Cystic Fibrosis
A Disease of Mucus Dehydration

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Richard C. Boucher Jr., MD, the 2008 Norma Berryhill Distinguished Lecturer, is recognized internationally as a preeminent leader in cystic fibrosis research, having made a series of seminal discoveries and pioneered the development of important therapies. He has built at the University of North Carolina a world center for pulmonary disease investigation and clinical care.

Dr. Boucher is William Rand Kenan Professor of Medicine, director of the UNC Cystic Fibrosis Pulmonary Research and Treatment Center, and codirector of the UNC Gene Therapy Center. He is former chief of the Division of Pulmonary Diseases, Critical Care, and Occupational Medicine in the Department of Medicine.

A native of Miami, Florida, Dr. Boucher received his undergraduate degree in psychology from Yale University and his medical degree from the Columbia University College of Physicians and Surgeons. His internship and residency in medicine at Columbia Presbyterian Hospital were followed by a cardiorespiratory residency at Royal Victoria Hospital in Montreal, a research fellowship at McGill University in Montreal, and a fellowship in the Department of Medicine at Royal Victoria Hospital.

Dr. Boucher joined the UNC School of Medicine faculty in 1977. He was recruited by Philip Bromberg, MD, then chief of the Division of Pulmonary Disease, Critical Care and Occupational Medicine, and now Bonner Professor of Medicine and scientific director of the Center for Environmental Medicine, Asthma, and Lung Biology. Dr. Bromberg was looking for a new faculty member with a particular interest in ion transport by epithelial cells. That described Dr. Boucher, who was eager to learn more sophisticated research techniques than were available in Montreal. At the time, UNC was already a leading center for salt transport research.

“"I came to Chapel Hill because there were two or three questions I wanted to solve, and then I planned to go into private practice," Dr. Boucher recalls. However, the more he became involved in the research and the more questions he answered, the more he found that he wanted to know about. Within three years, his work was focused primarily on cystic fibrosis.

“"Since then, it has been a continual push to understand how a defect in a gene could lead to a defect in a protein that could lead to a defect in salt and water transport that could lead to persistent bacterial infection in the airways," he says. "The big questions
were, what were the links connecting those pathways, and, ultimately, could we do anything about it?"

As recently as the early 1980s, it was not known whether cystic fibrosis was caused by a circulating blood factor or a problem intrinsic to the epithelial cells that line the airways. Dr. Boucher was the first to show definitively that it was not a blood-borne disease.

Ten years later, in 1992, he was a leading member of a group at UNC that created the first cystic fibrosis mouse model. Heading the project was Oliver Smithies, DPhil, Excellence Professor of Pathology and Laboratory Medicine and a corecipient of the 2007 Nobel Prize in physiology or medicine.

“There were 13 or 14 groups in a race to be the first to make the mouse model,” Dr. Boucher says. “Oliver and Bev Koller of his group made the mouse, and we characterized it. It has been very useful ever since.”

In the mid-1990s Dr. Boucher and his team discovered the extracellular signaling functions of adenosine triphosphate (ATP) in the lungs. Epithelial cells secrete ATP onto the lung surfaces, where, in Dr. Boucher’s description, “it acts as a master coordinator to keep the surfaces of the lungs properly hydrated.”

The discovery of ATP led to the inception of Inspire Pharmaceuticals for the development of a drug that mimics the function of ATP in the lungs of cystic fibrosis patients. That drug is now in the final stage of clinical studies.

Only within this decade, with the advent of the confocal microscope, have researchers truly begun to understand that mucus, a component of the thin film of fluid on the lining of the airways, must be perfectly hydrated to function effectively as the body’s primary defense against airborne threats. Disturbances of the complex interplay of salt, water, and mucus can cause the mucus to dry out and become so thick and sticky that it prevents the mechanical clearance of deposited materials from airway surfaces. Instead, the thick mucus molecules stick to the lung surfaces, where they become the perfect growth medium for bacteria.

“We now understand all of that at a level of detail that I never would have thought possible,” Dr. Boucher says. Much of that understanding, he says, derived from the collaborative efforts of the so-called Virtual Lung Group at UNC, which includes physicists, polymer biochemists, and applied mathematicians as well as medical researchers.

Disruption of the lungs’ self-cleansing mechanism plays a critical role not only in cystic fibrosis, but also in the development of other airway diseases, including chronic bronchitis and emphysema. In 2006 Dr. Boucher and his team developed the first specific therapy for cystic fibrosis. Given the complexities and highly sophisticated techniques of cystic fibrosis research, the simplicity of that therapeutic breakthrough was ironic. “It was an inhaled hypertonic saline solution—just very salty water—which probably costs no more than 10 cents a day,” Dr. Boucher says. “The concept is that the concentration of salt in the inhaled saline is much higher than in the blood, so it draws
water from the blood tissues into the airway secretions and rehydrates them.” Developed as a trial therapeutic concept, inhaled hypertonic saline therapy proved so effective that it is now widely used by cystic fibrosis patients in the United States.

Introduction of that therapy was followed closely by the development of a dry, powderlike molecule that also draws water to the airway surface. Another agent, which blocks sodium channels, has shown benefits in short-term studies. In all, the work of Dr. Boucher’s lab has led to the development of four different cystic fibrosis therapies in the past two years. All four are designed to hydrate and lubricate the airway surfaces and to thin the mucus.

Dr. Boucher also has led the search for previously unknown bacteria that can thrive in environments with almost no oxygen. He found that when mucus becomes very thick, it actually starves the airway surface of oxygen. That discovery brought the need to understand how bacteria can live in that mucus and to develop new antibiotics to kill them. “But at the end of the day, if we can rehydrate the mucus, the bacteria will come out,” Dr. Boucher says.

James Hogg, MD, PhD, emeritus professor and former director of the Pulmonary Research Laboratory at St. Paul’s Hospital of the University of British Columbia in Vancouver, trained Dr. Boucher at McGill University and has not been at all surprised by his success. “He is one of those people who, if you show him how to do something once, the next time he will do it better than you,” Dr. Hogg says. “Of the 100 or so fellows I have supervised or worked with in my career, there were maybe 2 or 3 I could spot right away as being smarter than me, and Ric was one of them.”

Robert Beall, PhD, president and CEO of the Cystic Fibrosis Foundation, describes Dr. Boucher as “a great basic scientist who is always looking to translate what he learns into new therapies. He has made major contributions to the field of cystic fibrosis, and he currently has drugs in the pipeline that could ultimately make an important difference in treating the basic defect.”

Dr. Bromberg calls Dr. Boucher the epitome of the academic physician. “On one hand, he is a scholar. On the other, he is a physician who wants to bring the bench to the bedside. He is always focused on the therapeutic possibilities. He operates at a level that is achieved by very few people. He combines intelligence, practical skills, charisma, the ability to recognize top notch talent, and the ability to motivate and galvanize people. All of those skills combined in a single individual give him a quality that is not often encountered.

“Ric always seems to be two jumps ahead of you, no matter what you are talking about,” Dr. Bromberg says. “He has thought about it. He knows the literature. He is able to talk to anyone, no matter how advanced they are in their careers, pretty much on their level and contribute to their thinking. He also has the ability to bring together people with all kinds of different skills to work in teams. He has translated that into many millions of dollars in research grants and has been very successful in obtaining private funding.”

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Dr. Boucher has held a number of editorial positions, including, for the past four years, lung section editor of the Annual Review of Physiology. “To be able to do that means you know and really understand what all the contributors are doing,” Dr. Bromberg says. “That is an example of Ric’s ability to master different fields. He has been able to keep up with modern cell biology, which is very complicated and moves ahead in leaps and bounds.”

Dr. Boucher has received numerous honors and awards, including the Cystic Fibrosis Foundation’s Doris F. Tulcin Cystic Fibrosis Research Award for 1989 and the Paul di Sant’Agnese Distinguished Scientific Achievement Award. In 2007 he received the Cystic Fibrosis Foundation’s first annual Champion for a Cure Award.

Dr. Boucher is known as an excellent teacher and mentor. While one part of mentoring is teaching the individual, the other part, as he sees it, is making sure the group effort is consistent with its goals. “One likes to see teams that have been created to solve a problem work creatively, efficiently and productively,” he says. “I have been blessed with a huge number of really great postdocs.”

Dr. Beall says Dr. Boucher has created at UNC “a great training Mecca” for both researchers and clinicians. “There are many wonderful, first-class people who have come through his program, a number of whom have stayed in Chapel Hill.”

Dr. Boucher continues to see patients, though not as often as he would like, on hospital rotations and in the Cystic Fibrosis Clinic. “Seeing patients is always stimulating and rewarding. It helps keep you oriented, and it teaches you what you should be thinking about.”

Over the years, Dr. Boucher has had many opportunities to move to other world-class medical centers, but he has chosen to remain at UNC. “This is an easy place to work, and that keeps a lot of people here,” he says. “It has always been very open, collaborative, and friendly; but it also is an efficient place to work. And as you go along in your career, you accumulate more and more colleagues whom you really value. I have always felt that our goals were best served by staying here, and I have never regretted it.”
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Well, thank you very much. It’s always an honor to deliver this kind of talk. It’s also a pleasure, because CF is one of these diseases that, the more you know about it, the more you’re going to be optimistic about it. I like giving lectures on CF because I like people to understand what the disease is about and what we can do about it. Thank you for the nice personal words, but I actually accepted, I think, delivering this lecture in the context of the CF Center and everybody in it, because it’s been, as I’ll show you, a collaborative effort. One thing I did learn, though, is that when you accept delivering a lecture like this, for the two or three weeks before you do it, you get all kinds of good advice on how you should do it. Harold Roberts, who has been through it, suggested that I go over the history of the CF Center, but I’m not sure I’m quite to that point yet. Bob Marriott thought that I should be funny for 35 minutes. After watching Obama and McCain doing the roast for the Alfred E. Smith Memorial Foundation in New York last month, I thought that it might be a good idea, but they had better writers than I do. However, the person who got my attention was Jim Bryan. Jim just came up and said, “I want you to tell me what’s going on in CF, and can I be hopeful?” For those of you who know Jim, he is one of the great humanitarian faculty on campus, but he also has a special interest in CF, with a grandchild with CF. So that was the suggestion that resonated with me, and that’s what you’re going to get. I’m going to try and tell you, in a way you’ll understand it, the science behind CF, as I’m most comfortable talking about science. But I’d also like to transmit to you the unbelievable optimism for this disease that we currently have, and how that has changed in last 20 years or so.

So I hope I won’t be too detailed, and I’ll try not to be too quick, but it’s one of these diseases that’s not that hard once you break into it. In brief, CF is a multisystem disease, and it affects, basically, all of the lining tissues of your body. It affects predominantly the lungs, as we’ll get into, but also the gastrointestinal tract, the pancreas, the reproductive tract, and the sweat glands. It’s the most common—I think we now use the word “potentially”—lethal genetic disease in North America and Europe. CF is the
disease that was diagnosed early by mothers by kissing the foreheads of their children and tasting the salty sweat. The CF gene was identified in 1989 by a consortium of essentially three people, two of whom now have very close ties to UNC. The first is Francis Collins, who actually trained here. When I first came here from New York as a moderately arrogant New Yorker, Francis was the first clinical fellow that I had down here, and I thought, “Wow—this place is really good!” Now, we also have Jack Riordan, who was one of the troika. Jack joined us through one of the Hooker fellowships about three years ago, and I’ll mention a bit of Jack’s work later. So, we’re basically two-thirds of the way to getting all the people here.

The CF gene—and again, CF is a genetic disease—codes for a protein. As I’ll show you, this protein acts as a channel that moves salt through membranes, and it regulates other channels that do this as well. The unfortunate statistic is that 95 percent of CF patients die of chronic lung disease. This is a terrible lung disease, because it is a chronic bacterial infection of the airways. It’s like having a cold in your chest, but a severe one, every day of your life. I’m an adult pulmonologist, and so CF lung disease was the target of our group. Indeed, CF lung disease has really been the mantra of the CF Center, which on one level is a center in the classic sense of the university, but in truth, it is really a research and development group designed to understand CF lung disease and cure it.

So, where do we stand now with CF? The CF survival curve, as a function of age, is shown in Figure 9.1. The year 1988 is about the time we started the CF Center here, when the average life expectancy was about 26. I think you can see, even though you can draw the line different ways, that there’s been a real improvement in the lifespan of CF patients over the last two decades. The nice thing about it, if anything, is that survival seems to be accelerating. So, the good news is that we’re doing much better; the bad news is that survival ends at 36–37 years, not 77 years. The other leavening point in this analysis comes from examining CF patients’ lung function: 100 percent is best; 30 percent is where you end up being transplanted. The point is that, after the mid-teens, even though you’re living longer, your lung function is not good. Consequently, CF patients have to be made better with respect to lung function so that they feel better throughout their lives, as well as live longer. So that’s been the tack that we’ve taken. A key message in all of this will be that, unless you really know what you’re doing, it’s very hard to be inventive in the clinic and do things that are novel and effective for your patients.

The problem with CF in the most fundamental terms is that it reflects a failure of one’s own innate defense against inhaled bacteria depositing on your lungs. In the next 40 minutes or so, you are going to inhale in this room about 6,000–10,000 bacteria, you’re going to clear them all from your lung, and you’re never going to know it. So you have terrific innate defense against bacteria. CF kids do not. And so the question is, then, What leads to the failure of lung defense, which is the result of an abnormal CF gene and its abnormal protein product, called “mutant CFTR”? Over the past de-
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Cade or so, there has been something in the field that has been called the salt wars. It reflects two very different hypotheses that attempt to describe how the lungs in CF patients fail in bacterial defense. In the first, shown on the right in Figure 9.2, which is a midwestern version of CF pathogenesis, the notion is that normal airways lining cells, the epithelium, absorb salt, but not water, just like the normal sweat duct does. When you do that, you allow a group of antimicrobial proteins to protect the lung. If CF children can’t absorb the salt from their airway surfaces, like in their sweat glands, these antimicrobial proteins are inactivated by the high salt concentration. So the notion is, the chemical shield that protects your lung normally against infection fails in CF. Now, there are a lot of reasons this hypothesis probably isn’t true, other than the fact the physiology isn’t right. However, it doesn’t make sense in an evolutionary way, in that, as you all know, if you take antibiotics over and over again, you become resistant to them. So, on first principles an antimicrobial shield is probably not the way we defend our lungs, and hence this mechanism isn’t pertinent in CF.

The more attractive hypothesis, from the point of view of physiology and evolution, is shown on the left in Figure 9.2. This hypothesis posits that clearance of bacteria from the lungs is mediated by a mechanical process. This mechanical process involves the force supplied by cilia to move a sheet of mucus—a material a bit like flypaper—

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from the lung. The mechanical clearance of mucus traps and sweeps the 6,000–10,000 bacteria out of your lung, as we talked about a bit before. If you have CF, you remove too much salt and water from airway surfaces, you make the mucus sticky, and it no longer is cleared from the lung normally. This is called the hydration hypothesis; we are normally hydrated, while CF patients are not normally hydrated.

So, on a larger scale, this cartoon (see Figure 9.3) depicts your airways on the left, i.e., these are the lining cells of your airways, your bronchial tubes. The important function of these airway cells from a cleansing point of view is to move this mucus layer that Dean Roper talked about that traps bacteria—and, as I will show you, this is a very robust and dynamic system. Indeed, you move mucus pretty quickly, because you don’t want trapped bacteria in your lungs very long. Now the key for making this system efficient is to have sufficient water in the mucus. You have to have about 98 percent of mucus to be water, and only 2 percent solids, in order for this system to work. And as I’ll show you, CF is a disease that illustrates that we have very critical hydration tolerances with regard to the difference between health and disease.

The way you hydrate mucus is much like the way you control your total body salt and water with your kidneys. It is the same principle. Just as there is active transport of salt with the kidney to move water, it is the same with your airways. Because it is so important to have just the right amount of salt and water on the airway surface, you have opposing, balancing salt transport systems. You have a system that is mediated by an ion channel, called the epithelial sodium channel (ENaC), that allows you to remove salt from the surface; and, most important, you have a channel—in this case it’s the CFTR protein channel—that allows you to add salt to the surface. So it’s like you have your foot on the accelerator and the brake all the time. It is the net amount of salt on the surface that dictates the amount of water on the surface, as water moves through water channels in response to the mass of salt on the surface, determined by these transport systems. The key fellow here is the cystic fibrosis transmembrane conduc-
tance regulator (CFTR) protein. It sits right at the interface between you and the outside world, and it controls, by virtue of its functions as both a regulator and mediator of salt transport, the amount of water on the surface.

CF, on the right in Figure 9.3, is a disease caused by mutations in the CFTR gene, and they lead to problems in the protein. Just to give you a feel for it, the normal CF gene codes for a protein that is about 1,480 amino acids long. The most common mutation in CF is a three base pair, three nucleotide, “three letter” deletion that means you’re missing just one amino acid. So, 1,480, you’re healthy; 1,479, you have CF. As Jack Riordan has shown, if you’re missing this one amino acid, the protein bends. Cells don’t like bent proteins, and they chew them up and don’t allow them to go to the membrane. So in this case, cystic fibrosis is caused by a lack of a protein at the membrane. As a result, you can’t secrete salt and water to the lumen, you can’t control the excessive removal of salt from the surface, and what happens is that you strip all the water from the airway surface. This deficiency in regulation of salt transport leads to a removal of the watery layer around the cilia, so they collapse onto the cell surface. It also leads to a concentration of the mucus so it becomes sticky, and now it adheres to the surface of the cell. And so this is the problem in CF: you get adhesion of mucus to your airway surfaces. It’s a bit like having a splinter under your skin. You have a “foreign” body there that should be cleared from your body, but it’s not, and infection typically results.

So why was it so hard to understand this in the lab, and why has it taken us so long to understand this? The problem is that when we’re talking about these mucus layers, we’re talking about the thin films of liquids, either mucus or water, that are less than 1/1,000th of an inch thick. Your lung does not want to have thick layers of mucus on the surface, because air is supposed to get in and out of your lungs easily, so you don’t want to impose a lot of work on your lungs, moving air through narrow tubes.

So Figure 9.4 depicts the system that helped us understand what the nature of this disease was about. This is the cell culture system brought to us by Dr. Scott Randell

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from the National Institute of Environmental Health Sciences, and this is what the lining cells of your airways look like in culture, much like they do in health and in life. These are the cells that have those little hairs on the surface that move the mucus layer, called cilia, and they beat at about 10–15 times per second to move mucus along. There are the cells that secrete the “flypaper,” or the “mucins,” that trap the bacteria. The ciliated cells are the cells that move the water across the airway surface. Now, we can study these cells and how they hydrate the surface in a system that is much like a CT scan. You can cut through these airways, and you can actually look at the cells, and if you like red stains, you can stain the airway surface liquid with a red dye and measure the hydration properties of the surface online in living preparations. If you want to study the mucus properties, you can add little beads, and there are little popcorn-like beads in the figure that are about the size of bacteria. They are trapped in the mucus layer. If you look down at the culture laden with beads, this is what mucus looks like on your airways now. The amazing thing about these culture systems is that they actually not only look like airways, but function like airways. Indeed, they hydrate the surface well enough so that they actually transport mucus in a rotational way. If you just open the shutter on a microscope for five seconds, you can watch these mucus “hurricanes” move.

The CFTR protein, at the interface between you and the outside world, functions as the master effector of all hydration on the airway surface.

Mucus clearance is a dynamic cleansing system. If you’re looking down at one of these cultures with a very simple microscope, you can watch the mucus move. If you look at the edge of one of these mucus blankets, you can see how the mucus, in a very vibrant fashion, cleanses the nooks and crannies of your airways in a very efficient fashion. Any bacteria and any fungi inhaled into the lung are trapped by this mucus and swept out of your lungs. So it is a system that is perfectly configured for health.

Figure 9.4. The in vitro model system that enabled the study of airway surface liquid. Upper left, routine histology; upper right, x–z confocal image; lower left, en face view of mucus decorated with beads; lower right, rotation of mucus layer

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The way we study this system quantitatively was pioneered by Rob Tarran, who is here. Basically, Rob puts these cultures in a confocal microscope, where we can measure ASL heights with this system, and we can measure whether or not this height mediates efficient mucus transport. If you put a little bit of extra fluid on a normal culture, it doesn't like it, so it absorbs it, but then it finds the level that it thinks is about perfect, which is about 7 microns. Again, that's less than \( \frac{1}{1000} \)th of an inch tall. The reason it likes it is that if you look at it, the cilia can stand up straight, so they can move mucus in a very efficient fashion. So this is the physiologically adaptive height of liquid on the airway surface. And if you measure mucus transport, normal cultures transport mucus efficiently.

In contrast, CF cultures absorb the added fluid more rapidly, and they don't know how to stop. The cells absorb all the fluid, and now the cilia can't beat anymore: they are essentially just lying on the airway surface just sort of vacillating, trying futilely to move mucus. If you measure mucus transport, they can't do it.

If you look down on a normal culture, you see it can move mucus nicely over its surface. The amazing thing about us, i.e., normal people, is that we know how to regulate the amount of water on the airway surface. And you can do it for days and days and days in culture and, obviously, throughout your whole life in your lungs. Now, in contrast, if you have CF, if we add, at the beginning of this experiment, essentially all the water that you need to hydrate the surface, the CF cultures clear mucus very well. So the cilia work well, the mucins work well; as long as the surface is hydrated, things work fine. But if you wait 24 hours, the CF cultures don't know how to move mucus anymore. They anomalously and aberrantly absorb all the salt and water. Now you begin to get a feel for what the real problem is in CF: that is, mucus sticking to airway surfaces. We call this a mucus plaque or mucus plug. Now, it may sound sort of benign, but if you section through them, again, with a different kind of cutting system, and look at them with a different type of staining system, this is what those mucus plaques look like. They glow with a green fluorescence. We call this plaque stained as such “beautiful but deadly.” This is the site of the infection in CF patients, and this is the site of the inflammation that bothers CF patients. And so, if you look at normal cultures, bacteria would be floating effortlessly on this kind of mucus, and in you and me, it would be cleared. In CF, the mucus is stuck, and the trapped bacteria will grow in this adherent mucus plug.

And indeed, this is what happens in real life. In CF patients, the infection is in the mucus—it’s not in the tissues, it’s not in the cells. The extent of the infection is really amazing—there can be 100,000,000 bacteria in one milliliter—that's \( \frac{1}{30} \)th of a teaspoon—of airway secretions. So, it’s a high-grade infection, but it’s confined to the lumen of the airways. So to summarize to this point, when CF patients have a “problem” like a virus, they absorb all the salt and water from the surface and get a mucus plaque. Unfortunately, the cells in the airways that typically secrete mucins don’t know that the mucus isn’t moving, so they secrete more, so you build up these plugs, and
these obstruct the airways, so that the CF kids can't breathe. Bacteria invade these mucus plugs and chronically infect them, and the CF child's secondary host defense mechanisms, like the white cells that come out of your blood into the airway, can't penetrate this thick, sticky mucus and capture and kill the bacteria. So this is the cascade of CF, and again, the important thing is that it is initiated by having too little salt and water on the airway surfaces; that is, the surface is dehydrated.

Now, we don't know everything about this disease, or how airways work. When I don't know everything about something, I always tend to go to the physicists because they have this exactness in their nature, and they ask the toughest questions. And so we have this wonderful group of physical scientists at UNC, and again, this is one of the pleasures of working on a campus like this, where you have a mixture of a medical school and a liberal arts campus. So about three or four years ago, we began talking seriously to a group of physicists led by Rich Superfine, augmented by a wonderful group of physical chemists, led by Michael Rubenstein, and computational mathematicians, led by Greg Forrest. We started the Virtual Lung Project to deal with some of the really fundamental questions about what goes awry on airway surfaces (see Figure 9.5). I'm not going to spend too much time on this, because I could spend the whole session on it and probably bore you with it. But we have focused on some really critical questions, e.g., how does mucus really move and slide over an airway surface? The physical problem is that mucus is like flypaper—you want it to be sticky, you want it to trap all the bacteria and everything else you inhale, but you don't want it to stick to your own cells. And so we started trying to ask questions about what the mucins look like in the mucus layer. Indeed, these very large molecules, or “big babies,” as John Sheehan calls them, are organized as a mesh, so the mucus layer that traps bacteria looks like a fishnet, designed to trap anything and get it out of your lungs. Michael Rubenstein asked, “Why doesn't this fishnet extend down to the cell surface?” And from a thermodynamic point of view, these mucins should want to move from this region of high concentration to a region of lower concentration. Michael suggested that the reason the fishnet didn't extend to the cell surface is that we don't really have a watery layer there, but we have another mucus gel. When we started putting that notion to the test, i.e., looking for this layer, we actually found that there was such a layer, which has enormous importance for how water is traded between the near-cell surface compartment and the mucus layer, normally, and how we do it.

The second question we've tackled is, how do you know that you have enough liquid on your airway surfaces? Basically, you have very, very many different periods of your day. Sometimes you're exercising, you're losing a lot of water from your lungs, you want to hydrate your surfaces; at other times you're listening to a lecture for an hour, and you don't need to have as much water on your surfaces. So how does your body know that? It turns out that there is a master signal, essentially an orchestrator of the amount of salt and water on your airway surface, and this signal is contained in the airway surface liquid itself. This signal tells you whether you have enough ASL or not,
and this is a signal that Dean Roper mentioned. It’s called ATP. When we first started in this field, ATP was thought to be the conventional energy “coin of the realm” inside the cell, and it’s only been the last decade and a half that we’ve known that ATP is released from the cell in both a regulated and a constitutive fashion. The amount of ATP in the airway surface liquid dictates how much hydration you have on your surfaces. And so, in the extreme, if you have no ATP, your airway epithelium sucks all the fluid off your airway surface, because evolutionarily you need to protect your lung from drowning at last resort. But as you release more ATP, there are signals that are transmitted through a receptor on the surface, called the P2Y2 receptor. This receptor is a good friend of Dr. Ken Harden, who is sitting in the audience. This receptor has the ability to slow the absorption of salt from the airway surface and to speed the secretion of chloride, so it rebalances the amount of salt, and hence water, on airway surfaces. And this is an incredibly important molecule for therapeutics for CF, and Dean Roper touched on one scenario that I’ll talk to you in a minute about.

We need to improve the efficacy of therapeutic maneuvers that we believe are important. For example, many of you, when you think about CF clinically, think about that rhythmic clapping of patients’ chests that happens on the pulmonary wards. Up until now, that’s been the only way of getting mucus out. No one has ever really understood how it works, and it never made any sense to me that, if you’re pounding on a chest, the resonant frequency of the physical therapist somehow matched that of the mucus and mucus sort of scooted out of the lung. Well, it’s very likely that what is happening when you do physiotherapy is that you’re stimulating release of ATP onto the

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surface of the airways of CF patients. ATP works in CF patients because it activates a chloride channel that is distinct, and I’ll show you this in a minute, from the CFTR chloride channel. Unfortunately, it only works while you’re pounding the chest, and the current devices we have are not optimally tuned to release ATP. So a lot of people in the lab, including Brian Button, who is in the audience, are trying to help the physiotherapists, and manufacturers of the vests that we use that mimic physiotherapy, understand the now-predictive mathematical relationships between pounding on chests, ATP release, and effective clearance of mucus from the lungs of CF patients.

Now, it’s very hard to talk drug companies into making drugs for rare diseases, and CF is a rare disease by definition—there are 30,000 cases in the United States, and probably 100,000 worldwide. It’s also very hard to talk drug companies into making drugs that have an entirely new mechanism of action, so you really have to work on it. And to talk them into it, you have to give them proof upon proof upon proof that dehydration, in this case, is the problem. So one way we attacked it, again, as Dean Roper mentioned, is to say, “OK, maybe we can just make a CF mouse model, and show that the lungs are dehydrated and get CF-like lung disease”—and prove it to them that way. So I remember perhaps 15 or 16 years ago, on a cold winter day, schlepping up to Wisconsin and, with a little bit of trepidation, going in to meet a very famous scientist, Oliver Smithies, and asking him, would he really try to make a mouse with us that mimics CF? And I shouldn’t have been worried, because Oliver was extremely gracious, as you all know, and extraordinarily smart, and realized that this would be a great model for his own gene targeting technique. Fortunately for us, he agreed and put his best postdoc, Bev Koller, on the project. Three years later, in a horse race with 12 different laboratories, Bev won by producing the first CF mouse, with about three weeks to spare.

So what happened with these mice? If you’re a normal mouse, this is how well you live as a function of time after you’re born—normal mice obviously do fine (see Figure 9.6). CF mice died with regularity after birth, much like infant CF patients did 60 years ago. So we had that part of the phenotype right. Now, the interesting thing was, and this is where you get into the issues of whether the glass is half empty or half full—CF mice died solely of gastrointestinal disease. So this is a normal mouse, and this is a CF mouse—what happens is that since the CF mice could not secrete salt and water at the base of the lining cells of the gut; they could not flush out the mucus and antimicrobial materials into the gut lumen. Eventually, the gut lumens became full of mucus, and the mouse became obstructed and died. However, the CF mice had normal lungs! So here we had made a precise genetic model of CF by knocking out the CF gene in mice, we’d gotten the GI disease, but we had no lung disease. So the question was, why?

At one level, from the glass-half-empty point of view, it was a disappointment, because we didn’t have lung disease to work with. But about two or three years before we’d made the CF mouse, Shelley Earp and I had done some work, and we had found that in addition to the CFTR chloride channel, there was a second chloride channel

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that could be involved in hydration. And this is called the so-called alternative or calcium-activated chloride channel. This is indeed the channel that is activated by ATP. Importantly, and what Lane Clarke in the lab found, was that normal mice used these two chloride channel types variably in the different organs. So, in the intestine, the normal mouse really liked to use CFTR exclusively to hydrate the intestinal lumen, and it did the same in the large intestine. So now, if you remove the CFTR chloride secretion by gene targeting the CF gene, they've got a real problem—they can't hydrate. So this explains the severe phenotype of the CF mouse. Now, in contrast, in the respiratory tract and in the pancreas, the normal mouse likes to use the alternative channel rather than the CFTR channel to do most of the work. So now, when you remove the CFTR channel by gene targeting, you don't do much damage because you have something else to pick up the slack. So, from the glass-half-full point of view, this taught us that it didn't matter how you hydrated the lung, with either the CFTR chloride channel or the calcium-activated chloride channel; you were going to be OK as long as you could do so by either means. It also parenthetically suggested to us that ATP might be a good way of activating this alternative chloride channel and making the human lung more like a mouse. This was actually the science that led to the creation of Inspire Pharmaceuticals, which set about making an inhaled version of ATP that would be safe and long acting—and I'll return to that.

So the mouse lung modeling thing sort of simmered for a couple of years, maybe a decade, and I couldn't talk anybody in the lab into trying a different approach. But finally a very energetic scientist from Germany came to the lab named Marcus Mall. And the question was, if we couldn't do anything with chloride channels to make a dry lung, could we actually overexpress the other part of the system? So if we made the salt absorption pathway overactive in the mouse, could we dry the airways out, get dehydration, and get CF? In essence, what we did is take advantage of what we knew of the

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nature of the sodium channels in the lung. They are made up of three proteins, from three different genes. And so the simple idea was, if we just overexpressed, in a different kind of mouse protocol called the transgenic approach, more Na⁺ channels in the mouse airway, then could we dehydrate the airway surface by that action without doing anything to the chloride channels? So, just by mass action, we tried to put in more Na⁺ channels in mouse airways to produce more salt absorption, hoping that it would suck water from the airway surface. We looked at each of the subunits of this channel, expressed transgenically, but the most interesting was the so-called beta subunit. We overexpressed this channel subunit, which means that you put many gene copies in a cell. We also could do it specifically in the airways.

As we had hoped, we increased the rate of sodium absorption in these transgenic mice, as compared to their normal controls, both as babies and as adults. Also, as we had hoped, the amount of liquid on the surface was less if you had this accelerated sodium absorption than if you were normal. And as we had hoped, the rate of mucus clearance from these transgenic mice was slowed, because we had dehydrated the surface.

So now, did we produce CF-like lung disease by dehydrating the surface? The answer was yes. About 60 percent of these mice died before they were weaned from their mothers. We then asked why they died. In the βENaC transgenic mouse’s main airway, called the trachea, we saw mucus plugs that had detached from the lower airways and occluded the airway at the level of larynx. This is a concentrated, sticky mucus, and indeed it is a lethal mucus. For the animals that actually made it into adulthood, if you looked at their airways, they were full of this mucus. Totally occluded in some places, partially in others, but most important, if you looked at the mucus at high power, you could see that it was stuck to the airway surface, or adhered, just as we see in CF. And if you look with an electron microscope at this junction, you see that the mucus essentially pounds down on the cilia so that they’re flattened again. So you can produce a disease that looks just like CF—in fact, if you look in the airway lumen, you can actually find inflammatory cells and occasionally bacteria by dehydrating airway surfaces.

Normally, if you balance absorption and secretion, you have plenty of liquid on your surfaces, and you clear mucus well. In the case of the βENaC mouse, we disturbed the balance by raising absorption over secretion, we dehydrated the airway surface, and we got airways that were full of mucus, inflamed, and actually were remodeling and producing too much mucus. So this was helpful to us. This was helpful with companies.

But finally, at the end of the day, we are trying to treat humans, and the problem is, you can have cell culture models, you can have animal models, but the real evidence has to be found in people. And that’s hard to do—that’s the hardest thing to do, i.e., clinical research. And we’ve been blessed by having a large number of really great people at the CF Center who do this. But we have evidence that CF patients have a problem with hydration of secretions. We have collected secretions that we induce in
normal people by having them breathe a salty solution. If we measure the contents of secretions, we find that about 2 percent of the secretion is a solid material. That means 98 percent of it is water. So these are the characteristics of secretions from normal people. Now, if you look at CF patients, 92 percent of their secretions is water. So, the difference between 92 percent water and 98 percent water is the difference between dying at the age of one or two, if you’re untreated, and living a healthy life. So it is an amazing requirement for hydration. And actually, if you sample directly from CF lungs, and these are obtained at transplant, you sample the material they can’t cough out. It’s about 85 percent water.

Now, if you look at the old literature—and you can’t get this on the computer, you have to go find the old journals—you find one of the most instructive papers ever. This paper was written by a gentleman at Columbia, Dr. Wolf Zuelzer, who was interested in the notion that Dorothy Anderson had suggested, i.e., that CF was a nutritional problem that produced lung disease. In Zuelzer’s paper is a photomicrograph of a lung of an unfortunate CF child who died at the age of two days from intestinal blockage in 1948. The photomicrograph depicts a child’s small airway, and it contains one of those mucus plugs exactly like those in the mouse.

So there’s evidence both from patient secretions and from tissue that mucus is a problem and dehydration is a problem. An assay that we use very often in this field to ask if mucociliary clearance, or clearance of mucus from the lung, is abnormal is to have patients inhale a radioactive particle that is about the size of a bacterium, and we put them in front of a gamma camera that measures the clearance of those particles over time. So if you’re normal, you inhale particles, and you have 100 percent retained in your lung at time = 0, but you clear them over time. It’s a rather brisk process, and by six hours, you’d have them all out. Now, if you have CF and you have normal lung function, i.e., you have “early” CF, you’ve lost about half of your clearance capacity. As your disease progresses, you lose more of this capacity, and as you get more severe disease, you lose more and more clearance capacity. Now, I used to think that this meant that there was a veil of CF that sort of descended over the lung homogeneously, and so if you are at 50 percent clearance, every part of the lung was at 50 percent—but that’s not true. Seminal studies refuting this notion were performed by Scott Donaldson and Bill Bennett here at UNC. They showed that the lung disease in CF is like a checkerboard. It moves through the lung one zone at a time. So there are zones in the CF lung of a typical adolescent that are very, very damaged, where mucociliary clearance, instead of being normal, is almost absent. There are other parts of the lung, particularly in the smaller airways, where there is a battleground, i.e., where there is some disease smoldering all the time, where clearance is intermediate. But there are also vast parts of the lung that look perfectly normal. And this is a very optimistic finding for treating this disease, because this means that we can deliver novel therapeutics to the normal portion of the CF lung. If we do this right, we can arrest the progression of CF lung disease. There’s no reason we can’t do that.
So just, finally, how do you do it? If you accept the notion that dehydration is the key problem in CF, that we need to hydrate those plaques of mucus that are stuck to CF airway surfaces and get rid of the infection that is a part of those plaques, how do we rehydrate airway surfaces, and how do we do it all the time? There are essentially two ways to do it. This slide takes us back to those in vitro experiments, showing data from Rob Tarran (see Figure 9.7). What Rob did is, very simply, put hypertonic saline on the airway surface. Simply, hypertonic saline is a solution that is about 7 percent salt, and your blood concentration is about 1 percent salt—and to orient you, the Atlantic Ocean is about 4.5 percent salt. So, when CF patients inhale hypertonic saline onto their airway surfaces, the high salt concentration draws water osmotically into the secretions. Very, very simple therapy, but, at least in the test tube, i.e., our in vitro experiments, hypertonic saline worked very well. The other way to do it is pharmacologically, and that’s with a drug. And so this would be either the ATP or UTP I talked to you about—when you inhale these chemicals, they slow the absorption of sodium, and they trigger that secretion of salt through the alternative chloride channel. And actually, they work quite well—again, in a model system.

So where do we go with humans, and does it really work? This is, again, where Michael Rubenstein’s hypothesis suggesting that there are two gels on the airway surface is critical. This hypothesis projected that hydration would help mucus clearance rather than hurt it. Videomicroscopy experiments demonstrated that if you have a mucus that is 2.5 percent solids, so it is only about 0.5 percent less water than it should be, it
moves a little bit slower than it should. If you put hypertonic saline on the mucus and just add another 1 percent more water to the solution, it greatly accelerates the clearance. So, thus, we felt comfortable going into humans with the notion that this kind of therapy should be useful.

The first of the studies that really showed that you could hydrate surfaces and do, essentially, good, rather than harm, were the studies of Scott Donaldson and Bill Bennett here at UNC. And what Scott did was to measure CF subjects’ lung function for a couple of weeks before they came into the study, and then he measured their mucus clearance. Then he put them on inhaled hypertonic saline four times a day for 14 days and remeasured their lung function and mucociliary clearance. So, in brief, this figure (Figure 9.8) shows clearance. This figure uses a little bit different format for display, so up is good—it means you’re clearing those radioactive particles as a function of time. So, when CF subjects walked into the lab, they had a clearance rate that was lower than normal, i.e., were typically “CF-like.” However, after 14 days of hypertonic saline, they had restored their clearance rates into the normal range. Now, what did they get out of this? In the two weeks that they were observed prior to going on hypertonic saline, they actually lost lung function. In the two weeks that they were on hypertonic saline, they gained 6 percent of their lung function. So there was a direct translation between a simple maneuver of inhaling saltwater and gaining clinical benefit.

The drug companies didn’t really like that saltwater was going to be useful. But the

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companion experiment was crucial. This is what’s fun about CF and what’s fun about modern science, i.e., the ability to collaborate worldwide. We had a group of friends in Australia who decided to do the parallel experiment with us, communicating by e-mail and what have you. It’s now easy to do this. This group of investigators did the hard experiment: they had a group of Australian CF patients inhale hypertonic saline twice a day for one year. We coordinated our efforts so that they used the same strength HS that we did, and they used the same nebulizer, so we could compare data back and forth. If they just inhaled saline with the strength of their blood, i.e., normal saline, they saw a little bit of activity. But if they inhaled hypertonic saline, they got about a 3–5 percent improvement in lung function. Now the critical thing is that CF patients have periods of times in their lives when they’re doing great, and then they get hammered. This bad period is often triggered by a virus infection, or factors we don’t know, and it is called an exacerbation. Exacerbations are what drive CF patients to the hospital, and that’s when we have to give them antibiotics. We hate exacerbations because, very often, CF patients lose lung function during an exacerbation and don’t get it back. So the key issue with this kind of therapy is, does it prevent exacerbations? If you were on control (normal saline), you quickly plummeted, i.e., you were not free of exacerbations for long. By the end of 12 months, only 20 percent of patients were free of exacerbations. If you were inhaling hypertonic saline, you reduced the number of exacerbations by more than half. You also reduced the days lost from school, the days lost from work, and your quality of life improved. So the simple maneuver of inhaling saltwater twice a day significantly cut the rate of exacerbations.

So the next issue, then, is that it is always nice if you have one kind of therapy that works by a defined mechanism, i.e., hydration, to see if you can achieve the same type of therapeutic benefit by another route. And so, another route utilizes a stabilized UTP molecule, an analog of ATP, that is designed to hydrate airway surfaces by redirecting salt transport. All drug companies like to name their drugs—this “clinical candidate” really is an analog of UTP and is called denufosol. José Boyer, of the company that developed this agent, Inspire, is in the second row. Inspire conducted a very well-done study, a randomized, placebo-controlled trial, 24 weeks on denufosol, inhaling it three times per day. The study was conducted in relatively young CF patients, adolescents, who were relatively healthy. The idea was to keep them healthy. This was a very real-world study. Overall, most of these patients were on three different medications, so denufosol was added to the real-world type of complement of medicines. I’ll just jump ahead and say that the denufosol was safe. I believe that the first rule of all medicine is first to do no harm, and it was very reassuring to see that no harm was done. Now, what happened? If you were on placebo, your lung function dipped a bit early, but by the end of 24 weeks, you maybe were a tad better. Interestingly, the placebo is regular saltwater, so it’s an active placebo. But if you were on drug, your lung function did better than placebo, and this was the predetermined primary endpoint for an FDA Phase III study, i.e., a final registration study. An important difference in lung function be-
tween drug and placebo would equal approximately 50 ml or so by the end of 24 weeks, and denufosol met this criterion.

What was really exciting about this therapy, though, is the fact that most therapies work for a short period of time and then stop. With this one, after the 24-week period for efficacy, Inspire kept the CF subjects on the drug, in this case, for safety reasons, and followed them. They continued to get better, and better, and better, for the full 48-week period! Nicely, if you took the patients who were on placebo and now put them on drug to get an assessment of drug safety, they also got better. So we’re now in the last stage of trials with this drug—again, a hydrating drug. What Inspire has decided to do is, rather than just capture 24 weeks of therapy, to go for it. They’ve extended the trial period in the next go-round to 48 weeks to try to capture the full benefit, and then they’ll probably go another 6 months after that to see if the response still keeps getting better.

So for us, this is very exciting, in the sense that this disease appears to be attackable by hydration by at least two mechanisms: inhaling saltwater and inhaling drug. The last “science” thing I’d like to bring up is the fact that the studies I have been talking about were done on young adolescents and adults. I take care of these people, so, to me, these are the seminal studies. If we had the opportunity to arrest their lung disease and let them to live until they are 77, I think this would be a tremendous triumph. The nice thing about CF, though, is that we’re aggressive, and nothing that we do is quite good enough—and what we’d really like to do is translate these concepts to CF babies. CF babies have normal lungs, and they develop this mucus plugging and infection with life. So we would like to see if we can prevent the onset of CF lung disease with these kinds of therapies. So I would just like to close with the last science vignette of this talk, i.e., can we really prevent CF lung disease?

This paradigm has been explored by, again, Marcus Mall in collaboration with others in Heidelberg, Germany, and at UNC. He has taken that CF mouse model (βENaC) I told you about, and he has exposed it to a blocker of the hyperactive Na⁺ channel I told you about. The drug is named amiloride. It is a fairly crummy drug—it’s an early-generation compound, not very potent, too rapidly absorbed. It has been tested in CF patients extensively and has interesting activity from the point of view of telling us a little bit about the science of CF, but it is not a practical drug. But Marcus was asking the tough question: can you take a drug that isn't so good, but if it is easier to prevent lung disease than reverse it, might a drug like this actually be useful? So, what he’s done is, he’s taken βENaC mice, and a one-day-old mouse—these things are about 1.5 grams, so it is not an easy experiment—and he has asked, if I expose them to this sodium channel blocker, this specific drug therapy, if you like, via the nose three times a day, will I be able to prevent lung disease? In contrast, he asked, if I start the therapy after four weeks of life (and by then they have a lot of mucus plugging), can I reverse the lung disease? Again, this kind of experiment shows you the dedication of CF researchers, because this experiment meant that Marcus and his crew had to anesthetize
a 1.5 gram mouse three times per day, weigh it to make sure it wasn't losing too much weight, give it back salt and water if it needed it, and do this daily for two weeks.

The results were stunning. Figure 9.9 depicts survival curves of wild type mice, untreated βENaC mice, and then mice that are treated with the amiloride. So, if you start amiloride therapy in newborn βENaC mice, there is about an 80 percent reduction in mortality. When you look at the mice, what you see is that you've cleaned out these mucus plugs. When Marcus started the therapy four weeks after birth, when they had the mucus plugs, he could not remove them. So the message is, it's going to be easier to prevent CF lung disease than it is going to be to treat it. The nice thing for us is that I think we're going to do both.

So this study led to a study that is just starting—everything has an acronym and I can't remember all of the acronym names, but this one is something like ISIS, the Inhaled Saline Infant Study. It's a large study of inhaled hypertonic saline for prevention of CF lung disease in infants. It's a one-year-long study of inhaled hypertonic saline in 300 infants. The primary goal is to reduce exacerbations, and I'm proud that the lead investigator is Stephanie Davis of our Department of Pediatrics, who is an expert at measuring infant pulmonary function tests as well as doing this kind of large-scale study.

Finally, CF is in a most extraordinary time. We had an exhilarating time at the North American CF meeting, the national meeting held last week. You know, it was the first time I'd ever heard the words “geriatric care for CF patients” mentioned, because we now have over 1,000 CF patients over the age of 50. And the optimism, based on data, that now we have specific therapies for CF lung disease has permeated not only the research community, but also the patient community. I suspect it's much like HIV was 15 years ago, when the protease inhibitors came to the fore. This cartoon (Figure 9.10) depicts how lung function is getting worse as a function of age. The current therapies that we have all used—and I see Gerry Fernald, the former director of the
UNC pediatric CF Center, in the audience—all the antibiotics we've tried and tried and tried, have bought us a little bit of time. However, they don’t change the natural course of the disease because they aren’t treating the disease at its rate-limiting step, the dehydration step. Currently, with rehydrating therapeutic agents and the notion that CF is a checkerboard disease with lots of normal areas, we can honestly hope and believe that we will be able to arrest the progression of CF lung disease and help our patients live the lives that they want, with the quality of life they deserve. Finally, I think we can prevent lung disease in CF infants by starting therapy early. The dream for all CF physicians is to be like the docs who take care of patients with asthma—if you can take two puffs of a drug in the morning and two puffs in the evening, and control the hydration status in the CF lung, our patients should live a complete life.

So I'd like to just finish by touching on the issue of “how do you do this”? You do this with people, and there are three things, when I look back on the CF Center, that explain why it’s worked. It will take me a minute to get through the three, but the first and most important has been people. We have been blessed at UNC with a group of senior leaders who stay here. And, almost without fail, the crowd has been here for the duration. We did lose Tom Boat, to Cincinnati—that's acceptable—we did lose Larry Johnson, who has become a division chief at the University of Arkansas, and unfortunately, we lost Tony Paradiso to lung cancer. But we’ve had this wonderful, marvelous group of people who have been here and worked and worked and worked to deal with this disease. I could bend your ear on all of them, but I’ll just mention two very briefly. One, Mike Knowles, last week won the Paul di Sant'Agnese Award at the CF Foundation. Mike is the quintessential clinical researcher, and we are delighted that he won the award for the top CF researcher of the year. I'd also like to mention briefly Dr. John Sheehan. John was our first Hooker Professor, of Biochemistry and Biophysics, who was funded by an incredibly generous donation “from the West.” John was so keen that he could help us with CF that he left his homeland in the United Kingdom to come and work with us. I think many of you know that John is now fighting a tough disease, but the passion in John's life is to make sure that everything he knows goes into CF and to help us over that final hurdle. John is typical of the inspirational people we see in this field.

The good news is, when the old crowd leaves you, we are going to leave you with a spectacular group of young investigators. This is a field that attracts the best and brightest. This is the crew that you need to get you over the hump.

When you operate a research group, you need to be efficient, you need to go fast, people all have to use the same reagents, people have to be able to compare experiments, and the most thankless job in the CF Center belongs to the people who run the Cores that provide the tissues, histology, the molecular work. This is just a wonderful group of people who do it day in and day out, and make us work.

And finally, the administrative staff. We're a bunch of scientists, and we know nothing about money, nothing about how to count, and we certainly know nothing about the endless regulations we have on campus. And I think we have the fewest adminis-
trative staff/faculty members/dollars of anybody on the campus. Again, the secret for success has been how long they have stayed with us. My own personal secretary, Judy Mar, has been with me over 20 years; she has a thankless life of trying to keep up with my endless changes in travel, etc., and I could talk about all of them, with time. In Rebecca Owen, we clearly have the best senior administrator on campus.

Second point: to make this work, we have to be able to do things off-campus as well. We can make mice, we can make measurements of water, we can make measurements of mucus clearance. However, if you’re going to make a drug, you have to do a lot of chemistry, and you have to do a lot of expensive, boring toxicology to get it through the FDA. You need companies to do this. Early on we were able to get our friends up the street at Glaxo to be heavily involved, and they were great to us in testing that “early” drug I talked about, amiloride, liposomes for gene therapy, and indeed, they provided major financial support for us, including a couple of million dollars for the Thurston-Bowles Building. We’ve also had the real privilege of having two biotech startups come out of the CF Center—as Dean Roper mentioned, Inspire Pharmaceuticals, and a second one, Parion Sciences. I’m really proud of both of these, for two reasons. Firstly, both have kept the focus on CF. It’s not easy, when you’re a in biotech company with a lot of venture capitalists wanting an early exit for money, to stay with a disease like CF, which is going to take 10 years to do, is hard to do, and, at the end of the day, comprises “only” 30,000 patients. So I’m proud of the fact that Inspire, as evidenced by the denufosol data I showed you earlier, has stayed the course for the 13 years it’s been in existence, and I’m proud that Parion Sciences, with now a very nice collaboration with Gilead Sciences, has moved their drug forward. Parion’s P680 will first be tested in humans next week. Secondly, I’m proud of the CEOs of both of these companies—Christy Shaffer, who is well-known to people in the UNC community, and Ross Johnson. Both of them are very loyal to UNC, both of them have a lot of UNC interactions—Ross, I believe, is on the external advisory board of the Department of Chemistry. Both of them have conducted their activities and those of their companies in ways that uniformly will reflect well on the university.

Thirdly, I want to thank all the CF faculty members—they know I thank them. But I also want to thank CF faculty family members. The CF faculty is an extraordinary group, they work very hard; I continually think about someone like Scott Randell, who has come in in the middle of the night for 15 years, getting all the CF lung tissue out of the operating room, so he can supply us with CF tissues, and what his wife must think about it. This thought translates to all the wives and children, and spouses, of CF Center faculty, because this is the kind of effort that is easy for us to do, but is tough on the families. My kids are in the front row, and I’m sure they could give you many stories about being the last one to be picked up from dance, soccer—sometimes not even picked up from dance recital—and I know my wife could tell you many stories about seeing the back of my head reading grants and papers and whatever, but, to my family, the CF faculty, and to all of their families, a big thank you.