JCI The Journal of Clinical Investigation

2015 Association of American Physicians George M. Kober Lecture
A doctor's dilemma: choices amidst change

P. Frederick Sparling

J Clin Invest. 2015;125(9):3330-3334. https://doi.org/10.1172/JCI83584.

AAP George M. Kober Lecture

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My title is derived from George Bernard Shaw's 1906 drama *The Doctor's Dilemma* (1). This short satirical farce spoofs arrogant doctors talking about their research successes and failures and their difficult choices. There is a surprising timelessness to the discussions.

Role models and mentors are important for any aspiring physician-scientist. For me, they included a kindly pediatrician who made frequent house calls when I was a boy. Other examples included several former Massachusetts General Hospital (MGH) house officers, including fellow intern and subsequent Nobel Prize winner Mike Bishop; assistant resident Phil Majerus, a former Kober lecturer; assistant resident Ken Shine, who became president of the Institute of Medicine of the National Academies; and John Parker, yet another assistant resident, possibly the most inspirational physician-scientist of them all. In training and in building a lab or a department, it is always good to surround one's self with very smart people!

My principal mentor was Mort Swartz, division chief for infectious diseases at MGH, a brilliant, kindly, and dignified man whom we all wanted to emulate. It was not an accident that three of our group of twelve interns became infectious disease people.

In building a career, serendipity helps. As an intern struggling to balance every second night on call with a burgeoning romance with my now wife Joyce, I missed the deadline for applying to the NIH and instead had to try for a position with the USPHS. There were many choices, including the Epidemiology Intelligence Service and the Indian Health Service. I took a phone call during morning rounds from an official from the Venereal Diseases Research Laboratories (VDRL), my last choice. He offered a position in their labs in Chamblee, Georgia, outside Atlanta. The choice had to be made right then or else I would be subject to the doctor's draft. I accepted!

Arriving in Atlanta, the VDRL labs were unimpressive, rows of old World War II Quonset huts set amidst weeds on blistering hot red Georgia clay. The choices for research were few and included either syphilis or gonorrhea. I became a clinical "short arms inspector," culturing prisoners in the county jail or in schools for wayward adolescent girls. It seemed the most boring job in the country. There was no ongoing research that interested me. But this was a fabulous time to read in the library, and I realized that it might be possible to create a genetic system to study gonococci. What seemed bad fortune was actually an unparalleled opportunity for thinking and personal reflection. Genetic transformation of gonococci turned out to be easy under the right conditions, which included using recently isolated human specimens that still retained their in vivo phenotype. Further in vitro passage resulted in loss of competence for DNA uptake (2). I was hooked on the daily work and excitement of bench research. Forgoing senior residency, after consultation with Mort Swartz, I started what today is the short track, a deep immersion in the world of *E. coli* genetics in the lab of Bernie Davis at Harvard Medical School. The rigorous tutelage of Bernie Davis taught me to set high standards, to try to do important work.

Another problem that turned out to be an opportunity occurred at the end of my clinical training, a year as an ID fellow at the MGH. It was time to support our family. I had multiple publications, including two single-author papers (2, 3), and was confident it was time to try my wings. A university faculty job was committed, only to be later withdrawn, to our great dismay. Fortunately, another position was soon found in the Department of Medicine Division of Infectious Diseases of the University of North Carolina (UNC). Joe Pagano, a superb virologist who valued basic science, was the division chief. There was a tradition of joint appointments between clinical and basic science departments. Sight unseen, lab space and a joint appointment in microbiology were given by Chairman Phil Manire. The environment was far bet-

Conflict of interest: The author has declared that no conflict of interest exists.
Reference information: *J Clin Invest*. 2015;125(9):3330–3334. doi:10.1172/JCI83584.
This is the text of the 2015 Kober Lecture to the Association of American Physicians, delivered at the annual meeting of the ASCI/AAP in Chicago, Illinois, USA, April 25, 2015.

ter at UNC for my growth as an investigator, for I had ample protected time and enjoyed the company of excellent basic scientists.

Our work at UNC on the genetics of *E. coli* ribosome structure and antibiotic action started well, with an RO1 funded and renewed, but other bigger and deeper labs in Berlin and Madison seemed bound to prevail. An undergrad student appeared at my office and offered to work for no pay — he had problems with grades due to a bit too much cannabis — and I put him to work on genetics of gonococcal antibiotic resistance. He found an interesting mutation designated *mtr* for multiple transferable resistance (4). Before long, we switched entirely to work on gonococci, just before NIH funders decided that sexually transmitted infections were understudied. More serendipity. This vignette illustrates two more general lessons: the most important choice young or old investigators make is what problem to work on. The other, less-discussed choice is when to persist and when to step away from a problem, in poker terms when to hold them and when to fold them.

Once we made the shift to focus on the gonococcus, progress came rapidly, although we worked in relative isolation in the first years. Applying genetic tools to study pathogens was in its infancy. This was a time when it was necessary to give a talk, "Is genetics really necessary?" We showed that resistance to antibiotics was the result of additive interactions between multiple mutations (5). Penicillin-resistance mutations included those for the penicillin-binding proteins Pbp1 and Pbp2 (6), and also the principal outer membrane porin (7, 8), but the phenotype of these mutations was amplified by the *mtr* mutation (9). The *mtr* mutation was incorrectly postulated to block entry (10), but my excellent postdoctoral fellow Bill Shafer and his colleagues later showed that it encodes an efflux pump (11), increasing resistance to many structurally dissimilar drugs. Emergence of plasmids encoding resistance to tetracyclines as well as penicillins (12) finally spelled the end for these therapies. Fluoroquinolones had their day, but resistance emerged rapidly (13). They were supplanted for a while by newer oral cephalosporins until higher level resistance to them showed up (14). Curiously, similar resistance has never emerged in meningococci for unclear reasons, but nevertheless fortunate.

The Mtr pump also increases efflux of many toxic hydrophobic compounds, including those that are part of our innate immune defenses (15). The result is increased ability to resist normal mucosal immune defenses. Ann Jerse, Shafer, et al. showed that the efflux pump increases fitness of gonococci dramatically in a female genital-infection mouse model (16). Thus, pressures for emergence of resistance are not only our new antibiotics, but include natural antimicrobials in plants and animals and throughout nature.

Therapy for gonorrhea currently is threatened by the recent emergence of cephalosporin-resistant strains in Japan and Europe, creating worries of untreatable gonorrhea (14). Alarm about these so-called superbugs may be premature, since the resistant clones have spread very little in the three years since they were first documented. This strongly suggests that these highly resistant strains are less fit. Nevertheless, we desperately need new drugs for resistant gonorrhea, especially ones that are effective when given once by mouth.

The ceftriaxone-resistant strains have acquired a novel cassette carrying multiple mutations in the gene for Pbp2 (17), the principal target for all β-lactams. When expressed in a gonococcus that contains the *mtr* and porin mutations, the result is high-level resistance to all β-lactam antibiotics and many others. The source of the cassette probably was horizontal transfer from a nonpathogenic *Neisseria* species in the oropharynx, as documented previously for earlier Pbp2 mutations (18). Analogous genetic events are common in the microbial world; one of the important evolutionary ideas is that microbes are moving DNA across species barriers frequently, challenging dogma about the definition of species.

My life has been characterized by recurrent ten-year itch. Studying genes that affect pathogenesis, principally involving the structure of the outer membrane, became a new focus. We became interested in the porin protein, the functions of lipooligosaccharides, and receptors for binding human iron ligands. We left the field of genetics of antibiotic resistance, which later served as excellent sources for important work by others. Another major branch point in my professional life emerged, when I surprised myself and others by taking the chair in microbiology at UNC. This was a challenge because I would be the only non-PhD in the department, as my students occasionally reminded me. Clinical work continued, but on a limited basis. I terminated my continuous care outpatient practice, but continued to serve for a month every year on the inpatient consultative service. The lab grew larger; excellent students and postdoctoral fellows joined.

Many interesting stories emerged, but time only permits brief description of one here. The problem is, how do bacteria acquire essential iron in vivo? Iron is bound tightly in humans to serum transferrin (Tf), mucosal and neutrophil lactoferrin (Lf), and red blood cell hemoglobin. Gonococci do not secrete small molecules that can strip iron from host proteins, as is common among other pathogens (19). Rather, they have evolved receptors that specifically bind human Tf or human Lf as ligands (20, 21). The Tf and Lf receptors are very similar (22, 23). The Tf receptor is encoded by a small operon containing two genes, one for the lipoprotein TbpB and another for the integral membrane protein TbpA (24). These two proteins work cooperatively to bind human Tf. After binding, they are activated by interaction with energy-transducing TonB (24, 25). The result is changes in conformation of both the Tf receptor and Tf (26). Iron is released from Tf and is then imported through an interior channel in TbpA to the periplasm and eventually into the cell.

Whereas all gonococci make the Tf receptor, only one-half make a functional Lf receptor (20). That is surprising, since gonococci infect mucosal surfaces where Lf is the dominant iron source and are attacked by neutrophils that literally spit Lf at gonococci. A less closely related two-protein receptor binds hemoglobin (27), a third source of essential iron.

How do these receptors function in infection? For two decades, the UNC group utilized male volunteers in experiments to test which gonococcal genes make a difference in early mucosal infection (28). Women were not studied because they are more likely to develop serious complications. There are a variety of animal models, but gonococci naturally infect only

humans and, to a limited extent, chimpanzees. Volunteers were inoculated in the Clinical Research Unit through a small catheter inserted into the anterior urethra and were housed there for five days to see what happened. All experiments were cleared by the institutional review board (IRB), and informed consent was obtained. Subjects were treated before release, and none developed complications. The questions were, which gene products really make a difference in early infection? Ultimately, we hoped to safely and cheaply test as proof-of-principle gonococcal vaccine candidates.

Experiments were conducted in which the inocula consisted of carefully constructed isogenic derivatives of a well-studied gonococcus expressing either a Tf or a Lf receptor or neither. The double-knockout was noninfectious even with high inoculum size, as compared with its siblings, which were infectious (29, 30). Interestingly, these iron receptors were the only gene products tested among many candidates that made an absolute yes/no difference. In order to test the relative importance of the Tf and Lf receptors, we did another experiment in which volunteers were simultaneously inoculated with a mixture containing equal amounts of a strain making both receptors and another making only the Tf receptor. The strain making both Tf and Lf receptors quickly outcompeted the one that only made the Tf receptor, saying that acquiring iron from Lf provides an advantage in the male urethra (30). So why are one-half of clinical isolates unable to use Lf as an iron source? Is there an advantage to being unable to use Lf as an iron source under some circumstances (30)? And why has an identical deletion of the *lbpAlbpB* operon spread horizontally to otherwise diverse genomic variants of gonococci (30)?

Many questions remain. Because of emergence of very resistant gonococcal strains, there is need for a vaccine. There is preliminary evidence that antibodies to PorB, TbpA, MtrE, and several other antigens, including a common core epitope in gonococcal lipooligosaccharide, are bactericidal and have properties suggesting their utility in a vaccine (31). Sadly, there is very little commercial interest in developing a gonococcal vaccine, especially when compared with other successful efforts for a vaccine against capsular type B meningococci (32). The need is great, but the market seems too small.

My main point is to illustrate choices in academic medicine. You may understand my angst when presented with yet another dilemma, the opening of the chair of the Department of Medicine at UNC. After refusing to be a candidate and serving on the search committee for two years, a comfortable niche in basic science was exchanged for chairing a big clinical department. This was a very difficult choice, since I was very happy where I was. Why take on such burdens? One reason certainly was concern for academic medicine and for our department of internal medicine, which appeared to be foundering with no leader after two years of searching. Perhaps it was deep imprints by my first chair, Walter Bauer, who walked the floors at 5:00 am and once offered congratulations when told that I had not slept. No 25% effort chairman, he was all in. I loved teaching, sometimes offering noncredit seminars to our best medical students for their and my fun. Maybe it was the allure of working closely with our talented residents. It certainly was the joy of walking one of our residents down the aisle at her wedding. And the joys of morning report, of stump-the-chair rounds. Of trying to build a department and to protect the lives of extremely talented physician-scientists who remained some of our best clinicians, including Ron Falk, Ric Boucher, David Clemmons, Stan Lemon. Of recruiting stars such as Mike Cohen, Bev Mitchell, and David Brenner, Sid Smith. The pleasures of working with other leaders in the school to make it the best it could be. Mistake it not, however, everything has a price, and when I no longer attended Gordon conferences and devoted only 10% time to being in my lab, something was being given up.

In the end, the business side of running a clinical department weighed too heavily. After ten years as chair of medicine, there was another transition, leading to more time for the lab, working on principles for a gonococcal vaccine (33), leading our sexually transmitted infections research center. More time for clinical work and for family and personal adventure.

An unexpected new research opportunity occurred, aimed at defense against bioterrorism, occasioned by the anthrax attacks of 2001. An unforgettable moment was a meeting of the Institute of Medicine Forum on Microbial Threats in November 2001, when Kenton Alibek, once the leader of the former USSR bacterial bioterrorism unit, presented the scope of their programs. He pounded the table and declared that they had not six weapons but 30. It takes only two years to make a weapon, but ten years to make a defense. He had been telling us this for years, but we had done nothing. A few months later, President George W. Bush announced a new program to help protect the country. We were required by the NIH to create regional teams and to work across academic boundaries in order to develop new drugs and vaccines and diagnostics. A group was assembled, initially headed by Bart Haynes of Duke, but for its last nine years, by me. I became someone who assembled and managed a team of the very best people drawn from multiple universities, analogous to a baseball team's general manager. Our group, nicknamed SERCEB for the Southeast Regional Center of Excellence for Emerging Infections and Biodefense, was an all-star team, and we actually had tryouts to select the players. It required me to learn the virology and immunology that had evaded me before, to use my experiences serving on pharmaceutical science advisory boards, and especially my ability to get people to agree to sacrifice for the common good. The camaraderie of the group was exceptional, as was their talent and commitment. Suffice it to say that really important work was done and the team idea worked, despite initial concerns and suspicions.

So what lessons can be gleaned from this series of experiences? Admittedly, times are different now. Yes, there are not enough jobs, and NIH grants are very hard to get. The average age for initial RO1 awards is nearly 45. Academic medicine is being squeezed by state government cutbacks and health care reimbursements. Shift work in clinical care threatens to undermine our historical sense of professionalism as well as continuity and excellence of care.

For many physician-scientists, one of the biggest dilemmas is whether to focus almost exclusively on either science or clinical medicine. My example does not answer the question. I straddled the divide

throughout an entire career and both profited from and immensely enjoyed the varied opportunities that arose. Rewards from working with people do not show on a curriculum vitae, yet they are gratifying and important. Nevertheless, many advise young investigators to focus on their science because modern science is competitive and moves rapidly. Such a choice is entirely personal.

To our youngest colleagues, I say be encouraged and not discouraged. See the glass as half full. Problems hide opportunities. Be optimistic and persevere.

Choose research problems carefully, looking at those that are at their beginning and not at their end. Learn when it is best to continue to press onward and when it is best to leave to work on a better opportunity.

The best strategy always is to do what you love, for you will do it better and will succeed more often.

Surround yourself with the most talented people possible, and be generous in your relationships with them.

The environment matters. Seek an institution that needs you and gives you time to grow and that protects your time.

Tell the truth. Honesty is your friend. Truth leads to trust, as exemplified for me especially by my former dean Stuart Bondurant, who never overpromised.

Do not lose sight of the advantages of short-track training in basic science laboratories, which launches young people into faculty positions without interposition of additional clinical years, as is typical for combined MD-PhD training.

Choose your life partner wisely. My wife Joyce has helped in so many ways, doing more than her share in raising our family and yet getting her PhD in mid-life, pursuing her own successful academic career, starting a company. We paddled the same canoe down some beautiful but occasionally turbulent waters. The life of a physician-scientist is a demanding one. Be thankful; your partner needs your love too.

Don't forget your roots. Patient care is rewarding personally and is the source of many ideas about which research problems are important. Translational research is an increasingly important focus for many reasons, and opportunities are vast.

The world of science is exploding around us. Just look at what high-throughput sequencing technologies and availability of much greater computer power have done for studies of genomics. Such advances underlay the million-person study being considered by the NIH. In my own disciplines of infectious diseases and microbiology, they also enable our understanding of how microbial flora (our microbiome) influence our gut development and the maturation of our immune systems, our weight, our metabolic profiles, our susceptibility to autoimmune and inflammatory diseases, cancers (34). Because of these powerful new tools, we know that overuse of antibiotics has great costs through inadvertent effects on our gut microbiome (34). These advances are revolutionary, enabling new questions that depend not on knowing about a single microbe as the cause of disease as in Koch's postulates, but rather on the relative proportions of great varieties of organisms, many of which we still cannot grow. This is a very young field, and it touches all of medicine. We are on the edge of discovering new therapies, better ways to prevent disease, of changing health for great numbers of people. Another example: new techniques for isolation of large numbers of specific human monoclonal antibodies and for understanding molecular structures have greatly enhanced epitope discovery and vaccine development. We know very little compared with what there is to know.

Would I do this again? Absolutely! The world of the physician-scientist is an exciting one, with possibilities for deep joy in learning something new. Nothing is more satisfying than helping others, either directly by personal care or indirectly by advancing the science of medicine. The culture of science, the rewards of working with very bright colleagues and collaborators is underappreciated sometimes, but it is seductive.

You are fortunate to live at a time when science is providing such incredible tools.

Be positive, choose wisely, and good luck.

Thank you.

Acknowledgments

I thank the UNC School of Medicine, which provided me opportunities to explore my potential as both a scientist and clinician, and also the National Institutes of Health, which funded my work continuously from 1969 through 2014.

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