlying assumptions in their design that had not been affirmed, mainly that the receptor and cyclase enzyme were separable macromolecules and not a single molecule with allosteric sites for hormone regulation, as imagined earlier by Sutherland & Robison (10) and later proposed by others (11). Fortunately, Marc Caron had by then joined Bob Lefkowitz's laboratory as a postdoctoral fellow and mentored me in the many tools for protein isolation and characterization that I had not learned during my accelerated PhD degree. Andre DeLean, another postdoctoral colleague, helped me realize the power of computational modeling to focus experimental efforts on discriminating experiments. Finally, Charles Tanford, who had taught the biophysical chemistry course I took at Duke to complete my PhD, mentored me in the properties of membrane proteins, including how to handle these proteins experimentally once they have been seduced into biological detergents. Ultimately, my studies led to the demonstration that the β-adrenergic receptor and adenylyl cyclase are indeed separable macromolecules (12), consistent with concomitant genetic (13) and cell fusion studies (14) being reported by other laboratories, and that the recently

LESSONS LEARNED

- Emphasize the joy. Research is fun—hard work, yes, but fun! And the ability to change directions and move forward based on new and unexpected data is unmatched in most other professions. Faculty bemoan a diminished interest of graduate trainees in research as a career—perhaps that's because we give too much verbal time to the hard work involved. Anything of value involves hard work.
- For any project, start first with repeating the work that is the basis for your next-step questions, and reach out sooner rather than later to the authors of the original findings if early experiments fail to replicate their results.
- Regarding training research assistants, focus on the question, not just the manual skills. This approach creates a partnership where your research assistant also becomes an effective troubleshooter.
- Data are the currency. No amount of networking or discussion of promissory findings can match data. Published data.
- Find a way to continually learn beyond the specific area of your research—both in content and in conceptual frameworks. It refreshes one's soul and recharges one's scientific batteries!
- Have exciting new and unexpected findings replicated by others in the lab before publishing. This lesson was learned painfully. Needing to retract papers resulted in painful introspection on my part, redirection of other trainee's projects in the lab, temporary challenges in acquiring grant funding, and diminished lab morale, which took too long, at least for my soul, to reboot.
- To inspire your colleagues, be they trainees or faculty colleagues, emphasize what they have accomplished rather than their "not-yets" (70).
- We need to bolster our overall training environments to create a just and equitable place for all young scientists to thrive. What we might have been willing to endure in a different time and different context may not be an environment sufficient to bring out the talents of every trainee, in this time and in our current contexts.

discovered separate guanosine-5′-triphosphate (GTP)-binding protein preferentially associated with an agonist-occupied receptor (15), an interaction that could be reversed by pretreatment of membranes with agonist plus GTP analogs before detergent solubilization (16).

A six-year postdoc, due in part to the duration of my husband's residency training in orthopedic surgery at Duke, allowed me to develop more than my laboratory skills. I had written proposals to support my independent development, but early efforts were thwarted by reviewers who mentioned that funding me would merely add more money to the Lefkowitz lab, then generously supported by the Howard Hughes Medical Institute. Thankfully, however, one National Institutes of Health (NIH) reviewer of my Young Investigator Award, Mark Entman, MD (Baylor College of Medicine), apparently countered another reviewer's argument that if I really wanted to be independent then I would go get a job elsewhere by saying, "Don't punish her because she wants to sleep with her husband!"—yet another advocate in my professional development. With my own funding, I was able to hire a technician, and I got my first experience training someone (see the sidebar titled Lessons Learned).

My most enduring memory of Bob's lab, as he too reports (17, 18), was that we had fun! We learned about the intersection of data and personalities through Bob's end-of-day stories shared from the perch of a lab step stool; discovery came alive in that storytelling. Yes, we all lamented that we could be angry with Bob about something and, yet, within 30 seconds of being in his office, he had us laughing about the same thing that had made us fume. We also all shared the frustration

that access to Bob paralleled your having data worthy of piquing his interest (see the sidebar titled Lessons Learned).

VANDERBILT UNIVERSITY

I moved to Vanderbilt University as Assistant Professor of Pharmacology in the summer of 1979, taking a one-month hiking trip with my husband while waiting for small equipment and reagents to arrive, all of which was facilitated by having my own funding as a Young Investigator awardee of the NIH. I selected Vanderbilt because of the breadth of research in areas related to signal transduction—I knew I still had so much to learn. For one thing, I did not know any classical pharmacology.

In selecting a professional home, I was fortunate to have found a place where I could grow, an environment where I could pick myself up after temporary failures, and a boss who judiciously knew when to place a thumbtack on my chair and when to throw me a life-preserver ring. Joel Hardman, who recruited me to Vanderbilt, had trained with the Nobel Laureate Earl Sutherland; his own research had identified and characterized guanylyl cyclase. But as Chair, Joel had devoted himself to the mentoring of students and junior faculty. Lucky, lucky me. From Joel, I learned about the principles of pharmacology by attending his lectures in a receptor theory course, which I later inherited. His mentoring formed the groundwork for a short book I wrote: *Cell Surface Receptors: A Short Course on Theory and Methods* (19). Joel also sat in on lectures for graduate and medical school courses; his postsession feedback was sometimes longer than the original class time itself! Despite the pain of this persistent feedback, I did learn how to consider the variety of content confidence that students may bring to class and introduce new material accordingly.

I grew to rely on Joel Hardman's breadth of knowledge. Without his considerable background in pharmacology, I would have never had the opportunity to serve as associate editor of *Molecular Pharmacology* or accepted Al Gilman's invitation to coedit the ninth and tenth editions of *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. I should mention that when Al Gilman asked me to consider editing "the book," as he referred to it, I reminded him that, from my point of view, I really didn't know a lot of pharmacology. "Oh yes, I know," he said, "but what a great way to learn!"

At Vanderbilt, I participated in journal clubs with Stanley Cohen, who later was awarded the Nobel Prize for his work on epidermal growth factor and its receptor-embedded tyrosine kinase activity. Stan was a model of discriminating thinking about experimental design. Keenly aware of my ignorance of in vivo pharmacology, I also attended weekly noon seminars of clinical pharmacology led by the department's then-Director, John Oates, MD. John modeled how to think like a scientist. Here is what occurred at most sessions: A clinical pharmacology fellow would present their work of a year or so for approximately 30 minutes while John ate his sandwich and apple from home. Then, John would carefully and slowly fold his paper bag, just so, and begin to ask questions. With John's characteristically southern grace, he would reveal the many untested assumptions in the experiments, the inherent flaws in the design, and redirect efforts to more fruitful and discriminating strategies. Like Bob Lefkowitz, John Oates was a master of focusing on the most critical experiment—not busying oneself with correlative data collection (see the sidebar titled Lessons Learned).

The Conceptual Thread of the Research We Pursued in My Laboratory at Vanderbilt

My goal is not to summarize, aloud with feeling, the experimental findings from our Vanderbilt lab but rather to discuss the why of our questions, hoping these insights will be of some value

to emerging scientists. I also regret that I cannot name all my wonderful student and postdoc colleagues but hope the reader will consult the references to learn of their individual contributions.

Determining the Generality of Agonist Occupancy of G Protein–Coupled Receptor (GPCR) Stabilizing Interactions with G Proteins

The last series of experiments I engaged in as a postdoctoral fellow in Bob Lefkowitz's lab demonstrated, in collaboration with the late Michael Gill at Harvard, that agonist occupancy of the β-adrenergic receptor led to an increase in apparent receptor size (15) due to the coelution of receptors with GTP-binding proteins, identified by cholera toxin–catalyzed ADP-ribosylation (16). Agonist occupancy of the receptor thus appeared to foster or stabilize receptor–G protein interactions that could be captured experimentally.

In my own laboratory at Vanderbilt, we first queried whether agonist-facilitated receptor–G protein interactions would be a general property of adrenergic receptors. Though our first experiments explored muscarinic receptors, an emerging literature revealed a plethora of muscarinic subtypes—and associated experimental challenges. Instead, we turned to α_{2A}-adrenergic receptors and human platelets as a model system based on two criteria: (*a*) They had a single adrenergic receptor subtype, which would simplify both biochemical and functional experiments, and (*b*) a Vanderbilt colleague, Jacek Hawiger, had perfected a method for platelet isolation that retained responsiveness to so-called weak activators of platelets, like epinephrine (20). Indeed, we demonstrated that agonist occupancy of α_{2A}-adrenergic receptors, which resulted in inhibition rather than stimulation of adenylyl cyclase, also resulted in stabilization of receptor–G protein interactions, albeit with a distinct G protein, dubbed $G_{i,}$ which can be tagged by pertussis toxin–catalyzed ADP-ribosylation (21, 22).

A Transition to the Role of Na^+ in Regulating GPCRs

Perhaps because I was a competitive swimmer, I do a lot of my thinking in water. To this day, the bathtub is my favorite place for scientific reading, thinking, and planning talks. I remember the day I asked myself while in the tub, "How long am I going to just focus on receptor–G protein interactions? There are wonderfully talented people in this field—they will find the answers. What can I contribute that is not already being aggressively pursued?" This musing was not due to a fear of competition, as I thrive on competition. Rather, I believe that taxpayers are investing in new knowledge when they fund biomedical research, so we should not all be asking the same questions.

But why pursue regulation by Na^+? I was intrigued by the ability of monovalent cations to mimic the effects of GTP on ligand-receptor interactions at G_i-coupled GPCRs: Na^+ and H^+, like GTP, decrease receptor affinity for agonists, less so for partial agonists, and increase receptor affinity for so-called inverse agonists (23, 24). Our question then became whether Na^+ could be binding to a distinct and not-yet-characterized regulatory protein in the overall GPCR signaling architecture.

Purification of the α_2-adrenergic receptor to homogeneity, however, revealed that the allosteric regulation of receptor interactions by Na^+ and H^+ as well as by inhibitors of Na^+/H^+ exchange was retained by the receptor itself (25), within the hydrophobic tryptic core (26). Because recent cloning of the β-adrenergic receptor had revealed a seven-transmembrane-spanning topography of GPCRs (27), we wondered whether—like some seven-membrane-spanning microbial rhodopsins (28)—the α_2-adrenergic receptor might also transport ions. Since binding to the purified α_{2A}-receptor is allosterically regulated not only by Na^+ and H^+ but also by amiloride analogs that inhibit Na^+/H^+ exchange, we postulated that the receptor itself might possess antiporter

activity. Despite our excitement about this hypothesis, introducing the purified receptor into unilamellar liposomes revealed no cation transport activity (29).

In parallel studies, we had shown that removal of extracellular Na^+ from platelet preparations eliminated α_2-receptor-mediated platelet aggregation and secretion due to eliminating receptor activation of novel phospholipase A_2–dependent priming of signaling for weak platelet agonists (30–37). However, we later had to retract the findings because an enthusiastic graduate student had misled us to conclude that the α_{2A}-adrenergic receptor activated a Na^+/H^+ exchanger (38, 39) (see the sidebar titled Lessons Learned).

What, Then, Was the Functional Relevance of Allosteric Regulation by Monovalent Cations?

We began a series of single point mutations of the α_{2A}-adrenergic receptor to eliminate allosteric regulation, aided by lessons learned during a six-month sabbatical with David Russell in the laboratory of Michael Brown and Joe Goldstein at UT Southwestern (40), a turbo learning experience for which I remain eternally grateful. It seems that I am someone who cannot troubleshoot experiments effectively unless I have had hands-on experience with the methods. This is, by the way, not a strength, as it slows the pace of the laboratory, but it is just the way it is with me, which is why we ultimately collaborated with other laboratories so frequently, because there is only so much one can master (another lesson learned).

We determined that the aspartate at the base of transmembrane 2 in the α_{2A}-receptor is responsible for the binding of Na^+, and its mutation to asparagine (D79N) eliminates allosteric regulation of the receptor by the monovalent cations Na^+ and H^+. Subsequent crystallization of the receptor ultimately revealed the structural basis for this regulation more rigorously (41). However, for us, the D79N α_{2A}-adrenergic receptor mutant allowed us to potentially discern, in cellular and in vivo experiments, the functional relevance of this allosteric regulation.

The α_{2A}-adrenergic receptor is coupled to both biochemical and electrical signaling pathways: inhibition of adenylyl cyclase, activation of K^+ channels, and suppression of voltage-gated Ca^{++} channels (42). By collaborating with electrophysiologists, we were excited to discover that the D79N α_{2A}-adrenergic receptor could not activate K^+ channel opening, though it could still inhibit cyclase and suppress Ca^{++} channel activation (43). This was particularly exciting to us, because it became clear that if we could substitute the gene encoding the D79N α_{2A}-adrenergic receptor subtype at the wild-type locus of the mouse, we could explore which of the two electrical signaling pathways was important for the myriad behavioral, cardiovascular, and metabolic effects of this receptor. A courageous student undertook these studies using a not-yet-achieved in vivo use of hit-and-run targeting—a more than two-year experimental sojourn.

Many investigators prefer postdoctoral trainees to graduate students in their laboratories because there is a delay in achieving experimental mastery for those early in training. However, my experience is that students, who will earn a degree whether the answer to their questions of mother nature is yes or no, are willing to take on technically challenging lines of questioning that may not yield informative insights.

The D79N α_{2A}-Adrenergic Receptor Had Its Own Surprises In Vivo: Turning Lemons Into Lemonade

We successfully achieved the D79N α_{2A}-adrenergic receptor mouse, but, alas, the steady-state receptor binding was only 20% of that found in control mice (44), which later we learned was due the instability of the D79N receptor at the cell surface (45, 46). Our earlier studies compared wild-type and D79N-expressing cells normalized based on the density of receptor binding, not

RNA expression. In the mice, the RNA expression was equivalent, but the steady-state receptor density was low. Though we could not use the D79N α_{2A}-adrenergic receptor mouse to discern between reliance on receptor signaling by K^+ channel opening versus Ca^{++} channel closing in vivo, we explored the possibility that the D79N α_{2A}-adrenergic mouse could serve as a functional knockout of the α_{2A}-receptor and at least permit the identification of in vivo functions mediated by the α_{2A} subtype, distinct from those mediated by the α_{2B}- or α_{2C}-adrenergic receptor subtypes (47). Thus began a series of wonderfully informative collaborations with experts in so many areas. We learned that the α_{2A}-adrenergic receptor subtype was responsible for sedative, analgesic, and anesthetic-sparing responses (48), lowering of blood pressure in response to adrenergic agonists (44), protection against depression and anxiety (49), spinal analgesia and synergism with opioid agents for pain suppression (50), antiepileptogenic effects of endogenous norepinephrine (51), and enhancing working memory performance by guanfacine (52). I am grateful for the patience and great fun that our collaborators offered in the continued education of the "Limbird Laboratory."

Our findings with the D79N α_{2A}-receptor mouse showing loss of function with 80% diminished receptor density called to mind findings by Jurgen Wess's lab demonstrating that various cardiovascular responses to muscarinic agents require different levels of receptor availability, revealed by comparison of findings in wild-type, muscarinic-receptor knockout, and heterozygous mice (53). Our own studies in mice heterozygous for the α_{2A}-adrenergic receptor revealed that some responses, like sedation, required greater than a 50% receptor density to be induced by adrenergic agonists, whereas cardiovascular and some behavioral responses could be achieved in heterozygous animals (54). These findings were exciting, because one of the undesirable effects of clinically administered α_{2A}-receptor agonists is their sedative side effect; our findings affirmed why partial agonists at α_{2A}-adrenergic receptors are effective in cognitive enhancement of working memory (55) and suggested targeting drug development toward varying levels of agonist efficacy. Though useful conceptually, this therapeutic target strategy has now been superseded by a more exciting focus on development of agonists biased for one of the G protein–dependent versus arrestin-dependent signaling outcomes of activating a GPCR (56–58).

How Do Receptors Know Where to Go and When to Stay There?

Our studies of the α_{2A}-adrenergic receptor in vivo using D79N mice reminded us of the dictum of Paul Erhlich (1854–1915), "*corpora non agunt nisi fixata*," or agents cannot act unless they are bound. Since α_{2A}-adrenergic receptors are often expressed in polarized cells, like epithelia and neurons, they need to be precisely located to appropriately respond to endogenous catecholamines. How is it that the receptors know where to be targeted, and what retains them in those locations to allow the receptors to mediate the physiological responses they mediate?

Our studies on receptor trafficking began with an investigation of receptor targeting in renal epithelial cells, using Madin-Darby canine kidney cells as a model system. We learned that the α_{2A}-adrenergic receptors are directly targeted to the basolateral surface (59), in contrast to α_{2B}-receptors, which are randomly targeted to both surfaces but only retained on the basolateral surface where they accumulate (60, 61). Since the greatest distinction between these two subtypes' structures, from a superficial examination, is the length and sequence of their predicted third intracellular (3i) loop, we postulated that the 3i loop region might serve a role in both targeting and retention in microenvironments essential for functioning. Demonstration that the 3i loop was critical for the stabilization of the α_{2A}-adrenergic receptor on the basolateral surface also revealed, by examining the surface retention of a mutant receptor expressing an inverse structure of the 3i loop, that it was likely the overall net charge of this region rather than a particular sequence that was critical for basolateral retention (62, 63).

We identified a series of proteins that interacted with the 3i loop, including 14-3-3-ζ, spinophilin, and arrestin-3 (also called β-arrestin 2) (64–67). Although we failed to demonstrate a functional relevance of the 14-3-3-ζ interactions (though that does not mean there are none), we did identify an exciting interaction between the α_{2A}-adrenergic receptor and spinophilin versus arrestin, suggesting that binding of spinophilin to the α_{2A}-adrenergic receptor antagonizes arrestin interactions and thus arrestin-mediated events (68). Arrestin, originally named due to its role in desensitization, is now appreciated to be multifunctional, supporting not only autologous desensitization but also receptor internalization and coupling to non–G protein–mediated signaling (69). Because spinophilin is preferentially expressed in the dendritic spines of neurons, I still wonder what are the consequences of spinophilin competition for arrestin at the soma of the neuron compared to arrestin's unchallenged effects on α_{2A}-receptor-regulated functions at synaptic terminals in vivo? Perhaps this will be ingeniously explored someday!

Despite the excitement we had over these findings concerning the functional relevance of spinophilin interactions with the α_{2A}-adrenergic receptor, this article in *Science* (68) was the last major paper to appear from our Vanderbilt laboratory.

LEADERSHIP OPPORTUNITIES, LIKE BENCH SCIENCE, CAN DRIVE THE FUTURE TENSE, TOO

Chairing the Department of Pharmacology at Vanderbilt School of Medicine

Once our two children, whom we adopted after moving to Nashville, were becoming little people with their own interests and activities, it was clear that I could no longer drive a research project with my own hands. This was hard for me, as I truly do love doing experimental work. It had also become abundantly clear that my just overseeing the lab was driving folks crazy, as I would of course remember my finest hours of productivity in the lab and expect that level of forward progress daily from others. While pondering how to gracefully take on a new role in the lab, I was asked to Chair the Department of Pharmacology at Vanderbilt. I saw complementing direction of the lab with leading the department as a potential new sandbox where I could have a positive impact by mentoring new faculty, by fostering collaborations among faculty who were unaware of their shared interests, and in rejuvenating the graduate program to embrace greater interdisciplinary and interdepartmental perspectives. One of my reflexes has always been to serve as a scientific yenta—introducing scientists who might work in different areas but had a lot to say to one another on a larger, conceptual scale. Though I continued leading our lab, leadership roles at Vanderbilt provided many such matchmaking opportunities.

My appointment as Chair was hard for many of my colleagues; I was younger than most, I was not formally trained in pharmacology, and, well, I was female—the first female Chair of a basic science department in Vanderbilt's School of Medicine. Later, I learned from colleagues nationally that my gender was perhaps an issue, and I am grateful to John Perkins, who reached out from UT Southwestern to recommend ways to modify some of my behaviors to not let my gender or personality interfere with developing a departmental intellectual campfire, where it was not only safe but expected to test new ideas. I reflect on the topic of gender in science below.

A Strategic Plan and a New Role as the First Associate Vice Chancellor for Research

As Vanderbilt anticipated new leadership of the Medical Center, I was asked to co-chair a strategic planning exercise with Hal Moses, then-Director of the Vanderbilt-Ingram Cancer Center and

Chair of the Department of Cell Biology. We reached out to the other schools at Vanderbilt beyond the Medical Center and to Meharry Medical School, a historically black institution a few miles from Vanderbilt. I found this strategic planning effort both informative and invigorating, as I shared my faculty colleagues' desire to see greater recognition of faculty research and funding as well as contributions to teaching and a need to invest in greater infrastructure for discovery. A new role, Associate Vice Chancellor for Research, was proposed, and I was delighted to take on that role. Working with Harry Jacobson, MD, Vice Chancellor and CEO of the Medical Center, was a delight, as his idea of a typical day was keeping 100 balls in the air. Importantly, when a ball dropped, as does happen, Harry's response was to remind you that the other 99 balls were still in play, so look at it positively (70, 71) (see the sidebar titled Lessons Learned)!

A mantra in the Vice Chancellor's office was "never give up success for control." Lesson learned. We had such extraordinary creativity and talent among our faculty at Vanderbilt that it was not necessary to micromanage things—standing back as encourager allowed rich new ideas and outcomes to surface. As but one example, due to an engaged and driven faculty, Vanderbilt Medical Center's extramural funding of research grew by approximately 24% per year over five years, even faster than the generous pace of the 15% increase in NIH funding being supported during that same time frame. Vanderbilt's concomitant investment in core research facilities meant our eight-year Strategic Plan was completed in five years. It was time to explore what was next.

SO, IF MY ROLES AT VANDERBILT WERE SO MUCH FUN, WHY DID I RETIRE AND TRANSITION TO MENTORING OF AND ADVOCACY FOR THOSE UNDERREPRESENTED IN SCIENTIFIC DISCOVERY AND LEADERSHIP?

Ultimately, after 25 years at Vanderbilt, I chose to leave a research-intensive university for a different calling. I have wonderful memories and terrific stories to share about each of the wonderful graduate and postdoctoral trainees who joined our lab at Vanderbilt; I regret that space precludes my sharing accolades and fun remembrances of each of them. Most importantly, I must acknowledge that trainees often had a lot more to offer me than I them. It is not uncommon for those of us in academia to teach as we were taught and to mentor as we were mentored. There is no doubt that Bob Lefkowitz's mentoring style (17, 18) informed my own. But a see-one-do-one strategy can fail trainees who are not inspired and supported by the same mentoring strategies as you had been. Despite my commitment to mentoring others, I shudder when I think back to off-hand comments I made while leading our Vanderbilt laboratory; it is easier to talk about mentoring than to achieve the optimal supportive mentoring style that each trainee needs. I learned even more about differentiated mentoring later, after joining the minority-serving institutions Meharry Medical College and Fisk University, and I mention these below.

If I look back, I can see that for nearly a decade my antennae were growing in sensitivity to the disparities in virtually every area of US society. But my efforts to balance leadership and research efforts at Vanderbilt and the responsibilities I had to others in nonprofessional roles meant I could only engage angst, not action. I do remember being struck by a line in Mary Oliver's poem "The Summer Day" (72, p. 60):

> Tell me, what is it you plan to do
>
> With your one wild and precious life?

I had been asking myself just how long I could be aware of the inequities in our world and act like I didn't have to do anything about them. My day-to-day life did not leave much room for reflection, so I decided (two years in advance, as there were trainees to transition to the next

stage of their careers) to close my lab, retire from Vanderbilt, and spend some time exploring next steps. I was in the habit of isolating briefly to think through next steps; often these isolations were nonvoluntary excursions as an inpatient at Vanderbilt, thanks to a chronic autoimmune disease. After retiring from Vanderbilt, however, I intentionally spent time in silent retreats; because I am a chatterbox, I needed silence to keep from self-distracting. What I learned during my pause for discernment is that I don't have readily transferable skills, but rather what I have to offer others is that I love to learn, I love to share what I learn, and I am willing to speak out in advocacy for those who may not often be heard as they mean to be heard. That's it! That's all I've got!

A series of coincidences led me first to Meharry Medical College, invited by President John Maupin, who had worked with me on the Vanderbilt Medical Center Strategic Plan, and later to Fisk University, a primarily undergraduate historically Black college and university (HBCU), also in Nashville. I learned in working at both Meharry and Fisk that the students were at least as capable and creative as the trainees I was blessed to train at Vanderbilt; how these minoritized students often differ is in their level of intrinsic confidence—a consequence of the daily bruising of their souls due to living in our racialized society.

Working at HBCUs Has Given Me Unanticipated Insights and a Deeper Commitment to Advocacy

Working at HBCUs has given me a painful education in the institutionalized and systemic racism suffocating our society. I think that, like others privileged by the color of their skin, I was under the mistaken impression that racism is the sum of many individual errors in thinking; thus, I thought that addressing ignorance and countering stereotypes in our microenvironments would ultimately diffuse to the equitable reality we surely all wish to experience in society. What I have come to learn is that racial and ethnic suppression has been intentional. For those who want a crash course in the many ways that have laid the path to the current ills of our society, I recommend articles detailing the step-by-step legislation that fostered, among other outcomes, the Jim Crow era and sustained racial suppression (73, 74), or Heather Cox Richardson's (75) book *How the South Won the Civil War*.

We have in Nashville what I believe to be a profound example of the impact of systemic racism. Compare the evolution of Fisk and Vanderbilt Universities. Both institutions were launched with $1 million gifts: Fisk University, thanks to the Jubilee Singers, with an 1866 gift from Queen Elizabeth of England, and Vanderbilt in 1873, with a gift from the railroad and shipping magnate Commodore Cornelius Vanderbilt. Today, Fisk University hosts fewer than 1,000 students and, like other HBCUs, is always in the crosshairs of financial challenges. In contrast, Vanderbilt University is richly endowed and now boasts ten schools, including the undergraduate-focused College of Arts and Sciences exceeding 6,000 students, nearly 1,000 graduate students, and nearly ten times the faculty engaged at Fisk University. Absorbing the reality of the similar initial financial investment compared to the trajectory of these institutions should give anyone pause.

Despite having lofty academic titles, my principal contributions at both Meharry and Fisk boiled down to sharing strategies of resilience I learned over the years in response to rejected manuscripts and unfunded proposals. What I discovered was that the progress of HBCU faculty often was stalled because no mentor had ever taught them that there is no such thing as a rejected manuscript, there is no such thing as a grant that can't be successfully revised—lessons Bob Lefkowitz taught me early in my postdoc. At Vanderbilt, I also had been privileged to learn how to get institutional plans decided and enacted. Meharry and Fisk faculty were eager to learn the nitty-gritty of how science works in research-intensive institutions and in the sometimes combative world of biomedical research. I took pride in faculty achieving more funding, more

publications, and more collaborations and in observing graduate students' maturation to confident emerging investigators due, perhaps in part, to my rigorous but supportive input as a member of their thesis committees.

Data show that HBCUs are more effective than primarily white institutions in developing African American students who later earn PhDs in the sciences, probably due at least in part to HBCUs' second curriculum (76), a nurturing environment that combines academic rigor with mentoring in strategies to successfully navigate our fraught society. Though society may suppress students of color with under-expectations, such is not the environment at HBCUs. But the burden of beginning to purge our society of -isms and limit the power of those who push against social justice cannot be a burden borne only by the marginalized. Rather, it is us white people, privileged by the structure of our society, who must also commit to broadening our understanding and to pursuing excellence in a new way. To paraphrase Ibram X. Kendi, it is not enough to not be racist, we need to be antiracist—to affirm that racial groups are equals and promote policy, program structure, and microenvironments that reduce racial inequity. Engaging in antiracism is all of our responsibilities (77) and, in the end, since assuring opportunities is not a zero-sum situation, our world would be better for all of us (78).

Environment matters. Imagine my rude awakening when, while I was serving as Meharry's Vice President for Research, I was approached by faculty from Vanderbilt Medical Center to provide an immediate signature on a letter of institutional support for a collaborative proposal to be submitted that day to the NIH. First of all, none of us at Meharry had been aware of this collaborative venture before the last-minute flurry. Secondly, my former Vanderbilt colleagues were now talking to me more slowly and more loudly than when I was at Vanderbilt. Really? Such microaggressions (79, 80) are experienced nearly daily by trainees and faculty of color at largely white, research-intensive institutions. For those interested in seeing how our behaviors as citizens from privileged academic environments appear to others, it is worth reading Langston Hughes's (81) *The Ways of White Folks*, a parody of W.E.B DuBois's (82) classic *The Souls of Black Folk*. Said another way, sometimes, in our inappropriate confidence, us white folks are like human steamrollers.

On Mentoring Those Whose Life Experiences Differ from Our Own

Black students and faculty colleagues have taught me so much about unintentional biases in mentoring. As just one example, like my postdoc mentor Bob Lefkowitz (17, 18), I emphasized focus in the lab, which implied a 24/7 emphasis on experimental design, data interpretation and planning next steps. However, because many Fisk undergraduate and Fisk-Vanderbilt Master's-to-PhD Bridge trainees grew up in a community-focused culture, they are driven to leave the world in a better place than they have experienced to date and want to do so now. Trainees who have been minoritized to the fringes of society simply cannot be stopped from reaching out to younger folks to encourage their learning or their interest in science. Engaging in outreach is as much of a psychological booster for these trainees as is going to the gym for many others—it does not mean they are less passionate about their research.

But what do these insights mean for improving training environments for a diverse talent pool? In training any young scientist, the emphasis cannot be on perceived deficits but rather should be on shared aspirational goals. To foster trainees' highest aspirations requires a focus both on individual trainees and on creating welcoming environments for learning and discovery, achieved by broad institutional and programmatic change. First, as mentors, we need to share the joy of learning and the joy of discovery. It is not seductive in training the next generation of scientists to focus on only the hard work and the challenges involved in acquiring funding in a research career. Second, we need to remember that the attributes affirmed in traditional PhD and postdoc

training environments have their origins in the white male–dominated and familial culture of the 1950s. These environments may not support the diversity of individuals currently exploring careers in STEM: This diversity goes beyond race, ethnicity, and gender and includes differences in learning approaches, cultural expectations imposed by family, different scheduling for those with young children, and varying availability due to religious commitments. Third, we need to ask ourselves whether our current initiatives on behalf of equity and diversity are still suffocating science due to a forced assimilation into our preexisting and unexamined research culture (77). Beronda Montgomery (83–85) uses the compelling analogy of nurturing plants to the training of the next generation of scientists. When a plant droops, do we ask of the plant, "Is this possibly not the right place for you? Can you really produce? Can you thrive?" No. We water the plant. We move it to sunlight. We add essential nutrients (83) (see the sidebar titled Lessons Learned).

WHICH BRINGS ME, AS WELL, TO GENDER IN SCIENCE

We all stand on the shoulders of those who preceded us, or those who journeyed with us, and my journey was made easier by women scientists who preceded me and by female contemporaries as well. I was shielded from a lot of the suppression that some women find in biomedical discovery, and science writ large, by mentors and advocates who both advised me and ran interference for me without my knowledge. Perhaps my male mentors were intrinsically attuned to making pathways for women possible as they also had daughters. Despite this advocacy, I was pained by some encounters outside my home institutions that still invade my memories. For example, an internationally recognized scientist at a Cold Spring Harbor symposium asked me why I persisted in science, as there was no future for women there. Similarly, after being recognized by the John J. Abel Award in Pharmacology (named after the founder of the American Society for Pharmacology and Experimental Therapeutics), a colleague asked me whether I was recognized for my science or for being one of a few women in the field—an internal query that still haunts every female or minority scientist when they are recognized for their work. Another challenge is being seen as a scientist, not a gender: During a dinner following my receipt of an award, each scientist at the table shared their most exciting ongoing work, but when it was my turn, I was asked about my family, not about our science in the lab. I regret not responding assertively in these instances; perhaps I should have. But, I have only one life to live, and I chose to focus on being motivated by joy, not anger. I am gratified, however, that Vanderbilt's academic hierarchy is now rich with women leaders, that PhD graduates in biomedically relevant fields are nearing 50% women (86), and that more women enter and persist to tenure in academia. I cannot take credit for these changes, but nonetheless I do welcome them!

CONCLUDING THOUGHTS: GREAT MEMORIES AND ENCOURAGEMENT TO THOSE PUSHING FORWARD THE FUTURE TENSE

Looking back, I am fortunate that it is joy that supersedes my memories, though it may have been anger that fostered my mid-career transition to working in minority-serving institutions. I still love trying to solve problems, including designing experiments! I love parenting trainees as they evolve in their careers. I marvel at how choosing an academic career let me define my own pathway, have such a diverse set of responsibilities, and change directions (scientifically, administratively, and personally) multiple times. I loved traveling to scientific meetings and collaborating with folks around the world. And I still love bringing folks together who don't yet understand why we are gathering for coffee.

There is a stage in life, or a transition in focus, in which we begin to prioritize others over ourselves; I think it is this transition that allows us to contribute to a more transformative future tense. I will end on a personal note. As was common in the 1960s, my husband and I wrote our own wedding vows. They concluded with the end of an unpublished poem by Nancy Schiebner (87), shared by then Hillary Rodham during her valedictorian address from Wellesley College:

> Earth could be fair. And you and I must be free
>
> Not to save the world in a glorious crusade
>
> Not to kill ourselves with a nameless gnawing pain
>
> But to practice with all the skill of our being
>
> The art of making possible.

Those are my words of encouragement for readers of this review: to find your own joy and ways of "making possible."

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