MY LIFE IN SCIENCE

By R.L. Juliano Ph.D.

This essay outlines my long career in biomedical research. It may be of interest to young scientists who are just starting on their own career paths, since it highlights the effects of both hard work and of serendipity in research. A caution though, the highly individualistic approach to science that I was able to take may be difficult to duplicate in today's environment where team science is increasingly important.

Rochester (1966-70) and Buffalo (1970-72). Red Blood Cells and Liposomes.

In 1966 my wife Eve and I completed a two-year stint in the Philippines with the US Peace Corps and moved to Rochester NY. My career in science began with graduate studies in the Dept. of Radiation Biology and Biophysics at the University of Rochester. My Ph.D. advisor was Aser Rothstein, a Canadian scientist who had worked at Rochester for many years. Aser was both an excellent investigator and a scientific leader and administrator. At the time I worked for him, Aser was the department chairman. In many ways he became a role model for my future career.

Aser was a very busy man so much of the lab training I received came from Joob Hoogeveen, a visiting scientist from the Netherlands. When I started in the lab Joob handed me a pile of research papers to read, so I sat there reading all day. Next day the same thing happened. The third day my chair was gone! Time to start doing experiments!

Aser's lab worked on ion transport processes in the red blood cell membrane. However, I decided I wanted to do something different. This was the first instance of a pattern that has persisted my entire career, of wanting to go in a different direction from other people in the field. Sometimes this was productive, but sometimes not.

I decided to work on studying the proteins of the red blood cell membrane using a then new technique called polyacrylamide gel electrophoresis. We used buckets of outdated blood bank blood, released the hemoglobin by osmotic lysis, and then centrifuged the now white RBC membranes called 'ghosts'. While dealing with gallons of human blood we wore no masks or gloves and sometimes pipetted by mouth!! Lab safety procedures were very different then!

We used different extraction conditions to categorize several groups of proteins. Two very interesting proteins emerged from the studies. The first was a ~150,000 MW water soluble protein that could only be extracted from the membrane at high pH and in the absence of calcium; we called this protein band 4.1. Later work by others showed that this was an important and ubiquitous cytoskeletal protein now known as spectrin. We also found a 100,000 MW protein that required detergents to be solubilized; we called this protein band III. Later it turned out that band III was the major anion transporter of the RBC membrane and was thus the protein involved in the ion transport processes that Aser's lab studied. I was the first to detect these important proteins, but others went on to establish their functions. We published several papers based on these studies (PubMed ID numbers 24174191, 5141126, 5130057).

After finishing my thesis experiments but before actually writing, I left Rochester to take a postdoctoral position. I had two good choices. One was at a very prestigious lab at Yale, the other at Roswell Park Memorial Institute in Buffalo, a good but less prestigious research institution. The job at Buffalo paid a lot more, and since our first child had just been born, that is where we went! In 1970 I joined the Dept. of Experimental Pathology led by Leonard Weiss, a brilliant but somewhat eccentric Englishman. Leonard was interested in the possibility of RNA transport between cells, a visionary idea at the time that has now been validated in the form of RNA transport via exosome trafficking between cells. I worked with Eric Mayhew, another Brit, and did a series of experiments on RNA uptake by cells. The techniques we used were incredibly crude by contemporary standards and we didn't really learn very much. Nonetheless we managed to publish several papers (4778410, 4113945, 4734767).

Another group in the department was led by Dimitri Paphadjopoulos, a charismatic Greek American scientist. Dimitri studied liposomes, a type of artificial membrane comprised solely of lipids that could mimic many

properties of cell membranes. I was quite fascinated by this new technology and collaborated with Dimitri: for example, we examined the interaction of my band 4.1 (spectrin) with liposomes (5003695). Dimitri had several talented postdocs in his lab including Harry Kimelberg and Ken Jacobsen. We three became good friends and did things outside of lab. For example, I learned to ski with Harry at Holiday Valley resort south of Buffalo. Both Harry and Ken went on to distinguished academic careers, and many years later I renewed my interactions with Ken while we were both faculty members at Carolina.

Surprisingly, at that time Buffalo was a hotbed of membrane research led by Dimitri and by Prof. James Danielli, yet another Brit, who was famous as the co-creator of the Davson-Danielli model of lipid membranes. There were many excellent seminars and lively discussions. It was during this period that I developed a strong interest in liposomes that would have an important role in my future career.

Toronto (1972-78). Discovery of the P-glycoprotein

After two years of postdoc, it was time to look for a 'real' job. Fortuitously, Aser Rothstein came back into my life with a job offer at the Research Institute of the Hospital for Sick Children in Toronto. While I was doing my postdoc, Aser had taken a position as Director of that institute. Eve and I had visited Toronto a few times, and we really loved the city; so in 1972 off we went for what we thought would be a couple of years in Toronto. Sick Kids was located in the heart of downtown Toronto but with my princely salary of $15,000 we could only afford a townhome in Mississauga, a distant suburb.

Aser had a large group at Sick Kids working on RBC ion transport. I had a small amount of my own lab space and, of course, started working on things completely different from the rest of the group. Fortunately, I was able to quickly acquire my first independent grants from the Medical Research Council of Canada and the Canadian Cancer Society.

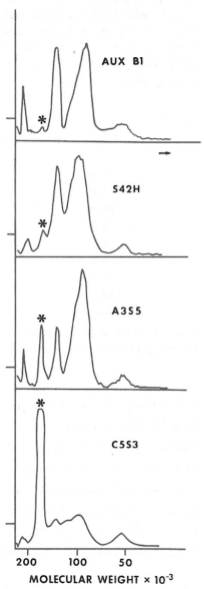
I pursued two research themes. One involved liposomes and their possible role in drug delivery. The other had to do with membrane proteins in mammalian cells and their role in cell-extracellular matrix interactions. I still am not sure why, but somehow the question of how cells adhered to ECM or to other cells fascinated me. Both projects went well and resulted in a number of publications, thanks to the very able people working with me.

On the liposome side I worked with Dennis Stamp and Nancy McCullough. Dennis was a technician but really had the makings on an independent scientist. Additionally, he was quite an athlete and taught me to play tennis on a little court behind Sick Kids during lunch breaks. Dennis and I did two basic studies that had quite an impact on the growing field of liposome research. First, we showed, that particle size and charge had a major impact on the clearance of liposomes from the circulation (1131256). Second, using lectins, we showed that liposomes could be 'targeted' to bind specifically to cells. This was the first example of numerous subsequent studies from many labs on the topic of liposome targeting. Although it was really a very simple set of experiments, its novelty earned me my first publication in NATURE (1272396). Nancy (my first postdoc), Dennis and I also worked on the use of liposomes for delivery of anti-cancer drugs and produced some of the first publications in this nascent area (PMID 619903, 7391983).

With the help of two very capable technicians, Monique Behar and Emy Gagalang, I also studied the mechanism of cell adhesion to proteins coating the tissue culture plate. Our main tool was the then new technique of surface labeling of membrane proteins using enzymatic addition of the radioisotopes ^{125}I or ^{3}H. This allowed us to define those proteins that projected outside of the cell from those that were entirely inside. We then used proteases to cleave some of those proteins to see which ones affected cell adhesion.

We published several papers on membrane proteins and cell adhesion, but the biggest impact of our techniques came from an unexpected direction. I had a visit from Victor Ling, a brilliant young assistant professor in the Dept of Medical Biophysics at Toronto. Victor was working on the problem of drug resistance in cancer. He had developed some interesting cell lines that displayed cross resistance to a number of anti-cancer drugs, and he had evidence that the cells had some sort of alteration in their membranes that prevented them from accumulating drug. Victor had heard of our surface labeling studies and asked us to look at his cell lines. We radiolabeled living cells, prepared membranes, and ran the membrane proteins on gels. I did the experiment

during the work week and then processed the samples over the weekend. I came in on Sunday morning to have a look and I was astounded to see a huge peak in the samples from the drug resistant cells that was not present in the controls. It was one of those rare Eureka! moments in science when you know you are on to something really exciting!



DISCOVERY OF THE P-GLYCOPROTEIN

CHO cell clones with different degrees of resistance to anticancer drugs had their cell surface proteins labeled with tritium using the galactose oxidase method. The membrane proteins were resolved by gel electrophoresis and visualized by autoradiography. A very prominent band (*) of 170 kilodaltons was observed in highly resistant C5S3 cells but was much less visible in non-resistant AUX B1 cells. Cells with intermediate drug resistance had intermediate levels of this protein. (adapted from Fig 1 of 990323)

Victor and I did additional experiments showing that a membrane glycoprotein of 170,000 MW was found in the drug resistant cells but not the controls and that the amount of glycoprotein correlated completely with the degree of drug resistance. Since the glycoprotein seemed to affect the permeability of the cells to drugs, we called it the P-glycoprotein. We thought this was a pretty exciting set of observations and tried to publish in a high impact journal. We tried NATURE, PNAS, JBC—all rejections. Apparently, the science establishment was not ready to accept this unexpected result from a couple of young, unknown researchers. We finally published in BIOCHIM BIOPHYS ACTA (BBA), a solid but not prestigious journal.

The modest BBA paper (990323) has now become a classic with about 3000 citations. Our P-glycoprotein turned out to be the prototype member of the large ABC family of membrane transporters that are not only important in cancer drug resistance, but that also play key roles in normal physiological processes such as maintenance of the blood-brain barrier. Victor and his colleagues went on to more fully characterize and eventually clone P-glycoprotein. That was the start of his distinguished career in cancer research that led eventually to him becoming director of the cancer center at the University of British Columbia in Vancouver.

Meanwhile I was becoming concerned about my career. I had a non-tenured faculty appointment in Medical Biophysics but I never really felt accepted. I had the sense that most of the senior faculty thought that I was a sort of glorified postdoc in the Rothstein group. So, even though Eve and I both loved our life in Toronto, I started looking for jobs in the USA.

HOUSTON (1978-86). Discovery of Integrins. Development of Liposomal Amphotericin B (Abelcet®).

I interviewed at several places but received the most enthusiastic reception from the Dept. of Pharmacology at the University of Texas Medical School at Houston. UT Houston was a brand-new medical school located on the campus of the enormous Texas Medical Center that also houses Baylor College of Medicine and MD Anderson Cancer Institute as well as several other major hospitals and institutes. Pharmacology was chaired by Al Robison an affable transplant from Alberta, Canada who had managed to pick up a distinct Texas drawl. Al had worked with Nobel laureate Earl Sutherland during the discovery of cyclic AMP and was quite well known in pharmacology circles. The department faculty included several relatively young and energetic scientists including Sam Enna, George Stancel, Peter Davies and Sam Strada each of whom would go onto a distinguished career

in research and in academic administration. The job came with an appointment as a tenure track associate professor and a very modest amount of start-up funding. I was impressed with this seemingly excellent professional opportunity. However, we loved living in Toronto, and Houston did not have a reputation for a high quality of life. Nonetheless, after much family discussion, we ended our six wonderful years in Toronto and moved to Texas.

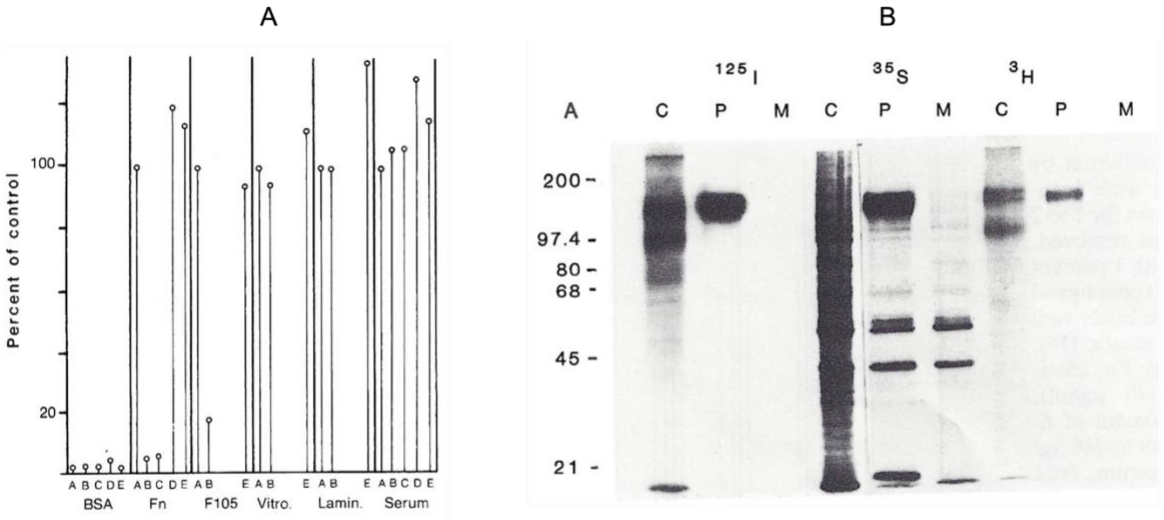
Patricia Harper, my first Ph.D. student, joined me from Toronto and we started to set up the lab. However, things didn't begin well. The first three NIH grants I submitted were all rejected. So, for a while we sat there with an empty lab and little ability to do experiments. After an initial period of profound depression, I went into grant-writing overdrive and fortunately the funding situation soon improved, and the lab started to function.

As in Toronto, we pursued two broad themes. The first concerned the role of cell surface proteins in cell-extracellular matrix (ECM) interactions and the second concerned liposomes and their possible therapeutic uses. Work on cell adhesion involved grad students Marty Schwarz and Esther Cheung and postdoc Patricia Brown, as well as Pat Harper. The liposome studies were led by postdocs Kapil and Reeta Mehta (a married team) and Ming-Jo Hsu and Mary Anne Kalp, two excellent technicians.

Cell-ECM Interactions/Integrins: the Discovery of the Fibronectin Receptor

At about this time, Richard Hynes of MIT and Erkki Ruoslahti of the Burnham Institute in La Jolla had identified fibronectin as a key protein in cell-ECM interactions. Pat Harper explored this topic by selecting cell mutants that were defective in various aspects of adhesion. In a series of studies, she showed that cells had two mechanisms of adhesion, one of which used fibronectin while the other involved additional (unidentified) ECM proteins. One of these studies resulted in my lab's second publication in NATURE (7207592). Marty, Esther and other members of the lab produced a number of nice papers on cell adhesion during this period, but the big question was- how do cells adhere to fibronectin?

DISCOVERY OF $\alpha 5\beta 1$ INTEGRIN (THE FIBRONECTIN RECEPTOR)



(A) Block of Fibronectin Mediated Adhesion. Adhesion of CHO cells to surfaces coated with different substances was measured. Monoclonal antibodies B and C (PB1, PB2) selectively blocked adhesion to fibronectin (Fn) or a fibronectin fragment (F105) but did not block adhesion to vitronectin, laminin or serum-coated surfaces. D, E are control monoclonals and A is untreated cells.
(B) Immunoprecipitation of the Fibronectin Receptor. CHO cells were surface labeled with 125I or metabolically labeled with 35S methionine or 3H glucosamine. Cell lysates were immunoprecipitated with monoclonal P2 . The 140 kilodalton band in the 'Pmnbvc' lanes is $\alpha 5\beta 1$, the fibronectin receptor. The 'C' lane is total cell lysate and the 'M' lanes are IPs with an irrelevant antibody. Adapted from Figs 2, 3 of 4012302.

Being in a pharmacology department meant that I went to lots of seminars that discussed ligand binding to receptors and subsequent signal transduction processes. I began to develop the idea that cell adhesion might involve specific cell surface receptors rather than just multiple non-specific interactions. This line of thought was supported by Ruoslahti's astounding discovery that a simple tripeptide sequence (RGD) was critical to the role of fibronectin in cell adhesion. So, we decided to go after the putative 'fibronectin receptor'. We did this using the

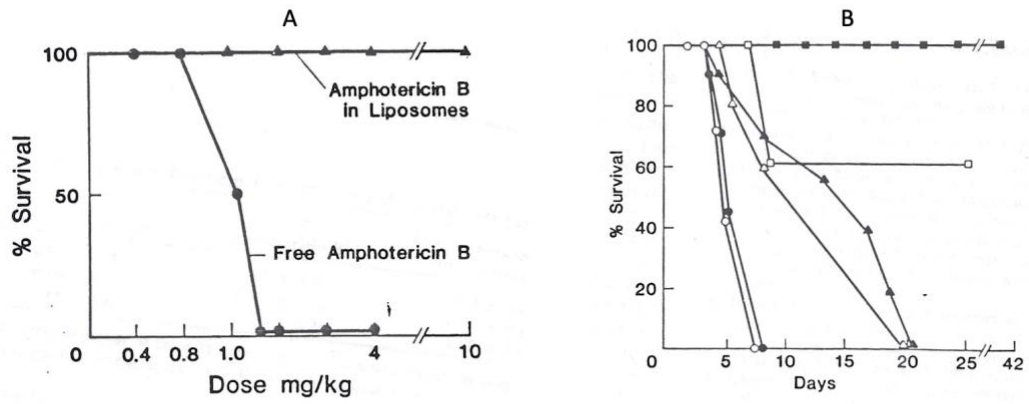
then new-ish technology of monoclonal antibodies. Pat Brown laboriously screened hybridoma clones for ones that produced antibodies that blocked cell adhesion to fibronectin, but not to other ECM proteins. We soon found antibodies that were exquisitely specific in their ability to block fibronectin mediated adhesion and further showed that they bound to a cell surface protein of about 140,000 MW. We published this study in SCIENCE (4012302). Almost simultaneously there was a study in CELL from the Ruoslahti lab that used affinity purification on fibronectin to identify the same protein. There was an additional report in CELL from Hynes and Ric Horwitz of Penn that also used antibodies to identify the fibronectin receptor and that went on to clone its cDNA. These three papers constitute the first identification of $\alpha 5\beta 1$, the fibronectin receptor, the prototype member of the large Integrin family of cell adhesion proteins. Eventually Integrins were found to play key roles in cell-ECM and cell-cell interactions in the immune system, in tumor biology, in blood clotting, in the maturation of the CNS and in many other contexts. As with the P-glycoprotein, our lab was there at the beginning of a whole new field of investigation,

Liposomes and Drug Delivery: the Development of Liposomal Amphotericin B

While this exciting work on cell adhesion was going on we were also steadily working on liposome technology. For example, I had developed a nice collaboration with Steve Regen, a professor in the Chemistry Dept. at Lehigh University, to study the properties of some unique polymerized liposomes.

However, our big breakthrough in the liposome area started with an unexpected visitor. Gabriel Lopez-Berestein was a young pediatric oncologist from MD Anderson Cancer Institute who wanted to talk with me about liposomes. He explained that the chemotherapy used to treat pediatric leukemia and lymphoma patients strongly impaired their immune function and thus the patients became susceptible to multiple infections. A particularly challenging problem was infection with fungal organisms such as Candida Albicans since the only really effective drug, Amphotericin B (AMB), was highly toxic to the kidneys. Gabriel explained that he would sometimes put a child's lymphoma into remission only to see the child die of a fungal infection. Gabriel wanted to know whether incorporating AMB into liposomes could make the drug less toxic to kidneys. We decided to take a crack at the problem and were soon joined by Roy Hopfer, a Ph.D. microbiologist from MD Anderson, and by postdocs Kapil and Reeta Mehta, to form a liposomal AMB team.

LIPOSOMAL AMPHOTERICIN B DISPLAYS A MAJOR THERAPEUTIC BENEFIT



A. Lipo AMB is Less Toxic Than Conventional AMB. Normal mice received intravenous injections of various doses of either 'free' (conventional) AMB (●) or liposomal AMB (▲) and survival was monitored.
B. Lipo AMB is as Effective as Conventional AMB. Mice were infected with Candida Albicans and then treated with various doses of 'free' or liposomal AMB and survival of the infection monitored. Doses in mg/kg. Free AMB 0.8 (▲), Lipo AMB 0.8 (△), Lipo AMB 2 (□). Lipo AMB 4 (■). The dark circles are untreated mice and the open circles liposomes only. Adapted from Figs 1,4 in 6842027.

I thought about the problem for a while, particularly in terms of how AMB might interact with various types of lipid bilayer membranes. Eventually I came up with a liposome composition that, at least hypothetically, would release AMB to fungal cell membranes but not to mammalian cell membranes. Initially, we tested this idea in cell culture to show that liposomal AMB (Lipo AMB) could kill Candida as effectively as AMB itself, but that Lipo AMB was

less toxic to mammalian cells (6372684, 6696909). That was a good result in cell culture: but what about the in vivo situation? Roy Hopfer proceeded to set up a model for systemic Candida infections in mice and the animals were then treated with conventional 'free' AMB or Lipo AMB. We were delighted with the results! The Lipo AMB was as effective as free AMB in treating the infection but was far less toxic (6842027). Our team also published several other interesting studies of liposomal AMB and other liposomal anti-fungal drugs in cells and animal models.

However, the big question was whether Lipo AMB would work in people. A situation arose that was both an opportunity and a tragedy. One of Gabe Lopez's patients was a little girl who was in remission for her cancer but who was dying of an aggressive Candida infection. Gabriel received permission to treat the child with liposomal AMB under a so-called 'compassionate IND'. We made a large batch of Lipo AMB in our lab, being as careful as we could to maintain sterile conditions. We put the material in an ice bucket and carried it over to MD Anderson hospital where Lopez used it to treat the little girl. Remarkably, after several treatments the fungal infection was controlled. Sadly, however, the child eventually died of her cancer. This direct lab bench to patient effort was very exciting, but it would be almost impossible to duplicate today because of ever-increasing regulations.

Following the single patient treated under the compassionate IND, Gabe Lopez and several clinicians from MD Anderson conducted a more comprehensive test of Lipo AMB in cancer patients. The 12 patients studied all had fungal infections that were not responding to conventional AMB. However, when treated with Lipo AMB 8 of the 12 showed complete or partial responses (3973417). These were quite remarkable results and our studies began to attract attention among oncologists and infectious disease physicians, as well as interest from the pharmaceutical industry. We had some discussions with Squibb, the producer of AMB, and at that time a major pharmaceutical company. However, large companies move slowly and we were frustrated with the lack of progress.

That period (late 1980s) witnessed the first explosive growth of the nascent biotechnology industry with lots of venture capital flooding into new start-ups. One of these was aptly named The Liposome Company (TLC) since its thrust was to develop liposome technology for therapeutic purposes. I was invited to be on the scientific advisory board of TLC and began attending meetings at the company's headquarters in Princeton NJ. During those meetings we discussed the work with Lipo AMB and eventually TLC decided to undertake pre-clinical development and clinical trials of the potential new drug. This was very gratifying since, despite the promise of our early studies, it would be a long road to attain FDA approval of Lipo AMB. Led by TLC's senior scientists Marc Ostro and Andy Janoff, development of Lipo AMB (now called Abelcet®) made rapid progress and was approved by the FDA in 1995. Although the market for antifungal drugs in immunocompromised patients is relatively small, Abelcet eventually reached sales of about $100 million per year. Abelcet® and the anti-cancer agent Doxil® were the first two FDA approved liposomal drugs and were the prototypes for a whole generation of 'nanomedicines' that are now in the clinic.

The development of Abelcet® was one of the most gratifying episodes of my career. Many years later, my younger son had become an M.D. specializing in infectious diseases. Now and then he would call me and say "Dad, I used your drug for one of my patients today." I was delighted and so grateful that I had had a chance to help people overcome a serious disease.

Although my scientific career was going well, I was getting tired of life in Houston. Despite the fact that we had made many friends there, we never became comfortable with Houston's sprawl and traffic and crime and its endlessly muggy weather. Besides that, I was developing an itch that needed scratching. In the back of my mind, I had always harbored the idea of following the path of my early mentor Aser Rothstein and becoming a departmental chairman. At UT Houston I had already begun performing some modest administrative functions including directing the pharmacology course for medical students and leading a joint UT-Baylor graduate program in pharmacology. Thus, I started looking for chairmanships and interviewed at several institutions. This led to some comical episodes as when I was considering a chairmanship in Buffalo and my wife threatened to leave me if I took the job! She had had enough of Buffalo our first time there!

Then the opportunity at UNC came along.

CAROLINA (1987-2015). Being a Chairman, Integrin Signaling, Delivery of Oligonucleotides.

I was invited to interview for the Chair of the Dept. of Pharmacology at UNC. They put me up at the Carolina Inn for a night's rest before the interviews. I left the hotel and walked around the campus on a luminous summer evening admiring the charming red brick buildings and the beautiful old trees. I was smitten- I knew that I could love this place. My meetings with the search committee were very positive. I was quite impressed by the committee members both as scientists and as really nice people and I think they were also a bit impressed with me.

However, there was a problem. I wasn't the first-choice candidate. That was a somewhat older and much more famous scientist from California. I went back to Houston expecting never to hear from UNC again. But after a few weeks the phone rang. It was Stuart Bondurant the Dean of the UNC Medical School who invited me for another visit to Chapel Hill. It seems that my more famous rival had opted to stay in California! Eve and I came to UNC, enjoyed Dr. Bondurant's kind hospitality, got to know the town a bit, and became totally convinced that this was the place for us.

Being a Department Chairperson

I started as UNC Pharmacology Chair on a part time basis in the autumn of 1986, while commuting almost weekly between Houston and Chapel Hill, and then started full time in January 1987. At that point Pharmacology was already a strong and nationally well-known department. It had been founded by Paul Munson who came down from Harvard with his then spouse Mary-Ellen Jones to trigger a lot of research energy at UNC. The immediate previous Chair, John Perkins, had also done an excellent job before leaving to take the Chair at Yale. There were several outstanding departmental faculty members with strong research programs including notably Ken Harden, Ron Thurman, Ken McCarthy, Gene Scarborough and John Gatzy. There were also several older faculty members with more modest activities and some faculty whose primary role was teaching including Barry Goz who ran the Dental Pharmacology course for many years, Ken Dudley who taught the pharmacology courses for the School of Pharmacy, and Curtis Harper, the only African-American faculty member, who did a lot of medical student teaching.

The first thing I did as chairperson was to organize a departmental retreat at a nearby hotel. I hoped that we would discuss our problems and emerge as one big happy family! Wow, how naïve!! There was lots of discussion, but Pharmacology was far from being a happy family. There were underlying concerns about fairness and how resources were distributed. Additionally, there was concern about me, as being both relatively young and also not a classically trained pharmacologist. Many of the faculty were disappointed that my Californian rival had opted out of the job. Clearly, I would have a lot of work to do to get the department running smoothly.

To make matters worse the long-serving departmental manager quit. She had been through two chairman and was not ready to deal with the quirks of a third. I quickly hired a new manager but that turned out to be a disaster. That person didn't think it was her job to help the faculty but rather to enforce UNC's rules to the max. Soon I had a parade of bitterly complaining faculty through my office. Fortunately, I was able to replace the errant manager with a capable young man named Chris Turner who took the situation in hand and started providing excellent service to the faculty, thus allaying many of their concerns. Chris stayed on as departmental manager for more than twenty very productive years and became my trusted ally and friend.

At the UNC Medical School, the Dean delegates a great deal of authority to departmental chairmen. That includes control of lab and office space, decisions on salaries, and assignment of teaching duties. With that authority comes a lot of responsibility. A good chairman can have a very beneficial effect on a department and its people, while a bad chair can cause chaos.

I hadn't had much training or mentoring in being a chairperson, but it was clear that there were some pretty basic concepts that I would need to follow in order to be a good chairman. First, the old Golden Rule of "Do unto others as you would have them do unto you" would be essential. Second, every chairman knows that it is vital to promote excellence in research and teaching. For me, an important part of that was to lead by example. I intended to be one of the most productive researchers in the department, to carry more than my share of the teaching load and

to do a good job with it. Third, a chair needs to be fair and be perceived as fair in terms of allocation of resources, salaries, promotions and all aspects of departmental life. Fourth, a chair needs to be accessible and to listen to the concerns of faculty, trainees and staff. Finally, a chairperson sometimes needs to put the department's interests ahead of his/her own. For example, to accept that departmental responsibilities may detract from one's personal research.

As I put these concepts into play over the next few years things began to go reasonably well. There were always problems of course, but in general the Dept. of Pharmacology became a functional and productive organization. In addition to the usual means of promoting intra-departmental cohesion, such as faculty meetings and a strong seminar series, we introduced some new social activities, including a Halloween party where labs competed for having the most outlandish costumes, and a X-mas season party. I particularly enjoyed the latter since I got to play Santa Claus. With the help of some pillows, my bony frame filled out a Santa suit and I had fun giving gifts to the little kids at the party!

One of the most important things a chairperson can do is to recruit excellent new faculty members. Fortunately, my start-up package included funds to hire five new junior faculty. My first recruit was Leslie Parise, a talented young biochemist who became an outstanding Pharmacology faculty member and later went on to chair the Dept. of Biochemistry & Biophysics at UNC. A second recruit was Rob Nicholas who has been a stalwart member of the Pharmacology faculty for decades. That early group of recruits also included Bob Rosenberg, Eric Lai and Barry Pallotta each of whom had productive years at UNC before moving on to other institutions. Over the next several years the department was also able to recruit Lee Graves, John Sondek and Channing Der, all outstanding scientists who have now been with UNC Pharmacology for many years. We also participated in important co-recruitments including Jude Samulski as head of the Gene Therapy Center and Fulton Crews as head of the Alcohol Studies Center. One of the most gratifying aspects of being a departmental chair is seeing the people you helped recruit advance and prosper in their careers.

Over the next few years our people worked hard, and a very good department became even better. UNC Pharmacology was consistently ranked in reputational surveys as one of the top two or three departments in the country, and for several years we had the highest NIH funding among departments of pharmacology. While there are always problems in running an academic department, overall, I was pleased with my efforts as chairperson and with the evolution of the department. The photo above shows PHCO circa 1990.

The guy on the left in the light pants, cowboy belt and plaid shirt is me. The gentleman seated on the left is Sven Toverud, a longtime PHCO faculty member, and the gentleman on the right is Paul Munson, the founding Chairman of the modern department. I won't try to identify the others. Most of them are long gone from PHCO, but you may recognize a few faces that are familiar.

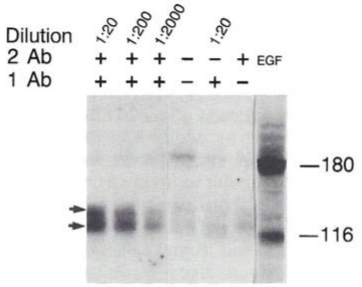
But while all this was going on there was still science to do.

After five years as Chair, I felt that things were going well but that my administrative duties were keeping me completely out of the lab. My bench science skills were getting rusty. To remedy that I asked Dean Bondurant if I could take a sabbatical and go work at the bench in another lab while still chairing the department remotely. Somewhat surprisingly he agreed, and I was able to arrange a six-month sabbatical in John Gurdon's lab at Cambridge University in the UK. At this point (1993) John was already quite a famous scientist. He was a member of the Royal Society and the Director of the Wellcome-CRC Institute for Cancer Research at Cambridge. John is an outstanding developmental biologist who had shown that nuclei from adult frog cells could give rise to intact animals when transplanted back into enucleated frog eggs. This finding basically gave rise to the entire stem cell concept, and in 2012 John shared the Nobel Prize with Shinya Yamanaka for discoveries about stem cells. I really enjoyed working in John's lab and interacting with his group of outstanding postdocs and brilliant Cambridge grad students. I also enjoyed typical British academic rituals like mid-morning tea accompanied by lively discussions of science. My lab project of trying to express cDNAs of human integrins in frog embryos didn't work out so well, but I still learned an enormous amount of cell and molecular biology in John's lab. Eve and I also enjoyed being in historic Cambridge and exploring the lovely English countryside. We were a bit sad to have to come home.

Integrins and Signaling

Back at Carolina I continued my investigations of integrins and their role in cell interactions. Now that it was clear that integrins were receptors, it seemed likely that they would have downstream signaling activity, so we began to look for that. Lori Kornberg, a post-doc in my lab, went to Shelly Earp's lab at the Lineberger Cancer Center to learn to do blots for tyrosine phosphorylation. We were excited to find that clustering of integrins on the cell surface using antibodies caused a sharp increase in the tyrosine phosphorylation of an intracellular protein of

INTEGRIN SIGNALING: CLUSTERING OF INTEGRINS ON THE CELL SURFACE TRIGGERS INTRACELLULAR TYROSINE PHOSPHORYLATION



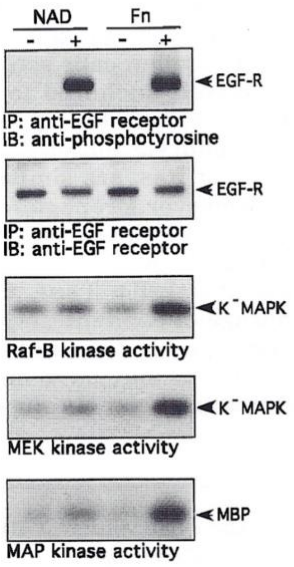
Human tissue culture cells (line KB) were held in suspension and treated with a monoclonal antibody that recognized the integrin β1 subunit, followed by a secondary antibody to promote clustering. Cell lysates were resolved on gels and then Western blots were probed with an anti-phosphotyrosine antibody. Increasing concentrations (lower dilutions) of anti-β1 caused an increase in tyrosine phosphorylation of a protein of approximately 130 kilodaltons (arrows). The pattern of tyrosine phosphorylation triggered by Epidermal Growth factor was completely different. Adapted from Fig 4 of 1717976 .

~130,000 MW. Clustering of other cell surface receptors did not do this. We published this finding in PNAS (1717976); it represented the first example of integrin signaling in nucleated cells. Meanwhile, Tom Parsons and

his colleagues at U. Virginia were working on a novel tyrosine kinase that was present at sites of cell-ECM adhesion; they called this protein Focal Adhesion Kinase (FAK). We collaborated with the Parson's lab and quicky showed that our 130 kDa protein was indeed FAK and thus that integrins activated FAK (1429685). Many labs went onto study the multiple roles of the integrin-FAK interaction in cell adhesion, migration, apoptosis and tumorigenesis, with hundreds of publications now in the literature.

These initial studies launched a series of investigations on integrin signaling in our lab that lasted over a decade. The studies involved several talented postdocs including Andrew Aplin, Alan Howe, Tsung Lin, Judy Varner, Carlos Rosales, Tonya Laakko and Vincent O'Brien as well as graduate students Quiming Chen, Sarah Short, Matt Edin and Jung Won Lee, and led to a large number of publications. Some important findings were: (a) that integrins could directly activate the key MAP-kinase signaling pathway (7929388); (b) that several integrins could modulate growth factor activation of the MAPK pathway (10531317); (c) that the integrin-dependent step in the signaling cascade was between the Ras GTPase and the Raf serine kinase (9082999). We first presented the Ras-Raf story at a 1997 Keystone meeting that I helped organize, much to the dismay of several other labs who were also working on integrin signaling but were not as far along. Additional key findings from our lab included a role for protein kinase A in integrin signaling (10980699) and the observation that integrin-mediated adhesion regulated the translocation of MAP-kinase from the cytosol to the nucleus (11309409). Our studies (and others) made it clear that integrins were far more than just 'sticky' proteins but rather were full-fledged cell surface receptors capable of engaging complex intracellular signaling pathways.

INTEGRINS MODULATE THE RTK-MAP KINASE PATHWAY



Serum-starved 3T3 cells were either held in suspension (NAD) or allowed to attach to a fibronectin coated surface (Fn). Cells were then treated with EGF (+) or not (-) and then lysed. Components of the Receptor Tyrosine Kinase to MAP Kinase signaling pathway were immunoprecipitated using specific antibodies and their kinase activities were measured using specific assays. As seen, cell attachment to fibronectin had little effect on the activity of the EGF-Receptor itself. However, it had major effects on the activation of the downstream kinases, indicating that the locus of the integrin effect on signaling lies between the EFG-Receptor and Raf, the first downstream kinase. Adapted from Fig 2 of 9082999.

In addition to work on signaling, the lab pursued many other aspects of integrin biology including roles in cell migration, ECM matrix assembly, tumor formation and vasculogenesis. The integrin deficient mutants we had isolated proved valuable in many of these studies leading to many publications. Some examples are: (1370495, 2531750, 1825619, 8314844). This work was ably done by postdocs Clara Schreiner, Melanie Sczekan and Yuri Danilov as well as by Jeff Bauer, an excellent grad student.

An interesting spin-off of the integrin story involved a cytosolic protein that we termed 'Nischarin', which means slow-moving in Sanskrit. We used a yeast two-hybrid approach to fish out proteins that bound to the cytosolic domain of the integrin $\alpha 5$ subunit. One of these was Nischarin, a large multi-domain protein (11121431) that turned out to have important roles in inhibiting the activity of certain protein kinases that affected cell motility (15229651). The Nischarin story was developed by postdocs Peter Redding and Suresh Alahari. Suresh went on to pursue further studies of Nischarin as an important part of his independent academic career at Louisiana State University.

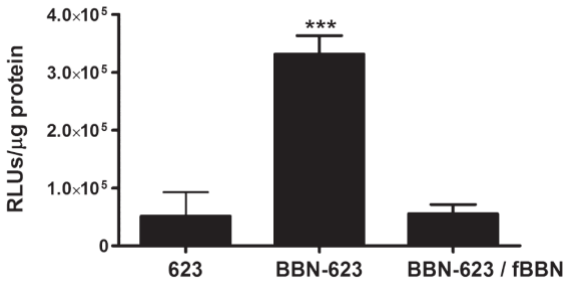
During those years our lab was a dynamic and generally happy place. We competed in departmental social activities including 5K runs, tennis matches, and the Halloween dress-up. Our nemesis was the Der lab who always seemed to beat us at those activities! Our integrin signaling studies also provided a good launching pad for career development, and several of our lab trainees including Andrew Aplin, Alan Howe, Jung Won Lee and Suresh Alahari went on to outstanding academic careers, while others pursued careers in the biotech industry.

Oligonucleotide Biology and Therapeutics

In the early 1990s the first wave of liposomal drugs was hitting the market. At Carolina I continued some liposome work with postdoc Sayed Daoud, however, I was getting bored with liposomes and wanted to do something else. At about this time the first studies on antisense oligonucleotides were being published. The idea that small snippets of DNA (oligos) could regulate gene expression with high specificity was exciting to both academics and to the nascent biotech industry. While there was great interest in antisense, little was known about the cellular uptake of oligonucleotides or about their interaction with membranes. We obtained oligos with different chemical characteristics from Mano Mahoharan, the chief chemist at ISIS (now IONIS) Pharma, the leading antisense company. Postdocs Saghir Akhtar and Yoko Shoji examined oligo interactions with cells and with lipid bilayer membranes (liposomes) (1658734,1945832). An important finding was that even oligos heavily modified with lipophilic groups could not diffuse across lipid membranes. This put to rest the then prevalent idea that making oligos more lipophilic would increase their intracellular delivery. A second key finding was that the major route of oligo uptake was active endocytosis rather than any sort of trans-membrane diffusion. These studies kindled a long-standing interest in our lab on the role of oligo uptake and intracellular trafficking in the delivery and pharmacological effectiveness of antisense (and later siRNA) oligonucleotides. A delightful side aspect of this work was that, many years after Yoko and Saghir returned to their home countries (Japan and UK), Eve and I were able to visit them and get to know their families.

Starting in the early 1990s our lab pursued a long series of studies on various aspects of oligonucleotide pharmacology including cell uptake, pharmacokinetics, hepatic metabolism, methods to enhance oligo delivery and (with chemist collaborators) the differential effects of various chemical modifications on oligo function. Several capable young investigators contributed to these projects including postdocs Jeff Hughes, Rob DeLong, Arno Nolting, Dong Xu, Anna Astriab, Dongju Ye, Hoon Yoo and technician Anna Avrutskaya. Particularly important were the manifold contributions of Mike Fisher our long-time lab manager and a skilled technician. We published many papers on these topics but their diversity make it hard to summarize them.

An important thrust of the lab was to bring ideas about receptor biology to the oligonucleotide delivery field. Postdocs Rowshon Alam and Osamu Nakagawa were talented oligonucleotide chemists who conjugated oligos to peptide ligands selective for certain integrins or G-protein coupled receptors. Xin Ming, a research asst. professor in our lab, led the way in characterizing the interactions of those conjugates with cells (18367474, 20551131, 20550198). Postdocs Hyunmin Kang and Kyle Carver were also involved. This was some of the



This assay makes use of a reporter gene (luciferase) that can be activated by intracellular delivery of an appropriate splice switching oligonucleotide (SSO)- in this case SSO623. Here PC3 cells incorporating the reporter have been incubated either with unmodified SSO623 or with SSO623 conjugated to a bombesin-like peptide (BBN-623). This peptide has high affinity for the bombesin receptor on PC3 cells, a G-protein Coupled Receptor. As seen, the BBN-623 has a substantially greater effect than the unmodified 623 and this enhancement is completely blocked by co-incubation with excess free bombesin peptide (fBBN) thus demonstrating receptor specificity. Adapted from Fig 2 of 20551131.

earliest work on receptor-selective delivery of oligonucleotides, currently an important aspect of the oligo therapeutics field. However, I then made a big mistake. I let myself be satisfied with our nice cell studies and failed to push on to the in vivo experiments that are always most important for therapeutic development.

Our studies on receptor-mediated uptake of oligonucleotides by cells led to an interest in their subsequent intracellular trafficking. Work from our lab and others had shown that after uptake by endocytosis, oligos trafficked through the various endomembrane compartments, but most of the oligo (~98%) was retained in those compartments and failed to reach the cytosol or nucleus. We started thinking about ways to improve the effectiveness of oligos by promoting their release from endosomal compartments. In collaboration with Bill Janzen, then at the UNC Drug Discovery Center, we undertook a high throughput screen to look for small molecules that would release the oligos. Postdoc Canhong Cao and technician Bing Yang laboriously screened more than 150,000 compounds and eventually found two compound families that we termed OECs (oligo enhancing compounds). In cells, these small molecules dramatically increased the effectiveness of antisense, splice-switching or siRNA oligos (25662226, 28703575). In collaboration with Silvia Kreda of the UNC Cystic Fibrosis Center we also showed that the OECs could be active in vivo, particularly in lung tissue (34107015). These observations raised the possibility that the OECs might be a valuable tool for improving oligonucleotide-based therapeutics and thus might have some commercial potential. More on this later.

In the late 1990s people were getting interested in the idea of designed transcription factors that could selectively regulate genes of interest. This would be very complementary to gene regulation by oligonucleotides but using proteins rather than nucleic acids. The designed transcription factors were created by using combinatorial library methods to find polypeptides that would bind selectively to particular DNA sequences. I decided to jump into the designed transcription factor field- but that turned out to be another mistake. Talented postdocs Victor Bartsevich, Dietmar Falke, Vidula Dixit and Dong Xu, as well as Xiaojun Cheng, an exceptional graduate student, worked hard on the project. They produced several excellent papers, including one in PNAS (9391163,10860921, 18426856, 12869633). However, we were outcompeted by larger, richer labs from places like MIT and never got any traction in the field.

As with our integrin studies, our work on oligos provided a good base for career development. Some lab members went on to academic careers, others went to industry. Notably Jeff Hughes and Rob DeLong did both, becoming tenured faculty members as well as having productive careers in the biotech industry.

Life After Being a Chairperson. The Roadmap, the CCNE and the School of Pharmacy.

By about 2000 I was tiring of being departmental chairman. I felt I had done all I could for the department and that some new resources were needed. Typically, the only way a medical school basic science department gets resources is when a new chairperson is recruited. Thus, I asked the Dean (then being Jeff Houpt) to search for

a new chairperson. The process took a while but in 2002 UNC was successful in recruiting Gary Johnson, an outstanding scientist from U. Colorado. Gary stayed on as PHCO chair for about 14 years, doing an excellent job, while continuing his pioneering work on signaling by protein kinases. The Pharmacology chairmanship has now passed to Henrik Dohlman who is also doing a great job and continuing his excellent research on G proteins.

My post-chairmanship life still involved quite a bit of academic administration and leadership. I got pulled into leading UNC's responses to two new initiatives from the National Institutes of Health. One was called the 'Roadmap' with the goal of promoting more inter-disciplinary interactions among medical researchers. The inducement of course was money, with a special pot being set aside for Roadmap grants. With the help of an able administrative assistant, I went around campus talking to clinicians, basic scientists, public health people, even dentists—trying to get them to form inter-disciplinary teams and apply for the Roadmap grants. Eventually UNC got more Roadmap grants than any other institution, but whether my efforts had anything to do with that is hard to know.

The second and more substantial task was to help UNC respond to a huge new opportunity from the National Cancer Institute. In 2004 the NCI had just announced its Centers of Cancer Nanotechnology Excellence (CCNE) program that was intended to bring the power of contemporary nanotechnology to bear on cancer diagnosis and treatment. Each Center of Excellence would be supported by a very large multi-investigator grant of about $4-5M a year for 5 years. Obviously, the competition for these grants would be fierce. UNC had several excellent nanotechnologists including Joe Desimone and Mike Ramsey in Chemistry and Otto Zhou in Physics. Additionally, there were many excellent cancer researchers, both basic scientists and clinicians, at the UNC Lineberger Cancer Center. The problem was that the nanotechnologists and the cancer researchers literally did not speak the same language!!—they couldn't communicate with each other. An NCI Center grant can't just be a hodgepodge of individual projects; it has to be an integrated, coherent entity. It fell to me to translate between the physical and biological scientists and to write the sections of the proposal that integrated the various parts. Things worked out and UNC joined Stanford, Northwestern, Harvard-MIT and UCSD-Caltech as a CCNE awardee. I served as Principal Investigator for the Carolina CCNE for 5 years. On a second iteration of the program Joe Desimone served as PI, and on a third Leaf Huang from the School of Pharmacy. The CCNE program brought several tens of millions of funding into UNC and supported years of outstanding research. I was pleased to have been part of its inception.

In 2008 I was asked to apply for the position of Associate Dean for Research and Graduate education at the UNC Eshelman School of Pharmacy. I had some concerns about the position since I was 67 at the time and had been doing academic administration for a long time. However, Pharmacy was in a rapid building phase and I thought it might be fun to help shape that process. Unfortunately, shortly after taking the job the 2008 recession hit with inevitable cuts to UNC budgets. Thus, instead of building up research at the School I spent my time dealing with all sorts of bureaucratic problems caused by budgetary restraints. Additionally, my relationship with Pharmacy Dean Bob Blouin was less than ideal. While I respected Dean Blouin's considerable abilities, our personalities were poorly matched. In 2011, by mutual agreement, I ceased being Associate Dean and returned to being a regular faculty member. I continued research and teaching at UNC until 2015 when I closed my lab and became a professor emeritus.

Initos (2015-2023:) The Last Chapter

While closing the lab at UNC I started one last push in science. It seemed to me that our OEC compounds might be able to improve many types of oligo-based therapeutics and thus might have commercial potential. Bill Janzen and I co-founded a small company called Initos Pharmaceuticals LLC with the intent of further understanding and developing the OEC technology. We were able to successfully complete for several NIH SBIR (small business) grants. We started by leasing so-called 'incubator' space from the School of Pharmacy but later moved to commercial incubator space at Biolabs in downtown Durham. We conducted a series of experiments in mice to evaluate the effectiveness and the potential toxicities of the OECs. We had some success, as with the lung studies with Silvia Kreda mentioned above, but we were disappointed to find that the OECs were less effective in other organs and that they had considerable toxicity. We needed to do chemistry to make new analogs of the OECs that would be less toxic. However, that requires a great deal of money, and we were never able to obtain

the venture capital investment needed to do the chemistry. Thus, as with many biotech start-ups, initial promise turned to disappointment. In 2023 I closed Initos thus bringing my 50+ year career in science to an end.

Perspective

In looking back at my long journey in science I remember good projects and not so good ones, good experiments and failed ones, all part of the complexity that makes doing science so challenging but yet so rewarding. Naturally I feel that I could have, should have, achieved more in research. Perhaps I made a fundamental mistake by dividing my efforts between integrins/signaling and liposomes/oligos/therapeutics. Certainly, that choice meant I had less impact on each field than if I had concentrated on one. However, I have always enjoyed doing both very basic work and therapeutically oriented work. Fortunately, I was able to maintain funding in both areas over many years and to recruit excellent students and postdocs. Thus, if I had the chance to do it all over again, I would want to do it the same way.

However, my career path would be difficult for a young investigator to replicate today. I pursued a very individualistic approach to doing science. I didn't go in the same direction as other investigators in the field and, while I had some collaborations, I didn't actively seek interactions with others. I also did not have an explicit disease focus in my research. Although some of it was relevant to cancer, my work was not strictly cancer research, nor was it directly connected to other diseases. In today's ultracompetitive biomedical research arena, it is clearly important for young scientists to network extensively, to be highly aware of what is going on in their field, and to seek opportunities for collaboration. Contemporary research has gotten so complex that it is almost impossible to have the resources to go it alone. Linking one's work to one or more diseases is also important. The NIH is still organized into disease areas and thus being connected with a particular institute, as well as with non-federal funding organizations like ACS or AHA, opens doors in terms of invitations to meetings, study sections, and editorial boards. The challenge for young scientists today, I think, is how to maintain their individual creativity in a scientific world that is increasingly driven by multi-investigator collaborations and by compilation of massive data sets.

At a mid-point in their careers many scientists consider the possibility of going into academic administration. That is a good choice for some but could be a quagmire for others. I don't consider myself to be a 'people person' but fortunately I was able to navigate the complex interpersonal interactions involved in running an academic department. I thoroughly enjoyed the sense of helping to build department, the feeling of participating in running the entire medical school, and pride in seeing the people I recruited advance in their careers. Being enmeshed in academic administration no doubt took a toll on my personal research, but in retrospect I feel that it was well worth it.

I hope this rather lengthy 'story' may be of interest to some young scientists. If so, it was worth the effort to write.