Silver Spoons and Other Personal Reflections

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Abstract

The intent is to tell a story—hopefully one that is at various times serious, light-hearted, or provocative—that describes my life in biomedical science, especially focusing on the 50 years from 1961 (as a college senior) to the present.

SILVER SPOONS

Many can tell Horatio Alger stories of their ascents into productive scientific careers, but I was fortunate to follow a more privileged path. I was born with a scientific/academic silver spoon in my mouth, or perhaps a pestle (but not the mortar).

Although my father was the son of a music store owner in Bridgeport, Connecticut, he somehow found his way to Yale as an undergraduate (BS 1928) and followed that with a PhD in physiological chemistry from Yale, earned just three years later. His thesis research on the regulation of gastric acid secretion was decidedly physiological, and most of his career was spent in a physiological/pharmacological domain. My father was well known in academic circles, especially for two accomplishments: conduct (with colleagues) in 1942 of the first clinical trial in cancer chemotherapy (treating a patient with terminal lymphosarcoma with nitrogen mustard) and creation, with Louis S. Goodman, of the "Blue Bible" of pharmacology, as it came to be known: *The Pharmacological Basis of Therapeutics.* Both the first edition of the book and I were born in 1941. As a tribute to my father's friend, colleague, and coauthor, I received the middle name of "Goodman." The name Alfred Goodman Gilman confused medical students for the decades when they still read books. And my friend and colleague Michael Brown humorously proclaimed that I was the only person ever named after a textbook. It was not easy for me to fly under the radar in pharmacological circles.

I was never pushed into science, but I have strong childhood memories of events that obviously made excellent impressions. These included visits to the Hayden Planetarium in New York, where I well remember signing up for a trip to the moon; visits to the medical school at Columbia University to watch the faculty demonstrate autonomic drug action using the elaborate dog heart-lung preparation; and trips to my father's lab at Albert Einstein College of Medicine to watch him do surgery in preparation for experiments relevant to renal physiology and pharmacology. In later years, I insulted members of my lab by stating that physiology was much more interesting to "watch" than biochemistry; pipetting is simply not that much fun—to watch or to do. Conversations at dinner also made impressions that lasted. When my father talked about a colleague and the brilliance of his work, my mother occasionally rolled her eyes and commented on the deficiencies of the colleague's personality. Subsequent personal observations taught me that my mother was almost always correct. I tried to pay attention to both sides of that issue when building a department. And when my father told stories that touched on politics and hierarchy in the medical school, my mother's eyes rolled again, and she followed with a sarcastic comment such as, "The biochemists shall inherit the Earth." (Some things have surely changed!) My mother obviously had a very strong personality. Also notable and memorable were my father's cutting his modest office in half to make room for a junior faculty member and closing his lab because another could use the space to better advantage.

Lesson: Pop off while you're popular, or at least move over and make room for others. A corollary: The present-day system is crowding out younger investigators who have energy, vision, and plastic brains. This is most unfortunate.

Back to childhood. My parents felt that my older sister had an inadequate experience at the local public high school, and I was shipped off to an all-boys prep school—The Taft School in Watertown, Connecticut. This was a strict, monastic, and frankly unpleasant environment in the 1950s: academic boot camp. I was not happy, but the education was superb and lasting. I learned to learn; I also learned to smoke. The latter was one of the few privileges offered to seniors and had to be enjoyed. After Taft, admission to Yale College was easy. Majoring in biochemistry, I encountered two extremely positive influences in my senior year. The first was Henry Harbury, who gave a half-course series of amazing lectures in the medical school biochemistry course. The

lecture hall was always standing room only because the clinical house staff and many clinical faculty would religiously walk across the street to attend. They lapped up basic thermodynamics, enzyme kinetics, and the details of photosynthesis! Can you imagine? Dr. Harbury set the biochemistry hook in me. I then enjoyed a great research experience with Mel Simpson, working part time and unsuccessfully on a problem in protein biosynthesis (which later was executed successfully by a collaborating team of senior investigators). The project was just a bit too ambitious, but I loved it, even including the many hours in the cold room and feeding samples manually into a planchet counter. I also spent a summer working with Allan Conney at the then Burroughs Wellcome & Company. Allan was thoughtful and generous; as a result, I was very pleased to publish my first paper in *The Journal of Biological Chemistry* (1). That became a happy habit.

Earl Sutherland [who earned the Nobel Prize for the discovery of cyclic adenosine monophosphate (cyclic AMP)] started a combined MD/PhD program at then Western Reserve University (Cleveland) in the late 1950s. The program was small, and Sutherland had recruited each student on the basis of personal contacts. Sutherland and my father were friends, my father liked to talk about his son, and I received an invitation from Sutherland to visit Cleveland during my junior year at Yale. He described a seven-year program in Cleveland that struck me as an eternity in purgatory, and I declined. But he wrote again early in my senior year, and for some reason that likely was less than honorable, I made the visit. He inspired me, the cyclic AMP story excited me (and Mel Simpson), and the other students impressed me. I told Sutherland that my only hesitation was that I really didn't want to get a PhD in pharmacology (too close to home). He immediately put his arm around my shoulder and said not to worry; pharmacology was "just biochemistry with a purpose." Another hook was set. I was sold on research but ambivalent about medicine. I vacillated during my clinical clerkships, at times completely fascinated by mechanisms of disease as manifested in individual patients, but ultimately decided to follow only the research path. I have never regretted that decision, nor have I ever regretted obtaining the broad education and insights that came from medical school.

Sutherland left Western Reserve for Vanderbilt shortly after I arrived, but I was able to pursue my fledgling interests in cyclic AMP by doing thesis research in Ted Rall's laboratory. Rall had actually done the cyclic AMP discovery work in Sutherland's lab (2), and he was the consummate mentor—always engaged, wise, and available. Many the evening would I come home very late for dinner with the apology that I had just stepped into Rall's office to ask a two-minute question that turned into a two-hour discussion. Whereas I worked on the thyroid gland, Rall worked on the brain, and his data were vastly more interesting than mine. That set the stage for the next move.

Of the many lessons learned in Cleveland, one has always held a firm spot in my memory. Ted Rall was fond of noting Earl Sutherland's determination to understand, at the molecular level, the difference between inactive and active liver phosphorylase. Sutherland had discovered this stable activation of phosphorylase while seeking the explanation for the hyperglycemic effects of epinephrine and glucagon. The inactive and active forms of the enzyme were purified and then analyzed (3). Luck had it that the difference was a phosphate group, which was detectable. It's tough to say how the future would have played out had it been a more esoteric modification (or even worse, a noncovalent regulator). Because addition of phosphate was apparently required to activate phosphorylase, ATP was included when attempts were made to duplicate this reaction in homogenates. More luck: ATP was required for two additional steps in the reaction pathway, and, as we will see, addition of ATP also supplied GTP as a contaminant. One of Efraim Racker's favorite lines was, "Don't waste clean thinking on dirty enzymes." Well said. For additional and similar wisdom, listen to Arthur Kornberg (4).

During this time and later, Ed Krebs, Ed Fischer, and their colleagues were pursuing a similarly determined approach that began with the muscle phosphorylase system and ended

with the discovery of cyclic AMP–dependent protein kinase: a seminal enzyme and event (5). The Sutherlands and Ralls and Krebses and Fischers were my heroes. How interesting: both Sutherland and Krebs emanated from the biochemistry department at Washington University, led by Carl and Gerty Cori, and both ended up as chairs of pharmacology departments—biochemistry with a purpose, said Sutherland. Joe Larner also belongs to that club. Others who emanated from that brilliant environment (but failed to see the pharmacological light) were Arthur Kornberg, Luis Leloir, and Mildred Cohn.

It was 1969 when I received my MD and PhD degrees. The Vietnam War was in full bloom, and a postdoctoral fellowship at the National Institutes of Health (NIH) as a "yellow beret" in the Public Health Service was thus a most attractive opportunity. Mandatory military service and two Asian wars (Korean and Vietnam) had an enormous effect on the quality of biomedical science in the United States because many bright young physicians sought research training at NIH in preference to the military. I surveyed the opportunities at NIH and was much intrigued when I discovered that Marshall Nirenberg (who earned the Nobel Prize for the genetic code and was a lab chief at NIH) had eschewed molecular biology and turned to neuroscience as the "last frontier" (led by Francis Crick and accompanied by several of his similarly minded colleagues). "Molecular biology was finished"—or so some thought, before the discovery of restriction enzymes. I applied to work with Nirenberg, I was accepted, and I drank the Kool-Aid: We could learn much of what we needed to learn about the nervous system by studying clonal cell lines in culture. Despite their deficiencies, my love of clonal cell cultures lasted for 30 years; they treated me well.

I didn't do much to advance neuroscience as a postdoc, but I did learn cell culture, I acquired an appreciation for genetics, and I accomplished one thing: development of a badly needed, simple, and sensitive assay for cyclic AMP—an assay that most anyone could do. Nirenberg and I had a most unpleasant disagreement over this. He had left me alone for a while after we had completed a truly boring project that mostly involved counting axons sprouting from cultured neuroblastoma cells, and during that time I gathered proof-of-concept data for a ligand-binding assay for cyclic AMP. When I presented the data to Nirenberg, he told me I must stop that project and continue looking at axons. I refused, and six tense weeks followed until I brought him a complete manuscript. He read it, complimented me, and, like a true gentleman, communicated it for me, without change, to the *Proceedings of the National Academy of Sciences* as a single-author paper (6). The work was a big hit at the first Gordon Research Conference on Cyclic AMP and soon became one of the most highly cited papers of its time. This provided a sweet path to job interviews. Joe Larner and Bob Haynes, both of whom had been in Earl Sutherland's department in Cleveland, were instrumental in recruiting me to my first faculty position as an assistant professor of pharmacology at the University of Virginia (Charlottesville), where I remained for 10 years before accepting the chair of pharmacology at the University of Texas Southwestern Medical Center in 1981. Twenty-four years as a department chair followed.

Lesson: Mentorship is better provided with high standards and great examples than with warm fuzzy advice and scheduled encounters (excuse the sarcastic tone). My father and mother set fantastic examples in different ways, in addition to creating a child with that silver scientific spoon. That spoon helped feed me a succession of superior teachers: Henry Harbury (Yale), Mel Simpson (Yale), Allan Conney (Burroughs Wellcome), Earl Sutherland (briefly), Ted Rall (Western Reserve), and Marshall Nirenberg (NIH). All of these men were rigorous and creative scientists with the highest standards. I had but one significant disagreement during this time, when Nirenberg thought it better for me to count axons than to develop the assay for cyclic AMP. There was absolutely no doubt in my mind that this superb scientist was wrong, and I am so glad that I stuck to my guns and that he appreciated the work. After this time, most of my best teachers were

the brightest and most dedicated of the students and fellows who graced my lab, along with a few notable colleagues and administrators who are mentioned below.

FOLLOW THE REDUCTIONIST BRICK ROAD: CHARLOTTESVILLE

I can't really say that I started my own research program in Charlottesville with the notion of becoming a determined reductionist, but the seeds had been sown in Cleveland when the question was posed thus: Is adenylyl cyclase the β-adrenergic receptor? This seemed unlikely because the cyclase would also have to be the receptor for glucagon, adrenocorticotropic hormone (ACTH), and a growing list of other regulators. Further suspicion was cast on the idea by Marty Rodbell's work on adipocytes (7), which strongly suggested the independence of receptors from the enzyme itself, but formal proof was lacking.

One of the more important consequences of Sutherland and Rall's cyclic AMP discovery work was the ability to detect the presence and action of a receptor by measuring the product of an enzymatic reaction, cyclic AMP, rather than by examining the functional performance of a muscle or gland. Rall convinced Sutherland to ignore the dogma that hormone action could be observed only in intact cell preparations. This was the first critical step in taking the system apart: from tissues with intact cells to broken cells and membranes derived therefrom. The next step—resolution of the receptor and the cyclase (if in fact they were separable)—would require independent assays for both and a means to take them apart.

Assaying adenylyl cyclase was apparently straightforward (unknown was the fact that this would not be true in the absence of the requisite G protein), but receptors were a different story. However, radioimmunoassays and similarly conceived ligand-binding assays were in vogue (e.g., my cyclic AMP assay), and receptors presented the same opportunity. Others proclaimed victory with ligand-binding assays for the β-adrenergic receptor using tritiated catecholamines, but this turned out to be incorrect (8). Gerald Aurbach and colleagues described the first assay that was believable (9), and we adopted it quickly.

How to attempt resolution of ligand binding and catalytic activities? Others were solubilizing the proteins that possessed these activities, and this was proving difficult. We sought a genetic approach, looking first for chromosome segregation in somatic cell hybrids and then for a mutation to do the job. The latter was handed to us in a most delightful fashion by the work of Henry Bourne, Phil Coffino, and Gordon Tomkins, who discovered the cytocidal effect of cyclic AMP on S49 lymphoma cells and isolated the so-called AC^- or cyc^- mutant (10). We collaborated to demonstrate the retention of β-adrenergic receptor binding activity by these cells (11); the human agent of that collaboration was Paul Insel, the editor of this volume of the *Annual Review of Pharmacology and Toxicology*, who was then a newly hatched faculty member at the University of California, San Francisco.

Elliott Ross, trained as a membrane biochemist at Cornell, was up to the task of reconstituting, biochemically, what nature had taken apart with a mutation. He eventually appeared to succeed by reconstituting detergent-solubilized adenylyl cyclase into cyc^- membranes containing β-adrenergic receptors (12). But control and other follow-on experiments quickly showed that he had in fact accomplished something much more interesting. The cyc^- mutant actually retained both β-adrenergic receptors and adenylyl cyclase. What was missing was a novel protein, eventually shown to be the GTP-binding protein Gs, that served as an essential intermediary in the flow of information from the receptor to the enzyme (13). The existence of this protein proved true the hypothesis of such an intermediate posed by Martin Rodbell and colleagues, who had demonstrated the essential participation of GTP in the system several years before (14).

The experiments that brought the major breakthroughs in this field—Sutherland and Rall's discovery of adenylyl cyclase and cyclic AMP and our discovery of G proteins—were surely aided by wonderful chance. Sutherland and Rall's discovery system was arranged to demonstrate a reaction that would activate glycogen phosphorylase in homogenates, and the reason for their inclusion of ATP in the reactions is discussed above. In our experiments, the cyc^- mutant was not lacking adenylyl cyclase at all. Good luck: The mutant cells retained the cyclase, which was much more labile and less abundant than the G protein, but highlighted the crucial importance of an unknown protein by its absence. So-called cyc^- membranes then provided the assay reagent for purification of the previously unknown G protein, Gs. Other bits of luck: There is but one gene for Gsα, facilitating mutational loss or alteration of function; furthermore, these cells behaved as if they were hemizygous for Gsα. Obtaining adenylyl cyclase–deficient cells is much more problematic because there are 10 such genes in mammals.

Lesson: You can expect to have some good luck if you are working hard on a tractable problem and taking reasonable approaches. The trick is to recognize good luck when it happens, embrace it, and then commit whatever it takes to extract its full value.

One reductionistic success whetted the appetite for more. It was clear to us that there was no choice but to purify the novel protein, using the reconstitution of hormone-stimulated adenylyl cyclase activity by cyc^- membranes as the assay. Happily, skilled postdocs John Northup and Paul Sternweis fully embraced that need and spent a difficult two years purifying the low-abundance membrane protein (15). Pretty much everything else flowed naturally from that success.

One of the first dividends was one of the more amusing. A purified G protein offered the opportunity once again to attempt to explain the mysterious actions of the fluoride ion. Fluoride was first added to relevant reaction mixtures by Rall and Sutherland for reasons buried in history. A remarkable stimulatory effect on cyclic AMP production was eventually observed. Sutherland burned more than one postdoc in his efforts to understand the phenomenon. He thought an effect that was so prominent and unanticipated must guard an important mechanistic secret. He was right.

First, we found that the effect of fluoride was exerted on Gsα, not on the cyclase. Next, a reasonably long series of wildly irreproducible experiments eventually led Paul Sternweis to a fanciful hypothesis: A cofactor was needed for fluoride action. This cofactor (or these cofactors?) was found in glass (but not plastic) test tubes and in commercial preparations of ATP, among other sources. In time, the cofactor seemed likely to be a metal, and it was purified and identified by neutron activation analysis. The very surprising answer: Al^{3+} (16). The eventual explanation: AlF_4^- binds to Gsα-GDP, mimicking the γ phosphate of GTP and stabilizing the transition state for GTP hydrolysis—a state that closely resembles the active state. The crystal structure of this complex (17), determined years later, answered important questions about the mechanism of GTP hydrolysis by G proteins—how it could be so slow and how it could be regulated by GTPase-activating proteins (see below).

KEEP YOUR EYES ON THE ROAD: DALLAS

If you purified a low-abundance, labile protein in the 1970s, you were pretty much stuck with its intractability. The 1980s brought to the masses like us technical advances that, of course, changed biological science forever. Our ability to take advantage of them was greatly enhanced by a change of scenery. Joe Larner and Bob Haynes had been superb and supportive mentors during my first years as a faculty member at Virginia, and Charlottesville was a delightful environment. But the University of Virginia seemed to have this attitude: "If they do well, they will leave; there's not

much we can do about it." And so I did. I was most fortunate to move almost everyone in my lab with me.

I arrived in Dallas at the University of Texas Southwestern Medical Center in 1981 to chair the pharmacology department (more on that below). Donald Seldin, the famous former chair of internal medicine in Dallas who correctly deserves much of the credit for building Southwestern Medical School, made the crucial first phone call; Kern Wildenthal, the new 40-year-old dean of the medical school (with whom I share a birth date: day and year) convinced me that I was not too young to take on some administrative responsibilities; and my new faculty colleagues, Mike Brown and Joe Goldstein, were instrumental in the recruitment. Happy coincidence or more good luck: Brown and Goldstein frequently went to the faculty club after 5 PM to sample a spirit. Their natural path to the bar passed my office door, and I often was invited to join them. Those 5 PM meetings became (among other things) my molecular biology classroom. After a few months of polite treatment of the "new boy" (a Taft expression), one of them said abruptly, "When are you going to bring your science into the twentieth century?"—meaning, when are you going to clone cDNAs for your proteins? I was insulted only momentarily and asked for help when the time was right.

We needed protein sequence for synthesis of oligonucleotide probes to begin cloning G protein subunits (antibodies for expression cloning were not available). Happily, we knew by then that Gsα, Giα, Goα, and Gtα (transducin) constituted a closely related family (18), although we were still oblivious to the intimacy of that relationship. Because quantities of Gsα were insufficient, we obtained partial amino acid sequences from the more abundant Goα and Gtα proteins in collaboration with Mel Simon at Caltech, where protein sequencing was quite routine. To our delight, we stumbled straightaway into a conserved portion of both proteins' GTP-binding domains that revealed striking homologies with ras proteins (19). Mike Brown offered humorous sympathies: "You're in trouble now; you're in with the oncogene crowd. They're a moving party. They'll come to your house, trash it, and leave you to clean up the mess."

Despite the dour prediction, the modest amount of protein sequence opened the door. With help from David Russell in Brown and Goldstein's department, Janet Robishaw, a new postdoc with a PhD in physiology, quickly learned the fundamentals of cDNA cloning, and she and Bruce Harris gave us our first detailed "look" at Gsα (20, 21). Technical advances—molecular cloning, construction of specific and general antipeptide antibodies to various G protein subunits (22), reasonably exuberant expression of G protein subunits in bacteria (23), and the ability to mutate proteins and express them in either bacteria or cultured mammalian cells—ushered in a productive decade from the mid 1980s to the mid 1990s, with a special focus on mechanistic studies of Gs, Gi, and bidirectional regulation of adenylyl cyclase activity. However, we had to do battle with one more dragon before the cyclase part of the story was approachable.

I once wrote in the annual progress report for my NIH grant, "Adenylyl cyclase is a hateful enzyme"; this continued a trend that had started with an earlier review article entitled "Frustration and Adenylate Cyclase" (24). It is a most difficult protein: rather rare, rather large, very hydrophobic, and very labile. There was only an occasional masochistic person in the lab willing to tackle the problem, and there was little to show for several years. Jack Krupinski finally made a determined effort to purify enough protein to obtain some amino acid sequence; multiple preparations of microgram quantities of protein were required over months. The final material, purified by SDS gel electrophoresis, was cut from the gel, proteolyzed, and fed to the protein sequencer. Several peptide sequences emerged, but the majority of them were a perfect match for casein. We stood in the hall pondering the mystery when another lab member walked by with a freshly prepared 4-liter flask of BLOTTO, having just weighed out large quantities of hydrolyzed milk protein in the same room where the electrophoresis had been done. The purification was repeated and

completed in a new location. Authentic peptides were obtained, and a full-length cDNA clone was eventually pieced together with enormous collaborative help from Randy Reed and his colleagues at Johns Hopkins. They had both a randomly primed bovine brain library and the skills needed for the tough job. The deduced structure of the enzyme was another great surprise; it included two sets of 6-transmembrane spans and two homologous, roughly 40-kDa cytosolic domains. It looked like a channel or a transporter. There were major skeptics, but it was an adenylyl cyclase (25).

This was the first of a group of 9-membrane-bound, G protein–regulated mammalian adenylyl cyclases to be cloned by us and others. They proved to be a treasure trove of regulatory diversity with a few common features—especially activation by both Gsα and the diterpene forskolin (26). But mechanistic studies were still severely stymied by the uncooperative properties of the proteins.

Wei-Jen Tang, a molecular biologist, succeeded Jack Krupinski, a biochemist, as the lead soldier in the adenylyl cyclase wars. With the gay abandon enabled by working with DNA rather than protein, Tang discarded all portions of the enzyme that annoyed him, especially the hydrophobic domains. He expressed what was essentially a 40-kDa fusion protein containing only the homologous regions of the cytosolic domains of the cyclase and, to our delight and surprise, produced an enzyme that was still activated by Gsα and, even more surprisingly, by forskolin—a greasy molecule that all logic suggested would act within the membrane (27). Even better, Carmen Dessauer found that the two cytosolic fragments of the cyclase could be expressed independently and in lush quantities in *Escherichia coli;* when simply mixed together, they miraculously reconstituted Gsα- and forskolin-stimulated adenylyl cyclase activity (28). If the correct isoform of the correct domain was used, inhibition of activity by Giα was also evident (29). This pleasant situation opened the door for Dessauer, who proceeded to characterize the enzyme's active site and regulatory binding sites for both small molecules and proteins by a variety of techniques, including equilibrium dialysis (30, 31). Who would have thought?

The delightful long stretch between the mid 1980s and the late 1990s brought many other pleasures from the lab via the minds and hands of several other talented students and fellows. There is no space for storytelling about all of these subjects, but high on the list of good memories are identification and purification of the Gi protein (32); studies of the regulatory roles of G protein βγ subunits (33); myristoylation (34), palmitoylation (35), and prenylation (36) of G protein α, α, and γ subunits, respectively; and demonstration that the newly discovered RGS proteins acted as GTPase-activating proteins for selected G protein α subunits (37) by binding to the transition-state complex for GTP hydrolysis (38).

Expression of G protein subunits in *E. coli* and in baculovirus-infected insect cells immediately brought what to me was the ultimate reductionistic dream—high-resolution crystal structures of the proteins as they traversed their regulatory cycles. We initiated what became a most enjoyable and rewarding collaboration with Steve Sprang and members of his laboratory. Maurine Linder, now a department chair at Cornell, was the first from our side to enter the fray. Fruits of her labor were slow to mature—frustrated by limits on the amount of protein that we could purify. An MD/PhD student, Ethan Lee, eventually took on the project and told me he wanted to improve expression levels. I told him just to get in the cold room and gut it out, but he fought back, prevailed, and succeeded brilliantly. We now had more than enough protein to saturate the crystal screens, and suitable crystals of Giα1 eventually emerged. However, despite data collection from two uranyl derivatives of the protein and analysis of the selenomethionyl-substituted protein, solution of the structure ultimately relied on the generosity of Joe Noel and Paul Sigler, who supplied coordinates from the structure of a Goα/Gtα fusion protein that they had solved previously in collaboration with Heidi Hamm (39). Both groups determined structures of GTPγS-, GDP-, and GDP-AlF_4^--bound α subunits and then the structure of a complete G protein

heterotrimer (17, 40–44). The latter gave visual life to Mel Simon's wonderfully named WD40 domains in the β subunit, whose repeats revealed themselves as a beautifully circularized sevenfold β propeller.

Thanks especially to fantastic work by John Tesmer from the Sprang lab and to David Berman and Roger Sunahara from my own, two structures of special interest emerged a bit later. One was the structure of RGS4 bound to the AlF_4^--activated state of Giα1, demonstrating how the GTPase-activating protein bound to the mobile switches of the G protein to stabilize the transition state (45). The other was of special significance to me: the structure of a complex of three proteins and three small molecules. The first protein consisted of GTPγS-activated Gsα bound to the complex formed between the two cytosolic domains of adenylyl cyclase (described above); the latter two proteins were glued together by forskolin, which bound in a spot symmetrical to the ATP-binding site of the cyclase. The substrate site was revealed by incorporation of a so-called P-site inhibitor of the enzyme (46).

Coupled with work by Carmen Dessauer, who next determined the location of the binding site on adenylyl cyclase for Giα (29), we suddenly thought we understood how adenylyl cyclase worked. The cyclase is a pseudosymmetrical protein. (Recall that the two cytosolic domains share a region of extensive homology.) The substrate binding/catalytic site is located at one interface between the two cytosolic domains (designated C1 and C2). Gsα, by binding to a cleft in the C2 domain of the cyclase, apparently improves the efficiency of the active site. Giα, binding to the pseudosymmetrical site on the C1 domain, impairs the efficiency of the active site. The pseudosymmetry of adenylyl cyclase is a clear structural correlate of bidirectional regulation of the enzyme by homologous G protein α subunits (**Figure 1**). However, I am reminded of one of Arthur Kornberg's Ten Commandments of Enzymology (4): "Do not believe something just because you can explain it."

Despite the Kornberg admonition, the ability to see this picture and explain 30 years of effort had a real impact on me. I had dreamed of taking the cyclase problem to the "seeing-is-believing" stage, but I never thought it would be possible because the dream was born before recombinant DNA, molecular cloning, and all that flowed therefrom. Having taken reductionism as far as I was able (and only there with enormous help), my thoughts turned more to how we were ever going to understand the way cellular signaling systems worked on a macroscopic level—in real cells and, someday, in real organisms.

Looking back with some attempt at objectivity, I would characterize my lab's work as mechanistic and reductionistic (as noted), very focused, and reasonably determined. We encountered difficulties and tried to solve them rather than skirt them. Henry Bourne, a friend and at times a friendly competitor, once described me as a bulldozer (47). Did he really mean that I roamed about, simply knocking over anything in my path? I hope not. I guess you'll have to ask him.

THE ALLIANCE FOR CELLULAR SIGNALING

The Alliance for Cellular Signaling was born of dreams of putting Humpty Dumpty back together again and was made possible by the so-called Glue Grants initiative of the NIH's National Institute of General Medical Sciences. It was a large-scale, multi-investigator, multi-institutional coast-to-coast collaboration, glued together by a common desire to understand the complexity of cellular signaling networks and enabled not just by money but also by modern communication technology: email and videoconference (48). The premise: Someday there will be a computer labeled "A Cell," and it will accurately predict all details of the behavior of a normal cell, as well as that perturbed by exogenous regulatory influences, drugs, mutations, and so on. I think I still believe the premise, but my time line for the prediction has expanded considerably.

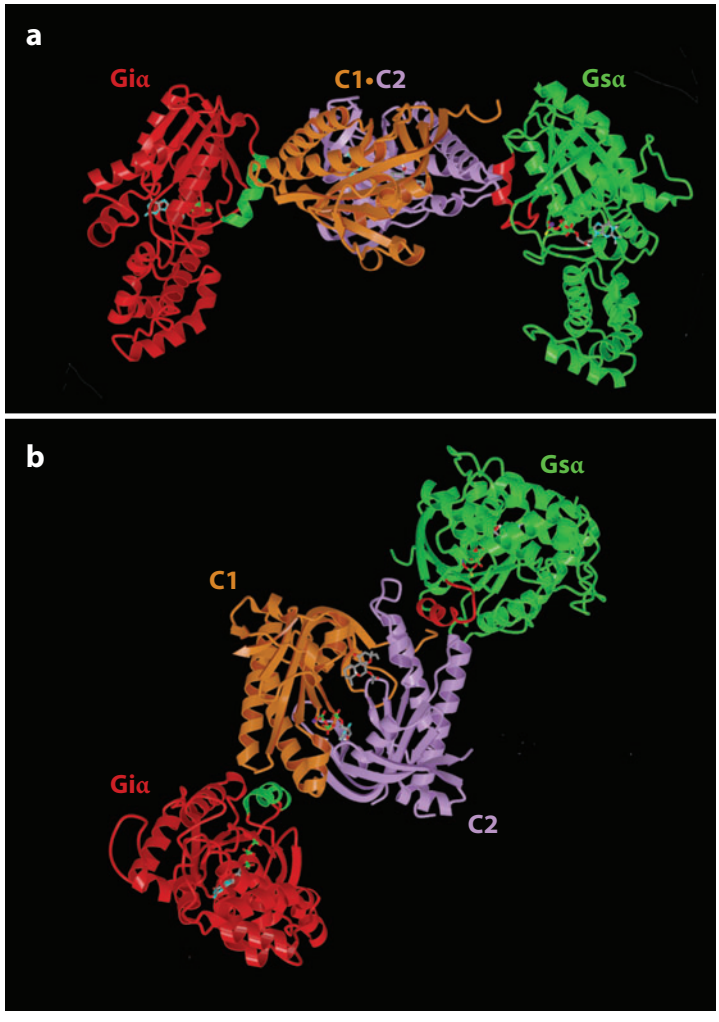

Figure 1

Two views of a theoretical complex between the catalytic domains of adenylyl cyclase (C1 and C2) and the stimulatory and inhibitory G protein α subunits, Gsα and Giα. The positions of Gsα, C1, and C2 are based on the crystal structure of that complex (46). The position of Giα is modeled on the basis of mutational analysis of the site of interaction and homologies. This research was originally published in *The Journal of Biological Chemistry* (29). © The American Society for Biochemistry and Molecular Biology. See Reference 29 for additional information.

The Alliance had growing pains, of course. We encountered problems and we solved problems. There were reasonable numbers of interesting findings and publications, which arose especially from extensive analyses spearheaded by Rama Ranganathan (49), and a lush grab bag of reagents was made available to the research community. However, there is no denying that we were falling short of both our own expectations and those of others. As a result, we were executed just when we thought we were turning a real corner. I'll grudgingly forgo the opportunity to comment on that especially unpleasant part of the process and what was wrong with it. Although we were premature and overly ambitious, I believe that the questions we tried to answer were correct and

that the approaches were generally valid. The need remains. Until we can do a much better job of predicting the behavior of complex biological systems, we will fall short of the desired application of knowledge acquired during the reductionistic heyday of the twentieth century.

Lesson 1: If you are not audacious, you may have to settle for being mediocre. If you choose to be audacious, you obviously should be able to withstand failure. And although I generally dislike the criticism of being "overly ambitious" (would you prefer "underly ambitious"?), there is a difference between being audacious and being truly overly ambitious. The goals and ambitions of the Alliance were off scale, and much of that was my fault. The voice of reason in that regard was Henry Bourne, a colleague and member of the Alliance who served it well.

Lesson 2: Large-scale collaborations are truly difficult to manage. I was overly idealistic. Individual ambition, be it motivated by ego or avarice or just the need to put bread on the table, is a powerful repulsive force that is not easily overcome by an opportunity to do collective "good." It is perhaps not too cynical to say that people will truly work together only if individual accomplishment or survival is clearly dependent on collective survival. Furthermore, you will never get anywhere if you are everyone else's second (or lower) priority. I believe that team science will become increasingly important in biology, especially as reductionism inevitably turns to systems biology. We will need to find better ways to do team science and reward it if we are to solve large overarching problems. Everybody on the team needs to get the same big gaudy championship ring (although some will still be paid much more than others).

THERE IS LIFE OUTSIDE A LAB

Basic research is, of course, essentially boundless. Each question answered (or each phenomenon "explained"—see Kornberg) leads to another. There are few opportunities to finish a book (or sometimes even a chapter). In part, for those reasons, I never had a completely single-minded approach to research. I enjoyed other scientific/academic/industrial activities and the opportunities they afforded to explore other areas, interact with a broader range of people, and sometimes even complete a project. Most such interactions were enjoyable (I hope mutually); a few were painful. The painful ones can consume a disproportionate share of one's memories unless one cultivates the art of selective amnesia.

Academic life was much simpler in the 1970s. The pace was slower, and competition was less ferocious, although we didn't appreciate that at the time. You pretty much said "yes" when asked to do something, and you could say yes to other things and still get your grants funded. Between 1971, when I started in Charlottesville, and 1977, when our research efforts really blossomed, I had also taken over organization and leadership of the medical pharmacology course and initiated a teaching program in which every member of the department met with groups of four to five medical students three times each week to discuss course material. I had become associate editor of *The Pharmacological Basis of Therapeutics*—a great deal of work but another mouthful from the silver spoon. And I had become director of the Medical Scientist Training Program and principal investigator on that training grant. I didn't feel like a hero or that I was doing more than was expected. That was the way it was, and it worked.

What Else Can One Do to Be Useful?

Chair a department. Chairing a clinical department in a medical school or an academic department with major undergraduate teaching responsibilities is a real challenge. You are running a business of sorts (especially in a clinical department), and you must take responsibility for it—providing a full range of needed services or teaching the needed spectrum of a discipline. Chairing

a basic biomedical science department in a medical school is much easier. Pick a theme or two or three; you can't cover the waterfront and don't need to. You need good taste, common sense, and either excellent resources or an excellent environment; having both of the last two named advantages is not so common. If you have neither resources nor environment, don't take the job if research is an important part of the mission. There are already far too many mediocre basic biomedical science departments. The following are some bits of advice about chairing a department.

- Hire only excellent people; this takes time, patience, and good taste. Do not be overly impressed with pedigrees or technical prowess. Read and listen between the lines of recommendations. Pay attention to personality, ability to get along with people, citizenship, and language skills. If you lose candidates to excellent places, you are in the game. If you do not, you are not.
- Protect new faculty members from non-research-related demands early on. This is especially important for women and minorities. The system demands representation and sabotages its own long-term goals by distracting women and minorities early in their careers. I always told female assistant professors to say no to requests to serve on study sections, blame it on me, and promise to serve as soon as tenured. This goes double for those with the common challenge of bearing and raising children while tenure clocks are ticking.
- When the time is right, expect proper citizenship. People can easily develop teaching interests that are not intimately linked to their research interests—especially for medical students. It should not be a challenge for a PhD biomedical scientist to stay ahead of first- or second-year medical students in some select area of a discipline. Medical students do not need to know what was discovered yesterday. They need to appreciate the most important of what was learned years, decades, or even centuries ago. I always told faculty that medical school teaching could be their moral or political mission in life, or both, but that they could not reject both premises:
- Two other crucial tips: First, you need a great departmental administrator, and you need to have a partnership with that person. Many make the mistake of hiring an administrator on the basis of perceived financial or mathematical skills. Budgeting in a basic science department is little more than managing a checkbook with some extra zeros. Cheap calculators have the necessary power. Your administrator should have advanced people skills and a sense of mission to you, the department, and the institution. Financial issues may slow you down, but personnel problems can bury you. This never happened to me because of 25 years of exceptional service from our departmental administrator, Wendy Deaner. Second, serve in an environment where the institution strives to make the chairs' jobs among the best, rather than among the worst. Look at the list of committee assignments for the chairs; it should be short. Your most worthwhile committee assignments will be search committees.

I think back on my days as a department chair with great satisfaction. We were near or at the top of NIH-funded departments of pharmacology for several years. Five members of the department were Howard Hughes Medical Institute investigators. Four were elected to the National Academy of Sciences, and more will follow. Members of the department served as dean of the medical school and dean of the graduate school (twice) and directed various graduate programs, including the biochemistry graduate program. I left the department in the great hands of two former recruits: David Mangelsdorf as chair and Rama Ranganathan as director of a newly formed Green Center for Systems Biology.

Lesson: Always hire people who do something useful better than you do, be it solving differential equations or managing difficult personalities. Life will be much more interesting and you will be much more successful, if you define success as actually getting something done.

Be a dean. Yes, I know all the jokes; nevertheless, it happened to me. UT Southwestern Medical School was blessed with a succession of highly regarded deans: Kern Wildenthal, mentioned above, who then became president of the medical center; Bill Neaves, a PhD medical school dean, who consistently won gold medals for efficiency and wisdom and then went on to be the first CEO of the new Stowers Institute; and Bob Alpern, who left Dallas in 2004 to become dean at Yale School of Medicine. I received a phone call from President Wildenthal just hours after the announcement of Alpern's departure. I was taken aback when he asked me to be interim dean. By coincidence, it was a beautiful week in the spring, and I had done needed yard work to an extreme over the prior weekend. My back was aching and I was taking Valium to relieve the spasms. Be dean? Sure, why not; what's the problem?

There were many positives to the job, such that I took it on permanently when asked after my trial by fire. It was a pleasure to work with Kern Wildenthal—a smart, analytical problem solver and decision maker—who quickly gave me sufficient rope to hang myself and resources to keep the ship of state on a quick but steady course. I was able to do the job because the UT Southwestern dean's position was almost completely academic; the clinical enterprise was under the direction of others. The president, the academic dean, and the overseers of the clinical business worked effectively as a team. I became convinced that this separation of church and state—academic mission and clinical service—was a good one. I quickly learned more about the entire medical school than I had in my previous 24 years as a basic science chair. I had the opportunity to help recruit and hire great people, reinforce old programs and establish important new ones, and interact with a broad spectrum of both academic and community leaders. It is a joy to see that several of these initiatives are thriving and making a difference.

So why do medical school deans survive, on average, only four or five years? I had roughly 50 direct reports, including approximately 35 department chairs and independent center directors. This is insane and means that you often just run from place to place putting out fires; it's tough to stay ahead of the game. Email is great, except when you get more than a hundred a day, with each sender expecting an immediate response. You are the leader of the faculty. You try to promote institutional as well as individual goals, but some see only the latter as worthwhile. At the extreme, some of the more entitled believe you are available 24/7 if they have a plumbing problem in their lab. And with a faculty of 2,000 and a comparable number of students, you are presented with a random sample of life crises or, more rarely, insane, immoral, or illegal behaviors. Bad actors consume way much more than their share of your time. At the end of it all, you wish that you had kept a diary and that you could write a book and name some names.

Contribute to your discipline—beyond research and teaching. I think it goes without saying that service on peer-review committees and editorial boards is mandatory payback. There are many flaws in the current systems that go well beyond anyone's abilities to fix them. Despite this, they work reasonably well and must be supported and nourished by the best among us. This is the pro bono side of academic life. Recognize it for what it is, and do it well.

Edit a textbook. Although all types of books and everything else that is printed on paper are perhaps doomed, I spent the equivalent of at least four full-time years as associate editor and then editor of four editions of *The Pharmacological Basis of Therapeutics* (1975–1990). For most of this time, I had no difficulty convincing myself that this was a worthwhile activity: The book was widely read by many types of students and physicians as the definitive source of information about drugs; academic pharmacologists and clinicians were honored to participate; and colleagues in the publishing industry were intimately involved in the production of quality products. One of the pleasures of this foray was the opportunity to work with both my father and my middle-name

namesake, Lou Goodman. Lou wrote beautifully and had strong, excellent opinions. He expressed these opinions colorfully, earning himself the nickname of "Louie the Lewd." He also had an enormous collection of reprints that he would share at the drop of a hat, as well as an infinite capacity to coax and cajole me into working harder and harder. My father died before I received significant accolades for my research. Lou filled in as proud cheerleader.

The only dark side concerned those who failed to meet their obligations—or worse. Again, when managing any reasonably large group, you take a random sample of life crises. You cannot delete a chapter in a textbook when a promised contribution is not forthcoming. You simply have to ghost-write it yourself or hire someone else to do so when the ultimate final deadline has passed. The worst of all was a major case of plagiarism—major sections of Goodman and Gilman text from several of our chapters were taken verbatim by one of our associate editors and contributed by him to another textbook. Bring on the lawyers.

So much about the printed page has changed. Students do not learn by consulting a reference work. They require a distillation, preferably delivered electronically in formats that can be fitted optimally into their life schedule. In the universities that I know best, most medical students do not attend lectures, but law students do! Once, frustrated, we put a matching multiple-choice question on an exam: Match the picture of the lecturer with the name. You can imagine the "amusement" of the students. Might it be that we demand more logic and reasoning from law students? Law is, after all, a rules-based system created by man. Biology is also rules-based, but our challenge is to discover the rules written by millions of years of evolution.

Students and physicians alike seek the answer to everything from Google and its equivalents, and they generally and uncritically accept the first answer encountered. On the other side of the fence, the publishers have become mere middlemen, issuing subcontracts for editing, indexing, graphics, printing, binding, and most everything else to a low bidder. Despite all of these issues, some classic textbooks survive. I am grateful first to Joel Hardman and Lee Limbird and then to Larry Brunton (who just happens to have been my first graduate student) for carrying on the tradition of *Goodman and Gilman's The Pharmacological Basis of Therapeutics*.

I am not a complete "old fart," blaming all or even many ills on the younger generation; its members are what we have made them. I love Google and instant gratification. I also appreciate a textbook or a brilliantly written review article in which there has been a sincere effort to synthesize a barrage of often contradictory information. Many of the ills lie with poorly conceived educational goals. I'll confine my brief comments to medical education, in which I have the most experience. During the first two or so years of the classic medical curriculum, we shove facts down increasingly resistant gullets. I actually once saw a biochemistry exam that asked the question, How many nucleotides are in the genome of phage lambda? Most of these facts are quickly forgotten or would not be useful if retained. During the clinical years, we too often teach technique but not reason and judgment. Wise teachers can extract concepts and make a joy of them. Remember Henry Harbury (above). When we find such teachers, we should hire them. It's not a common career path in a medical school.

Relationships with industry. Consulting activities can be most worthwhile intellectually and on both sides of this give-and-take relationship. To be frank, they also offer an opportunity to level the financial playing field, as most of us believe we are underpaid compared with (to cite but two examples) the most specialized physicians, who have an amount of training comparable to that of PhD scientists, and lawyers, who have much less. As a pharmacologist who spent his career at the more basic and mechanistic end of the discipline's spectrum, it has been both a pleasure and an education to serve on the board of directors of two pharmaceutical companies (Regeneron and Eli Lilly) and participate, even from a distance, in drug development from beginning to end.

Regeneron is an up-and-coming (we hope) biotech firm, founded more than two decades ago by Len Schleifer, who was my MD/PhD student at the University of Virginia. Eli Lilly is, of course, a venerable, large pharmaceutical company and provides an interesting contrast with Regeneron.

Len Schleifer is an exceptionally smart and extraordinarily creative physician-scientist—he has a PhD in pharmacology; is board-certified in neurology; and is an instinctive, self-taught, hard-driving competitor and entrepreneur. He has remained CEO of the company since its founding in 1988, despite the fact that its first profitable product will not reach the market until 2011 or 2012 (we hope again). This has required enormous deal-making and administrative skills; vision and infinite tolerance for risk; and the ability to acquire, retain, and inspire superb discovery scientists, especially George Yancopoulos and Neil Stahl. Commercial success, if it comes, is also owed to Roy Vagelos, chairman of the board, who came to Regeneron after retirement from notable roles as chairman and CEO of Merck. Roy Vagelos is the best example I know of the weaknesses of mandatory retirement systems.

The corporate cultures of New York–based Regeneron and Indianapolis-based Eli Lilly could not be more different. Despite this, both companies remain committed to innovation from science. Eli Lilly, led by John Lechleiter, PhD (chemistry, Harvard), maintains this commitment despite facing great challenges because of patent expirations—a common problem that has led to the demise of more than one major pharmaceutical player. Management of a pharmaceutical company is just plain difficult. Discovery cycle times are more than a decade, meaning that the late-stage product candidates in the pipeline are usually the brainchildren of former CEOs and executive vice presidents for research, whereas current management's ideas usually do not come to fruition in their corporate lifetimes. When challenged, some companies come to rely predominantly on in-licensing or grander acquisitions, which have a chilling effect on internal innovation.

The pharmaceutical companies were the darlings of the 1990s but have become the pariahs of the new millennium. Some of their new bad reputation stems from unattractive or illegal marketing practices and is deserved. Some stems from a dearth of valuable new products. Explanations for the latter are not simple to come by. The public lays the blame on the pharmaceutical companies for high costs of medical care, although drugs account for only about 12% of the nation's health care spending. More visible are pricing practices that take advantage of third-party payment systems that will pay a fortune for an approved drug with only modest benefits. The public does not understand risk, wants perfectly safe drugs, and expects heads to roll (and lawsuits to pay off) if uncommon side effects are not detected in clinical trials that cost hundreds of millions of dollars. I strongly believe that the current problems are not the results of either incompetence or inadequate investments. One continues to hope that the dramatically better approaches to drug invention that have been in hand for the past decade will soon yield dramatic results. Nowhere is this truer than in medical oncology.

THE CANCER PREVENTION AND RESEARCH INSTITUTE OF TEXAS

The people of Texas have done a great thing by approving a constitutional amendment that authorizes expenditures of $3 billion over 10 years: 90% for cancer research and commercialization efforts and 10% for community-based prevention activities. I had the opportunity to help frame the legislation that enabled a rock-solid, conflict-of-interest-free peer-review system for making these awards, and when this was secure, I agreed to leave the University of Texas Southwestern Medical Center after almost 30 years and try something new in what is my last job (I am quite certain) as chief scientific officer of the Cancer Prevention and Research Institute of Texas (CPRIT). The scientific advances of the 1970s, 1980s, and 1990s defined cancers as genetic diseases, and the technological advances of the past 10 years have enabled cancer research in a way that could only be

imagined just a few years ago. We speak of scrutiny of every individual cancer in molecular detail, including whole-genome sequencing. We dream of drugs exquisitely well targeted to disrupt signaling pathways that have been usurped by oncogenic mutations. We have countless major challenges to meet, but we believe we see a path to their solution. CPRIT enjoys the counsel of many of the leading cancer investigators of the United States—from the most fundamental to the most applied—led by Phil Sharp (MIT). We fund research, training, and recruitment of superb talent to Texas. We will enable major contributions, and we will facilitate their application and commercialization. Great accomplishments and satisfaction lie ahead.

PHARMACOLOGY THEN AND NOW

This last section is where the "old fart" comes completely out of hiding. But maybe it's not a terrible idea to let the elders reflect on the changes they have seen over a career.

Most modern students of pharmacology could not describe a kymograph; that's history. A fellow in my lab was mystified when told that a certain protein was expressed in tracheal smooth muscle; that's biology. We might have convinced him that the trachea was connected directly to the rectum. This isn't all bad, but it's not all good either.

Pharmacology departments used to be low on the academic totem pole: "The biochemists shall inherit the Earth." The science was very descriptive. Methods for drug discovery were primitive and empirical, although rather effective! (Low-hanging fruit?) Jobs in the pharmaceutical industry were taken mostly as consolation prizes. Pharmacology graduate programs did not, in general, attract excellent students. Those who measured enzymatic reactions or took cells apart, rather than performing bioassays with contracting muscles, were regarded suspiciously by many and judged not to be real pharmacologists. I am reminded of a superb postdoctoral fellow (now a long-term pharmacology faculty member) who was somewhat chagrined to find himself working in a pharmacology department. He admitted that (before the current experience) he thought pharmacology departments were places where they tortured cats. I could understand his opinion.

Clearly, changes have been dramatic. More rational, target-directed drug discovery came from the team of Gertrude Elion and George Hitchings and just as dramatically from Jim Black. A new journal, *Molecular Pharmacology*, was founded by Avram Goldstein in 1965 and spoke to and for the change. Real progress was made in understanding signaling from nicotinic cholinergic receptors and from what came to be known as G protein–coupled receptors. But to me, at least, molecular cloning really changed the game. Human proteins could be cloned, expressed, and targeted with drugs. Human proteins could be cloned, expressed, and used as drugs, with insulin and growth hormone leading the way. Genes could be overexpressed or knocked out to mimic gain or loss of function. In a real sense, molecular biologists were suddenly pharmacologists, worrying not only about pharmacodynamics but also about pharmacokinetics, drug disposition, and pharmaceutical preparations. Drugs were good, and biotechnology companies were the place to be (and get rich). Some were so enthusiastic that they even reinvented the discipline, calling it chemical genetics or chemical biology. I have stolen this description from the Web: A key aspect of chemical genetics is the identification of small molecules that can perturb a biological target or process of interest. Shucks; my daddy taught me that.

All of science and medicine has become specialized, subspecialized, and sub-subspecialized. Pharmacology is no exception. The lack of breadth of many current PhD curricula in biomedical science is troubling—again the victim of success: new knowledge and novel technologies. I have never been accused (at least to my face) of being unsympathetic to hard-core basic science or to science for the sake of science. I fully support the underlying premise—basic research forms the foundation from which most applied research and beneficial products spring. There is, however,

a gap in application of basic findings and a paucity of emergent products, especially drugs. We must pay attention to that gap, especially in light of current funding pressures that flow from completely legitimate concerns about financial deficits. The best defense of basic research is its successful application in ways that improve human lives. Pharmacology has always been a bridge science—perfectly positioned to both develop and apply basic knowledge. To do this, we must remember that the trachea is not connected to the rectum.

I should probably close on that anatomy lesson, but I have an urge to set forth a few issues that I believe threaten biomedical science. These are not novel observations, but they deserve much more debate and action than they receive. I have opinions but no solutions. The range of opinions on these subjects is as broad as the gulf between socialists and tea partiers.

- We do way too much begetting, reproducing ourselves many times—sometimes a hundred times or more. We pretend that most of our trainees will find positions as research scientists, even independent investigators. This is simply not possible. Training times lengthen because jobs are scarce. The vigor, enthusiasm, and creativity of youth are wasted. Perhaps we need to train a cadre of "supertechnicians" who can have productive and reasonable careers working in successful labs. We need a labor force rather than an infinite number of trainees.
- We allow labs to become enormous, further exacerbating the begetting. But these labs don't practice needed team science. After all, everyone has to prove himself or herself and get an independent position. At least many who oversee enormous labs acknowledge that they did their most interesting and creative work when they were younger, had a small lab, and stayed home to supervise it. That's the way they got famous and acquired a large lab!
- Despite obvious evidence that the federal deficit is out of control, we continue to lobby for more money. How else will our offspring survive, to say nothing of ourselves? Look at the countless journals. Is there a lot of money being spent on mediocre research? We must funnel precious funds to research that will have a real impact. We must take more risk to ensure more impact. It's tough to find low-risk, high-impact projects.
- The tenure system is intrinsically flawed, at least in science. Many universities guarantee eternal employment to 40-year-old assistant professors as they clear the first hurdle and are promoted. They may not even have obtained the first competitive renewal of a major research grant. This was a weak system when there was mandatory retirement; it is insane in its absence. We revere the aging researcher who still does useful work, and a few do. But most should at least contract and move aside. We need constructive activities for senior citizens. Teaching comes to mind.

There is my closing rant. I acknowledge that some of the opinions and suggestions are most unpopular. Unfortunately, the issues are very real, and they are getting worse. Someday we will pay the piper.

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Errata

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