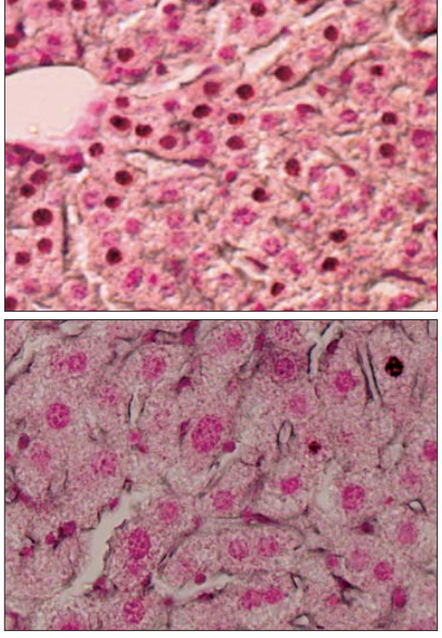
Jeanne Calment celebrates her 120th birthday in Arles, France, in February 1995. If you had been able to ask Calment, who is now deceased, "What's the secret to a long life?" she would most likely have said, "Laughter." Scientists are still trying to answer that question and a more fundamental one—that is, what makes us age?

A PITT RESEARCHER SUGGESTS SHE'S FOUND A MECHANISM AT WORK IN AGING | BY CHUCK STARESINIC

WHY DO WE AGE?

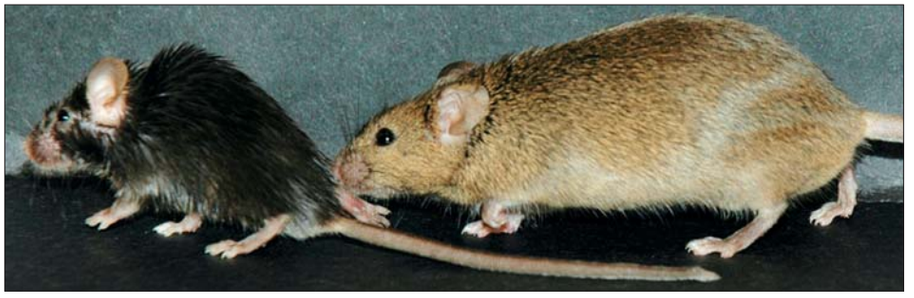
On February 21, 1875, a girl was born in the town of Arles, in Southern France. She began life like any normal baby—working her tiny limbs and fighting for breath in this strange new atmosphere. Her parents named her Jeanne.

She grew as expected and eventually lived a comfortable adult life, marrying at 21. Her husband, Fernand Calment, was prosperous, and she did not need to work. Jeanne Calment swam, played tennis, bicycled, and especially liked to go along on hunting excursions. She bore one child, a daughter, who eventually gave her a grandson. For the first 90 or so years of her life, the details of her day-to-day existence were no more and no less noteworthy than those of most women, but by the time she turned 100, she was a local celebrity. At 110, her notoriety extended beyond the borders of France.



COURTESY NIEDERNHOFER

Healthy tissue regenerates through cell proliferation, as shown with these liver cells from a young mouse (top). Proliferative cells are stained dark red. An engineered mouse fails to repair DNA damage, leading to cell senescence (bottom) and outward signs of premature aging. Only one proliferative cell can be seen.



A mouse that lacks a DNA-repair protein (left) wastes away, loses muscle mass, and develops osteoporosis and neurodegeneration in a matter of weeks, while its normal sibling (right) ages normally.

In 1995, newspapers around the world reported that Jeanne Calment had become the oldest living person the world had ever known. She was 120 years and 239 days old, and still she lived. Readers around the planet sifted through the details of her life, looking for the secrets to longevity. They learned that she treated her skin with olive oil, sometimes ate two pounds of chocolate a week, and rode a bicycle until she was 100. She favored port wine and reportedly quit smoking cigarettes when she was 117. But which, if any, of these nuggets were the gems that helped to explain her long life?

There are many theories of aging and no consensus on which is closest to the truth. Some argue that aging is the result of gradual damage to our cells, particularly DNA that goes unrepaired. Others believe that, though such cellular damage has significant health consequences, it is just a symptom of the overall aging process. Aging itself, they say, is probably orchestrated at a level higher than the cell, perhaps by the winding down of some sort of master biological clock. Uncovering the basic science of aging could dramatically change the way we live, not to mention the number of years that we live. To do so, some researchers are taking a counterintuitive approach: To learn more about longevity, they study those with short lives.

When Jeanne Calment was a mere 110, a 17-year-old woman in Afghanistan gave birth to her first child, a boy, fathered by her cousin. The boy, whom we'll call Kahlil, seemed healthy at birth, with one peculiar trait: He developed sunburns very easily, despite having the normal dark skin of an Afghani boy. By the age of 6, he showed mild learning disabilities and suffered some hearing and vision loss. At 10, Kahlil began to look old beyond his years. His features narrowed. The bones of his face protruded. By 12, he was a wizened boy, who not only failed to grow taller but began to lose weight. His spine curved, and he lost muscle tone. Desperate, his parents brought him to Germany for help, where doctors diagnosed him with an undetermined form of progeria, or premature aging. Kahlil was 15 then. His case didn't fit any of the known progerias neatly, but his face was like that of a grown man with a small head. He was 47 inches tall and weighed 39 pounds. He moved with an unsteady gait, and his knobby knees seemed to knock together. In a matter of months, he developed severe pneumonia complicated by acute respiratory distress syndrome. He died of multisystem organ failure at the age of 16.

What happened to Kahlil? His family had come to Germany for a cure. When that failed, they hoped to find some comfort in a simple answer. Uncovering the mechanism underlying their child's physical deterioration has led researchers to ask other, profoundly important, questions: Is the syndrome that this child experienced physiologically the same as natural aging, or does it only resemble aging? If it is analogous to natural aging, is it possible that this young man, by aging in fast-forward, could point the way toward slowing the aging process?

In a lab at the Hillman Cancer Center, Laura Niedernhofer pops open a shoebox-size "caging unit." She reaches in with a latex-gloved hand and gently grasps the tail of a mouse between her thumb and index finger. "Good morning," she chirps. "See how old they look?" she says to her visitor. Her eyes peer out from between a surgical mask and a sort of shower cap as she demonstrates the proper way to pick up a mouse. When momentarily held by the tail, she explains, most mice will spread their legs wide for balance and wait to be put down. But this one, when held for an extra second, curls its limbs asymmetrically and trembles. She sets down the mouse and watches it walk. It's unsteady on its feet.

They seem a little arthritic, she points out with wonder. "They have trouble getting up in the morning, but once you get them moving, they do okay." Niedernhofer turns to the lab tech, Andria Robinson, who is also clothed head to toe in sterile garb, and asks, "Do we have some soft mush?" referring to the food prepared for the mice as they grow older.

The arthritic-seeming mouse is small and squinty. It appears almost disheveled next to its sturdy, svelte companion sniffing about the same enclosure.

The curious thing about these two very different mice is that they are the same age; they are littermates, in fact. Although the living arrangement looks like that of an aging parent stuck with a grown child, it is more like that of Kahlil, who seemed to grow old before his time, and a normal sibling. By studying the two side-by-side, Niedernhofer, a University of Pittsburgh assistant professor of molecular genetics and biochemistry, expects to learn about more than premature aging syndromes. Niedernhofer believes that her observations are revealing something new about natural aging and cancer, as well. As Robinson goes about the process of weighing and observing dozens of mice being studied, Niedernhofer explains how she came to work with these engineered mice.

She never set out to study aging or progeria. She was more interested in cancer when she walked into Kahlil's case almost by accident. As an MD/PhD student at Vanderbilt University, she was interested in the ways in which our cells contend with damaging compounds that result

Is the secret to how and why we age linked to DNA damage? Laura Niedernhofer thinks so.

MARTHA RIAL

from normal cellular metabolism. When she exposed bacteria to one such compound (malondialdehyde), the bacteria mutated. Our bodies are making gobs of this stuff, she thought, and if it causes mutations in human cells, it's a potential cause of cancer. Sure enough, when she exposed human cells in the lab, they mutated. With her PhD adviser, Larry Marnett, she showed that this natural byproduct of our metabolism causes a devastating kind of DNA damage called interstrand crosslinks. No one had demonstrated this before. These crosslinks are strong bonds that form across the two strands of DNA. They can kill the cell if not repaired: For our cells to do anything with DNA, the strands have to be pulled apart and read. That's how DNA is replicated to produce daughter cells and transcribed to produce the proteins that are the workhorses of cells. If the genome is the book of life, crosslinks can be thought of as drops of spilled glue pasting whole pages together and rendering them unreadable.

Niedernhofer found this fascinating: When she exposed cells to their own byproduct, they developed crosslinks. Yet, spontaneous crosslinks are almost impossible to find in people. A lab in the Netherlands offered Niedernhofer a chance to explore this puzzle further. She went to Erasmus University in Rotterdam for a postdoctoral fellowship because Jan Hoeijmakers' lab there had engineered a mouse with a missing DNA-repair gene. This mouse could not repair crosslinks and died very young. Normally, to study crosslinks, researchers would have to induce them by treating an animal with a chemotherapeutic agent, but they had not treated these mice with anything, providing evidence that crosslinks form spontaneously. Around the time that Niedernhofer arrived, the lab discovered a connection between the engineered mouse and the boy with the unknown progeria.

A year earlier, Kahlil's doctors in Germany had sent a living sample of his cells to this same lab for diagnosis. They found that Kahlil had a mutation in a gene called Xpf, which was intricately linked to Ercc1, the gene they had knocked out of their mouse. The proteins these genes produce are like a pair of figure skaters spinning with all four hands locked together. As long as they hold on to each other, they are stable; take away one protein, and everything breaks down. In other words, if you don't have Ercc1, then you don't have Xpf, and vice versa. This protein duo is called Ercc1-Xpf, and, letter by letter, its name rolls trippingly off Niedernhofer's tongue, with the ease of a 10-year-old talking about R2-D2 and C-3PO. Ercc1-Xpf was known to be involved in DNA repair as a molecular switchblade that snips the ends of damaged strands of DNA after other proteins have identified them. But Niedernhofer and her colleagues had found something new: The protein duo appeared to be related to accelerated aging, too, possibly by virtue of its connection to crosslinks. Kahlil had a mutation in Xpf and had progeria. The Rotterdam mouse lacked Ercc1; it developed spontaneous crosslinks and lived only three weeks.

"It's difficult to study a mouse that only lives three weeks," says Niedernhofer, so she began experimenting with different versions of the Ercc1 knockout mouse. She came up with two knock*downs,* as she calls them, because they are able to produce drastically reduced but detectable amounts of the protein. One produces 10 percent the normal amount and lives six months. The other produces 20 percent the normal amount and lives 18 months. (Normal lifespan for a laboratory mouse is about two years.) As she and her colleagues watched these mice, they began to think they had more than a model for studying a rare genetic disease.

The knockdown mice hobbled around, their spines became a little hunched, they lost weight and muscle tone, and the collagen in their faces began to degrade, so they had bags under their eyes. Then they started showing solid tumors, spontaneously, which is unheard of in young mice. The mice looked like a model of human aging.

Human cells are bombarded daily with insults to our DNA, including ultraviolet light, cigarette smoke, and other environmental toxins. But some of what damages our DNA arises spontaneously as natural byproducts of our own cellular metabolism. Our cells use oxygen to create energy, for example. Wayward rogues among those oxygen molecules pick up loose electrons in the cell, become highly reactive, and scoot around the cell damaging DNA.

Scientists used to believe that metabolic rate could predict longevity, both in species and in individuals, because high metabolism seemed more likely to result in rogue oxygen molecule activity. Mice owed their short lives to high

metabolism, went the logic. Elephants were at the other end of the scale, and humans were somewhere in the middle. Birds, however, fell off the curve; they had exquisitely high metabolism and longevity. It turns out that birds have a very efficient metabolism that doesn't result in oxygen wreaking much havoc. So it appears that the amount of destructive, or reactive, oxygen that cells produce is a better predictor of longevity than metabolism.

Niedernhofer's hypothesis goes one step further. She's suggesting that the cell's proficiency at avoiding and repairing specific kinds of DNA damage is the real mechanism at work in these comparisons of longevity versus metabolism. In her model mice, she and her colleagues have made a strong case that what makes the mice look old before their time are interstrand crosslinks, those messy drops of DNA glue. Niedernhofer believes this process is directly related to natural aging, too, in mice and in humans. To strengthen that case, she'd like to find spontaneous crosslinks in living organisms, which is a challenge. As Niedernhofer has learned, the crosslinks appear to be so toxic that cells with unrepaired crosslinks don't stick around long—they simply die.

In the coming months, Niedernhofer will watch her engineered mice and their normal littermates to see how well they are able to repair crosslink damage, and how this ability relates to signs and symptoms of aging. In collaboration with Pitt biochemist Shivendra Singh, she's feeding some of her mice special diets—one high in fat and another high in phytochemicals from broccoli and garlic—to see how these variables might affect aging.

Don't expect to see antiaging pills that include DNA-repair proteins. It's not that simple. First of all, a large number of proteins work together in complex ways to repair our DNA. Second, DNA-repair proteins are very destructive; that's why the body destroys them when they aren't needed. To leave them in place, or to supplement them with a pill or an injection, would be like leaving several power saws running in your house at a time when you didn't even need any repairs. Niedernhofer says we're probably better off preventing DNA damage in the first place—by eating intelligently, for example.

In many ways, Niedernhofer still feels like her work is just getting off the ground. It takes time to raise and observe a colony of mice, some of which live two years, and she's been at Pitt only a year since completing her postdoc. She's tall, outgoing, and she laughs when anyone calls her Dr. Niedernhofer. (A security guard at the cancer center has been known to meet her halfway with "Dr. Laura.") She spends part of every workday down in the "mouse house"—not because the colony would fail to thrive or the study would lose data otherwise, but because she knows the mice have more to teach her, and she'll never know what that is if she's upstairs in her office. "When you come downstairs and see a mouse that looks old, that is just overwhelming. ... That takes it one step closer to home for me."

One of the possible problems with Kahlil's disease, or any progeria, as a model of natural aging is that neither mice nor humans with these syndromes mimic the process of aging exactly.

Richard Miller, a gerontologist at the University of Michigan, says progeria syndromes in general may look like aging, but they are probably just another illness. The way he sees it, animals with progeria simply remind us of what old animals look like.

Niedernhofer says Miller is a critic of her work, but one with whom she has an open dialogue and who offers his best advice in the interest of advancing the science. Her team has created a long list of physical and behavioral characteristics found both in natural aging and in her mouse models, but it's not extensive enough for Miller. The exceptions, she says, are partly because of the nature of aging—different tissues age through different processes.

"We call it segmental aging or tissue specific," says Niedernhofer. "It doesn't happen throughout the body, but just in certain tissues." Her mice are deficient in one particular aspect of a very elaborate system of DNA repair pathways. "I think it's reasonable to imagine that the DNA damage you get in your liver is different from that in your heart," says Niedernhofer. So a progeria like Kahlil's may not be a complete picture of aging but is still very relevant to aging, she and others hold.

Miller says that no theory of aging has been demonstrated to be correct, but he doesn't find the segmental hypothesis compelling. He imagines an underlying mechanism keeps time for a wide range of processes in the body:

"If I tell you, for instance, that I've got someone in my office right now who has got some cataracts, thinks a bit more slowly, reflex speed is down, they have broken blood vessels in their skin, and bones are a little bit porous, you know that's an old individual, but it could be an 80-year-old person, a 15-year-old dog, a 30-year-old horse, or a 3-year-old mouse. All of those factors change together. All of those systems decline together at a pace that is specific for the species' own aging range. And it's very hard to see how that might come to pass if all of these symptoms were all aging in an unsynchronized fashion. Similarly, a calorie-restricted diet slows all of those things down in mice and rats—all of them together. And that's almost impossible to imagine how that might occur by chance unless there's some common underlying timing mechanism."

Niedernhofer and others who believe in using mouse models of accelerated aging have their supporters. They contend that natural aging in humans is segmental. We all age a bit differently; various tissues age quickly in some people and slowly in others. Two researchers (Paul Hasty and Jan Vijg of the University of Texas, San Antonio), responding to Miller in *Aging Cell* last year, wrote that, though Miller "believes the scientific community does not yet have a sound idea of what causes aging... in our opinion there is such an idea and it is based on damage accumulation." They point out that more than 100 genes are involved in DNA repair and many more in overall maintenance of the genome. Therefore, knocking out one or two mechanisms of DNA repair should be expected to result in an animal that shows segmental aging, and certain progerias could model the way specific parts of our body age. Niedernhofer's knockdown mice are particularly promising in this respect, because they seem to age in so many different tissues and because the crosslinks they suffer from had never been shown to be related to aging before.

Science advances slowly, methodically, and rationally, yet scientists themselves are a bit like speculators. They stake out what looks like promising ground, commit to it wholly, and see what will come of it. Such a gamble might someday be seen as a paradigm shift or just a historical footnote. But unexpected outcomes will always occur in science as well as real estate. Back in the mid-1960s, for example, a lawyer in France made a deal with an elderly woman: He would pay her 500 francs a month for the rest of her life. In return for this regular income, he would take possession of her grand apartment in town when she died—a common transaction in France. He was in his 50s and she was already 90, so it might have seemed that he was taking advantage of her. "Sometimes in life, one makes bad deals," said Jeanne Calment, 32 years later. By then, the lawyer had paid her three times the apartment's worth and finally died without ever taking possession. His family was obligated to continue the payments until 1997, when Mme. Calment died, at the age of 122. ■