IN THE EYE OF A STORMY DEBATE

WORDS BY MARYANN BRINLEY / PHOTOGRAPHS BY JOHN EMERSON

To recognize that the lens of the eye holds awesome diagnostic keys to disease is to think outside mainstream medical research. Peter Frederikse, PhD, is at home pioneering new ideas. The lens is a transparent, crystallin structure made of one cell type at different developmental stages. However, the lens hasn't grabbed the attention of mainstream medical researchers for years. "What people don't realize is that the lens never stops growing throughout life. The cells at the center of your lens were laid down when you had been in the womb only a few weeks and yet these cells focus light for decades," this researcher explains.

"Look at these gorgeous electron micrographs taken 50 years ago," asks Frederikse. He's pointing to his computer screen and the side-by-side images of a lens fiber cell and a neuron. The black and white photographs look like classic Ansel Adams pictures but, art aside, what is remarkable is how similar the two cells are at every level.

These pictures have inspired him for decades. "I've stuck with it. What we've shown is that these two cell types not only look alike, but the molecular regulation to build them is remarkably similar. The degree of shared cell biology processes that create these elongated lens cells and neurons is striking. Here are the dendritic spines where neurotransmitter signals arrive They have the same construction as these spines on surfaces of fiber cells in the lens. The same molecular machinery is producing the same cellular biology to a degree that has scared me and my grad students at times." Yet, lens tissue had never been studied with regard to Alzheimer's Disease (AD). "No kidding, the lens is considered the undead tissue," Frederikse says, like some sort of voodoo thing, but it's not.

Spend an hour with this researcher and you come away understanding some of the little secrets about AD research as well as the key roles AD proteins play throughout the body. You also treasure the type of scientist who persistently thinks outside the box in his research. Listen to him: Degenerative disease in general is much like a "vintage car: you can't really undo any rust but you can prevent it." If you are among the millions of people worried about getting Alzheimer's or diabetes, this guy has some breakthrough answers.

"I am the latest in long line of PhDs and MDs," explains Frederikse. He's been on the Newark campus for 10 years. "Those family shadows are long, and hard to escape," he admits. His father was a super-semi-conductor physicist, a serendipitous fact that opened an important window in this medical researcher's mind about three years ago. His wife, Melissa Frederikse, MD, a former NJMS faculty member, is now a psychiatrist in private practice and at this point, just one of his children, his son who is a junior in high school, seems to be interested in science. "He's doing very well in Advanced Placement Chemistry though I tell him, 'Don't think you couldn't be a lawyer.' My daughter, who is 12, is the artist who will probably eschew Dad's business." Frederikse points to her drawings on his office wall. "She's really good." And in spite of the kidding, so is he.

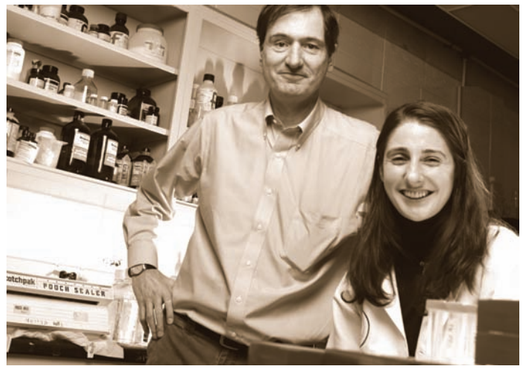
"My father was Dutch and I started school in the Netherlands," he explains. Always drawn to biology, on his undergraduate college application he noted an interest in psychology and chemistry, the perfect mix for brain research, in fact. "The brain, of course, is the most exquisitely sensitive, self-reporting organ. You'll notice loss of function. By comparison, if your pancreas is going, you only notice it when you've got a little bit left. But the eye is also an exquisite self-reporting tissue."

He earned his BS from the University of Washington in Seattle in 1979, his PhD at the University of Maryland, did post-doctoral work at Columbia University College of Physicians and Surgeons under Lucy Shapiro, PhD, a renowned biologist, and then went on to the National Eye Institute at the National Institutes of Health (NIH) for nearly 10 years. "I started off as a developmental biologist. Now, here I am doing Alzheimer's research."

PETER FREDERIKSE, PHD
IS A UMDNJ-NEW JERSEY MEDICAL SCHOOL (NJMS) ASSISTANT PROFESSOR IN THE DEPARTMENT OF PHARMACOLOGY AND PHYSIOLOGY AS WELL AS THE RUTGERS/UMDNJ INTEGRATIVE NEUROSCIENCES PROGRAM.

Every disease has a constellation of diagnostic indicators but the "Holy Grail," in AD according to Frederikse, has been to get a handle on those A, deposits seen in the brains of deceased Alzheimer's patients. The deposits of Alzheimer proteins, first discovered more than 100 years ago by Professor Alois Alzheimer, still remain central to the mechanism. "I think he'd be happy about that."

The Mini-Mental Status Exam, developed at Johns Hopkins in 1975 which is still a "stalwart part of any AD diagnosis, is an 11 question profile of five cognitive functions used to make a very difficult diagnosis," Frederikse says. "There are so many factors involved in cognitive function. As one of my students once said, if you had a bad case of the 'cocktail flu,' or a hangover, you'd fail that test." Meanwhile, all the biomarker methods to find the A, and plaques, from blood tests to cerebrospinal fluid to brain imaging scans "haven't panned out. Those clumps in the brain are probably the accident at the end of the road which don't tell us about the oil slick a half mile back up the highway" when the slide toward AD was just beginning in the body. What's been missing is the ability to look for the initial overproduction of Alzheimer A, peptides, and not just in the brain.



PETER FREDERIKSE, PHD WITH **CLAUDINE BITEL**, A GRADUATE STUDENT IN HIS LAB, IS JUST COMPLETING HER PHD.

"Everyone is sanguine about the idea of a blood test for Alzheimer's," Frederikse explains. But looking at blood is not so very different from looking at any tissue in the body. Lens tissue, in particular, has been considered a field of its own and never included for study of neural versus non-neural expression. But why not?

Since the 1990s, researchers have shown that Aβ, pathology is also a hallmark of other age-related diseases in the body. We know AD and diabetes are closely linked. Nowadays, "The question isn't so much: is Alzheimer's part of diabetes? But just as likely, is diabetes a part of AD?" And, for Frederikse, the point is: "Maybe the blood isn't the only canary in the coal mine." Could our early warning to the existence of systemic Alzheimer deposits be visible right there in the eye? Yes, he says. "We believe that this Aβ, protein builds up in a lot of places and very early on."

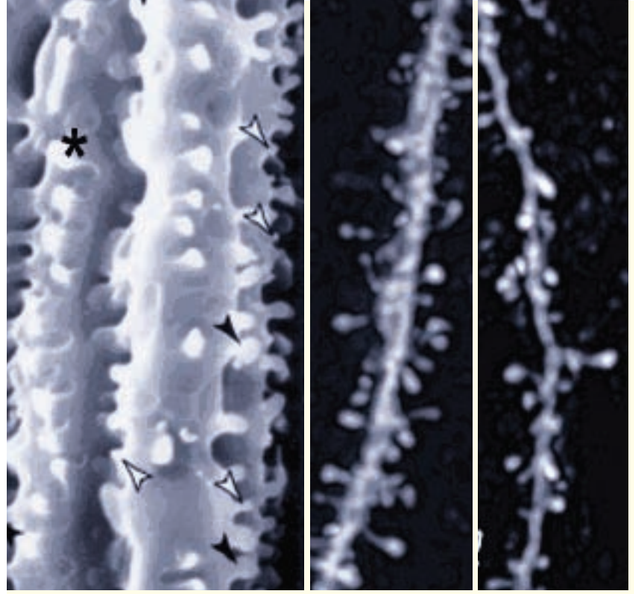
To test his theory that the eye can also be a window to the soul, or the brain, Frederikse needed an animal model, but not your ordinary AD research animal model. It had to be a mammal that really does produce human-like Alzheimer proteins. A surprising secret about Alzheimer research is how much of it is conducted on mice and rats, and "they don't get AD!" To work with these animals they must be genetically manipulated to carry a human Alzheimer gene. Going with the traditional way of using only a transgenic model wouldn't have let Frederikse's team see the effects throughout the body. "We wanted to use a genetically unmodified animal that responds in a normal manner, system-wide, so we could look at all tissues that produce and contribute to Alzheimer pathology, in addition to the brain." In addition, AD is so closely linked with diabetes, some call AD "type III diabetes." So they chose a classic model of diabetes in "wild-type" rabbits. Four months into their study, "Boom, we got gangbuster plaques in the brains of these rabbits. Their brains were littered with Aβ, deposits and we also got big-time coordinate production of Aβ, pathology in lens and muscle too, as we predicted."

All the physical chemistry he learned in college and never thought he would need came together when Frederikse turned his attention to a diagnosis of Alzheimer's. That was three years ago. "It dawned on me, thinking of my father, that spectrometry can be used in the transparent lens. Light can go in and out." A laser spectrometer can "give you a chemical signature in a crowded party of chemicals. Even in a complex mixture, those rotational vibrations of the chemical structures produce different wavelengths. So, we can identify the Aβ, peptides and quantify them as well. These chemical signatures can be seen in Aβ, in test tubes, and in Aβ, scooped-out plaques from diseased brains. But in the lens, we can do it all non-invasively." Alas, the signs of Alzheimer's are right there in the eye. When they examine spectra from intact diabetic lenses, the Aβ, signature intensities match Aβ, levels measured biochemically.

Frederikse's discovery of a method for early diagnosis of Alzheimer's, for which he holds a patent, will call for nothing more than leaning your forehead forward into one of those devices in an ophthalmologist's office and having your lenses read. Will it be frightening to be presented with this type of information? He doesn't think so. Ignorance is not bliss in this case, especially when preventive steps can be taken. "Just like heart disease, we can use this non-invasive tool to develop drugs, to get a better idea of what to do early on, and to understand what is going on with these Aβ, peptides."

Think of your body like that "vintage car," he suggests. It's hard to re-form rusty parts. You might someday replace them in the brain, but it's easier to prevent rusting before it starts. "I've never considered myself a holistic medicine kind of guy but at the end of the day, it has to be true. The body is one system and we have to focus on systemic pathophysiology to understand these effects. We can't just keep looking at a genetically modified mouse that dumps Aβ, into the brain and nowhere else and expect to understand AD, aging and the relationship to diabetes.

"I feel very lucky to be doing something that will advance this knowledge and can contribute practical applications to help individuals." ■



LOOK AT THESE GORGEOUS ELECTRON MICROGRAPHS. "PEOPLE" HAVE BEEN LOOKING AT PHOTOGRAPHS LIKE THESE FOR 50 YEARS," SAYS FREDERIKSE. THE BLACK AND WHITE IMAGES LOOK LIKE CLASSIC ANSEL ADAMS PHOTOGRAPHS BUT, ART ASIDE, WHAT IS REMARKABLE IS HOW SIMILAR THESE TWO CELL TYPES ARE AT EVERY LEVEL. ON THE LEFT ARE LENS FIBER CELLS AND ON THE RIGHT ARE NEURONS. THE PICTURES HAVE INSPIRED THIS RESEARCHER FOR MORE THAN 15 YEARS.

A Method for Identifying Disease Markers in the Lens

BY PETER FREDERIKSE, PHD

ALZHEIMER'S DISEASE (AD)) is by far the major age-dependent degenerative disease of the brain, and is rising with the increasing lifespan. Diagnosis is key, not only for treatment but also for affected individuals. Plans must be made, and early treatment is more effective. How do we diagnose AD? Like many diseases, we look for biomarkers. One hundred years ago, Alois Alzheimer identified deposits in brains of deceased demented patients. A century later we know these deposits contain "Alzheimer" Aβ peptides, cleaved from the "Alzheimer" Precursor Protein, and these proteins remain central to disease mechanisms investigated today. As a result, Aβ also remains an important focus in AD biomarker research. Aβ biomarkers have been examined in blood, serum and cerebrospinal fluids. Brain imaging tries to correlate deposits and damage with disease progression, and imaging enhancements are sought to detect plaques. Although these methods have been around for a while, they have had limited success.

Our goal is to take advantage of the long-time understanding that Aβ pathology also occurs outside the brain. Aβ deposits also provide clear hallmarks of major age-dependent disease in muscles, the pancreas, and from work I initiated as a staff scientist at the NIH in the 1990s, also in the lens of the eye. Here at UMDNJ, my group has worked in parallel on two questions: Why should the lens of the eye produce Aβ pathology similar to the brain? And more importantly, can coordinate production of Aβ in lens and brain provide a "window on the brain?" Many have mused that the eyes are the window on the soul, but can the eye provide diagnostic information about the brain?

Along with two graduate students, Claudine Bitel and Charley Feng, I have worked to measure and characterize the onset and progression of corresponding Aβ accumulation in the lens versus the brain. To test these ideas we needed a model. The model needed to be "wild-type," without genetic manipulation to allow comparisons about corresponding lens and brain Aβ accumulation due to "normal" responses to system-wide pathophysiology. In this way we could determine if Aβ in the lens could be used "like a blood test."

Diabetes turned out to be the link we were looking for. A large number of epidemiology studies closely connect AD with diabetes and hyperglycemia. Diabetes is also closely associated with lens degenerative disease. The system-wide physiological stress that occurs in diabetes has been identified as a good model of aging. My team examined Alzheimer and Aβ pathology in the brain in a classic model of diabetes and hyperglycemia. We showed for the first time in this wild-type model of system-wide pathophysiology in diabetes and hyperglycemia that protein deposits are produced and litter the brain cortex and hippocampus. Further, we also translated an important finding about Alzheimer Aβ and the insulin receptor that had been worked out in cultured cells, which showed that Aβ interacts directly with insulin receptors and aggregate together. My team found this is also true in the diabetic brain *in vivo*, and further substantiates this mechanistic link, in the *in vivo* brain, which goes far to explain the strong epidemiological links between AD, diabetes, and insulin receptor dysfunction. But what about our lens/brain hypothesis? Our study also demonstrated that diabetic lenses with obvious histopathology showed considerable Aβ accumulation as well. When we measured Aβ in the lens and brain, quantitative ELISA (a widely used diagnostic tool for determining the presence or amount of protein in a biological sample) antibody assays identified > 3-fold increased Aβ in brain cortex and hippocampus, and showed 3-fold greater Aβ in diabetic lenses.

Next, the question became: How can we use this information to measure Aβ in the transparent lens? Light can enter and leave the eye and lens, and thus is amenable to laser spectrometry. Spectrometry is widely used to detect chemical signatures in light emitted back at a detector, even in a complex mixture. Laser spectrometry measures chemical signatures of Aβ and in this way it is more similar to antibody detection of signature epitopes. Our team, aided by a NJ Technology and Commercialization grant, obtained a laser spectrometer. We showed that Aβ chemical signatures are detected and measured in intact lenses, and critically, we could measure Aβ increases in the intact lens that closely matched antibody ELISA assays *in vitro*. Today, links between diabetes and AD are becoming stronger and more precisely defined. Diabetes and hyperglycemia, AD and vision loss are major health concerns that also drive up costs. Development of non-invasive diagnostic instrumentation can aid early detection and the ability to design and study drugs to treat these devastating conditions. ■