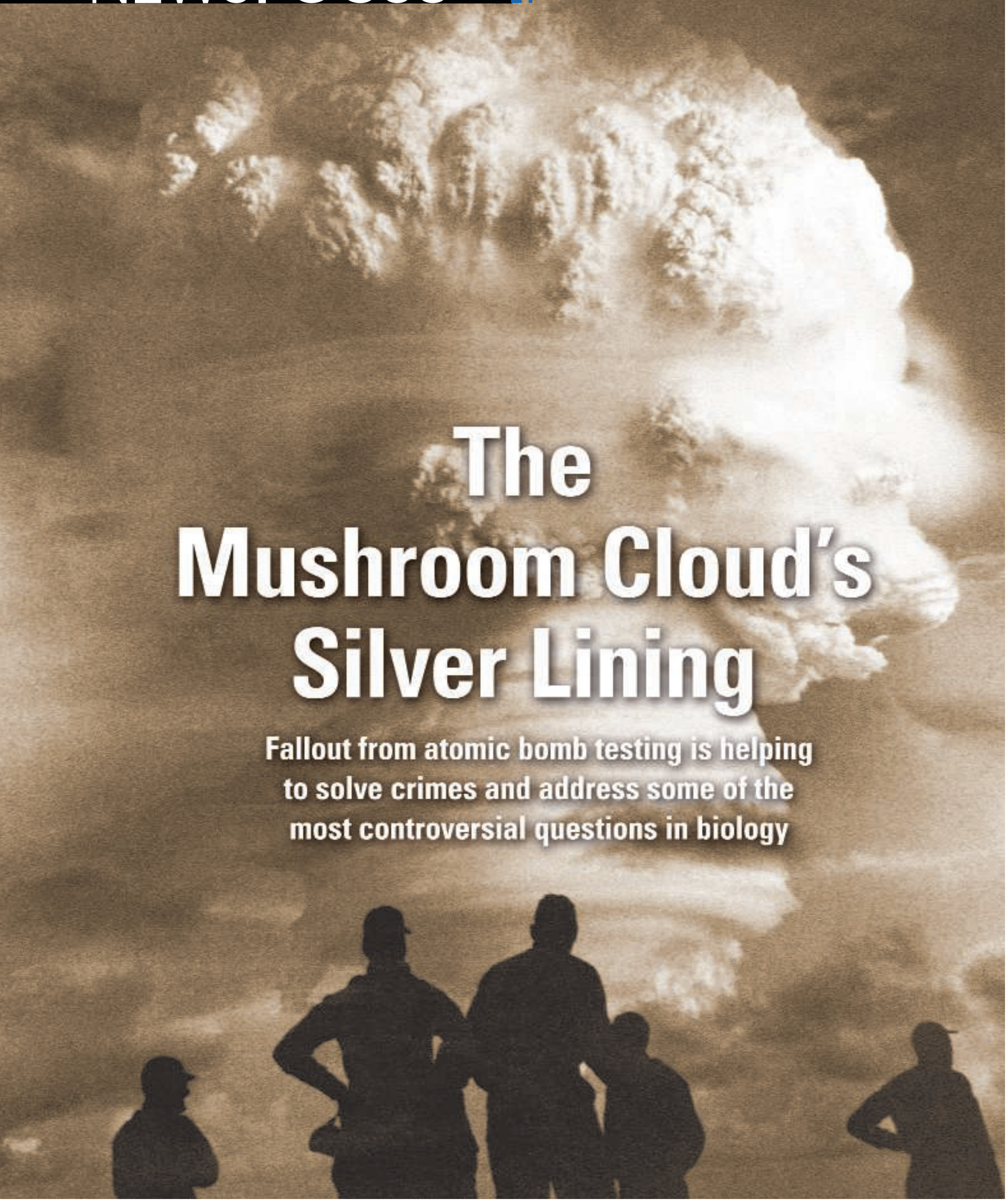

Bomb boom. Hundreds of aboveground nuclear tests, like this one carried out in the Pacific in 1958, seeded the atmosphere with excess ^{14}C.

The Mushroom Cloud's Silver Lining

Fallout from atomic bomb testing is helping to solve crimes and address some of the most controversial questions in biology

THE TWO MUMMIFIED BODIES IN THE Vienna apartment told a sad tale. The reclusive elderly sisters had clearly been dead for several years, but no one had noticed; neighbors in the upper-middle-class complex believed they had merely moved away. Stale bank accounts finally tipped off the police, who discovered the remains in December 1992.

Investigators found no evidence of foul play, so they focused on the question of who died first. Both sisters had large pensions and separate life insurance policies, and the insurance company of the woman who died last would collect the bulk of the funds. "There was a lot of money at stake," says Walter Kutschera, a physicist at the Vienna Environmental Research Accelerator at the University of Vienna in Austria. Not long after the bodies were found, a scientist from the university's forensics department approached Kutschera and his colleague, Eva Maria Wild, to ask if they could help crack the case. The forensics expert knew the pair had been using radiocarbon dating to determine the age of archaeological samples, and he wondered if the same technique could shed light on the year each sister had died.

It couldn't. Radiocarbon dating is a blunt instrument that relies on the slow decay of a form of carbon known as carbon-14 (^{14}C), which is incorporated into animals during their lifetime. The method works well for samples that are tens of thousands of years old, but it's only accurate to within a few hundred years.

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Wild and Kutschera had another idea. Aboveground testing of nuclear weapons after World War II had injected ^{14}C into Earth's atmosphere, creating an abnormally high level of the isotope that has been tapering off since then. If the researchers could measure the amount of ^{14}C in something carbon-based that the sisters had generated just before death—fats in the bone, for example—and compare it with historic levels of ^{14}C in the atmosphere, they should be able to tell which year each sister expired.

It worked. Wild and Kutschera found that one sister had died in 1988 and the other in 1989. "One sister lived for some time next to the dead one," says Wild. Investigators closed the case, and Wild and Kutschera returned to dating ancient bones and seeds. But it would soon become clear that the "bomb pulse" technique had much more to offer. In the past decade, thanks largely to the pioneering work of an Australian postdoc with a taste for trying new things, groups have begun using the strategy for diverse causes such as identifying disaster victims, authenticating wine vintages, and tackling some of the most controversial questions in biology, including whether the human brain generates neurons throughout life.

From pet shop to slaughterhouse

2001 started well for Kirsty Spalding, but by the end of the year she would be knee-deep in a failing project. The 29-year-old had just finished her graduate work in neuroscience at the University of Western Australia in Perth, and she was planning on spending a year in Europe as a postdoc before moving to the United States. On her way to interview at a couple of prospective labs at the Karolinska Institute in Stockholm, Sweden, Spalding caught a talk by Jonas Frisén, a prominent stem cell researcher there. "It wasn't what I had planned on doing," says Spalding, referring to Frisén's work on the formation of new neurons in the brain—a process called neurogenesis. "But I found him very personable and the work very interesting."

A few months later, Spalding was in Frisén's lab, trying to map neurogenesis in the zebrafish brain. But neither she nor her labmates had worked with the animal before, and they weren't aware that technical suppliers provided fish specially bred for laboratory study. Instead, Spalding biked over to a local pet shop and brought a few zebrafish back to the lab. Needless to say, the experiments didn't work.

Her mentor didn't lose faith, however. "I could tell that Kirsty liked challenges and that she was extremely entrepreneurial," says

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Frisén. That made her perfect for a new project he had in mind. Familiar with the bomb-pulse work done by Wild and Kutschera, Frisén wondered if it could be applied to DNA. When a cell divides, ^{14}C in the environment is incorporated in new chromosomes, and thus the DNA effectively takes a snapshot of the amount of atmospheric ^{14}C—and hence the birth date—of the cell. If Frisén could exploit this, he might be able to show whether humans generate new brain cells throughout life—a central question in neuroscience. But no one would take on the project. Postdoc after postdoc turned him down, calling the work too risky and too difficult. When Frisén saw Spalding with the zebrafish, he knew he had found someone who wouldn't be daunted.

Spalding agreed. "I liked the problem-solving aspect of it, and I didn't have the burden of knowledge to know how difficult it would be," she laughs. Spalding's planned 1-year sojourn in Europe suddenly became an indefinite commitment.

To address neurogenesis in humans, Spalding needed brains from an animal with a similar life span, so she turned to horses, which can live more than 25 years. That meant trips to the local slaughterhouse. "I would watch them walk the horse in, … and then they would chop off its head and hand it to me," recalls Spalding, who had to excavate the skulls herself. "It's not so easy to hack your way into a horse's head. … It was not pretty."

Brains in hand, Spalding still had challenges to overcome, such as measuring a scarce isotope. ^{14}C makes up only one part per trillion of all of the carbon in the atmosphere. Most comes from cosmic ray collisions with nitrogen, but when the United States, the former Soviet Union, and other nations detonated more than 500 nuclear warheads aboveground in the 1950s and '60s, the atmospheric ^{14}C level doubled. It only began to dissipate when the Limited Test Ban Treaty of 1963 moved atomic tests underground (see illustration).

Despite these elevated atmospheric concentrations, only about one atom of ^{14}C incorporates into every 15 cells. So relatively huge amounts of tissue—up to 5 grams, depending on the part of the body it comes from—are needed for even the world's most powerful isotope detectors to spot it. Horse brains were big enough to provide that amount, but Spalding also had to find a way to sift through a custard of fat, glia, and fibroblasts for the neurons she needed. After taking nearly a year to develop a technique, she was ready to pin ages on neurons and enter the ongoing fray over neurogenesis.

The brain war

Pasko Rakic is a five-star general in a conflict that's been raging for more than a decade in the neuroscience field. The Yale University neuroscientist, who did pioneering work in how the primate brain forms, has famously established the beachhead position that the human cerebral cortex—a region key for memory, language, and consciousness—does not make new neurons after development. He's often made the point that such adult neurogenesis would be counterproductive, disrupting already formed memories, for example.

But in 1998, a research team found evidence to the contrary. It gave people with terminal cancer a synthetic compound called bromodeoxyuridine (BrdU), which inserts into newly synthesized DNA and thus serves as a marker for new cells. The compound was supposed to gauge tumor growth, but it also showed up in the hippocampus, the brain's learning and memory center (*Science*, 6 November 1998, p. 1018). A year later, Princeton University neuroscientist Elizabeth Gould bolstered the case for ongoing neurogenesis in the brain by giving adult macaques BrdU and finding it in the neocortex, a region responsible for language and consciousness in humans. But 2 years after that, Rakic injected a different DNA marker into monkeys and saw no new neurons in the adult brain. The field has been divided ever since.

"The clinical implications are huge. … There are hundreds of great biological questions that can be answered [with this technique]."

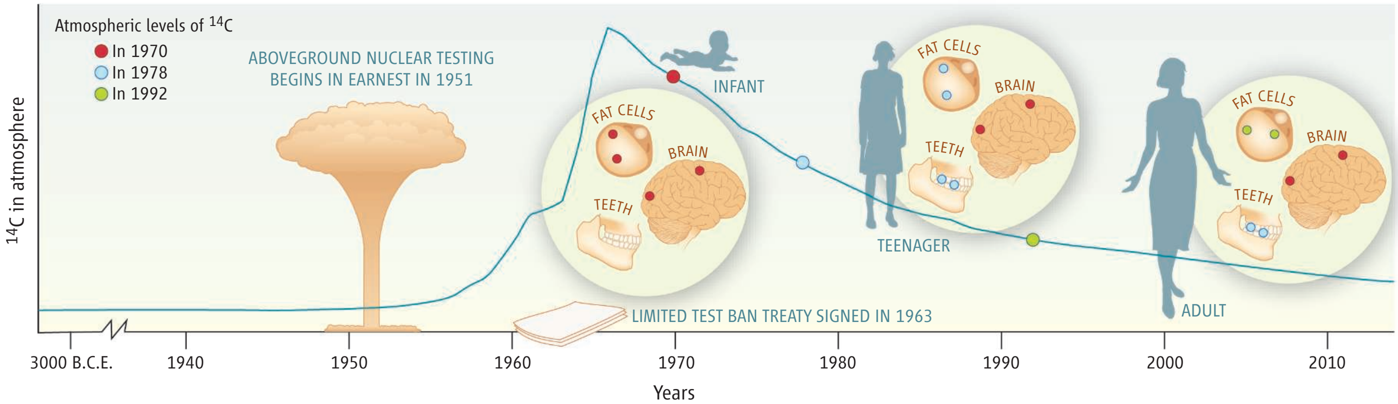
—YUVAL DOR, THE HEBREW UNIVERSITY

"It's been extremely difficult to get any information in humans," says Gerd Kempermann, a neurogenesis expert at the Center for Regenerative Therapies Dresden in Germany. BrdU is toxic, so it can't be given to healthy people, and Rakic has expressed concern that the compound confuses cells into dividing, leading to false positives.

^{14}C doesn't have that problem. It's not toxic, and like it or not, we've all absorbed it. "All of humanity is labeled," as Kutschera puts it.

As the salvos continued in the neurogenesis debate, Spalding had proved that she could use the bomb-pulse technique to date brain cells in horses. She shipped her first human samples—from the brain's visual center, the occipital cortex—to Bruce Buchholz, who runs an isotope detector the size of a basketball court at Lawrence Livermore National Laboratory in California. Although nonhuman studies had suggested that the occipital cortex was a hotbed of neurogenesis, the ^{14}C data collected by Buchholz indicated that human neurons from this region had the same birth date as the people they came from. That meant no new visual neurons for adults. A year later, Spalding and colleagues found similar results in the human neocortex.

"It's really extraordinary work, and it's extremely clever," says Kempermann. "I think many people will take it as the final



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Atomic child. ^{14}C levels in DNA from visual and memory neurons stay the same throughout life, indicating no neurogenesis in these brain regions. Levels in fat cells change, suggesting constant fat cell turnover. ^{14}C levels in tooth enamel remain constant and can be used to calculate a person's birth date.

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word in the debate." Still, Gould notes that other regions of the human brain—such as the hippocampus—have yet to be tested with the technique. And she says that because the bomb-pulse method doesn't target individual cells, it may not be sensitive enough to pick up a small population of neurons that does divide and could contribute to repair and learning. Spalding was in the midst of addressing those questions when disaster struck a continent away.

CSI: Sweden

"Total chaos." That's how Stockholm's former chief medical examiner, Henrik Druid, describes the scene as bodies piled up at the Karolinska Institute morgue in the wake of the 2004 Indian Ocean tsunami that killed more than 200,000 people, including more than 500 Swedish tourists. "The bodies were so badly decomposed, you couldn't tell the teenagers from the old people," he says.

Hoping to help, Spalding approached Druid with some intriguing findings from her days at the slaughterhouse. In addition to analyzing horses' brains, she had looked at their teeth, showing that because enamel is permanent and forms early, its ^{14}C levels give an accurate estimate of the animal's age. Spalding asked Druid if the technique might be useful to him.

"At first I was skeptical," he recalls. But Druid didn't have many options. In the confusion surrounding the disaster, identifying materials such as x-rays and DNA samples from relatives had not been shipped with the bodies. "If you have no clue to the identity of a person, age and sex are the most important way to limit the search," he says. Anthropologists are only accurate to within about 10 years when trying to determine age from a skeleton. So, aided by Spalding, Druid applied the bomb-pulse technique to the teeth of six tsunami victims. After adding the time it takes for human enamel to form (about 12 years for wisdom teeth, for example), they were able to predict the ages of every victim to within 1.6 years, as borne out by the identifying materials that eventually arrived at Karolinska.

With further refinement, Druid has shaved the accuracy down to 1 year, and he's now using the approach to help Swedish investigators crack two unsolved homicides. "This is going to be very, very valuable for criminal investigation," says Druid. "In a year or two, you're going to begin seeing cases in the newspaper that were solved with this method." Spalding too has begun working with Swedish police—as well as with investigators in Canada—and she eventually hopes to set up a company to perform the tooth analysis. In preparation, she has taken business classes at night, all while forging ahead with her brain work—and a new project that would send her spinning in an entirely different direction.

> *"In a year or two, you're going to begin seeing [criminal] cases in the newspaper that were solved with this method."*
> —HENRIK DRUID, KAROLINSKA INSTITUTE

The fat offensive

In 2005, Spalding was presenting her brain findings at Karolinska when a member of the audience approached her. "A Ph.D. student came up to me and said he thought the ^{14}C work was something his dad would be interested in," she says. The father—a prominent researcher at Karolinska named Peter Arner—was grappling with a debate not unlike the one faced by the neurogenesis community.

This time the issue was fat. "If you go to any textbook, it will tell you that once a fat cell is born, you've got it forever," explains John Prins, an expert on fat-cell turnover at the University of Queensland in Brisbane, Australia. But there were some who believed that the blubber on our bellies and hips is constantly dying and being replenished. It's not just an academic debate: If you can make the body destroy more fat than it creates, you've got a ticket to weight loss.

But no one could conclusively address the question. "The techniques we have for measuring fat turnover are insufficiently sensitive and fairly inaccurate," says Prins. The best researchers could do was have volunteers drink heavy water, which contains elevated levels of an isotope of hydrogen known as deuterium, and look for that isotope in fat cells. "Not too many people want to drink heavy water," Prins says.

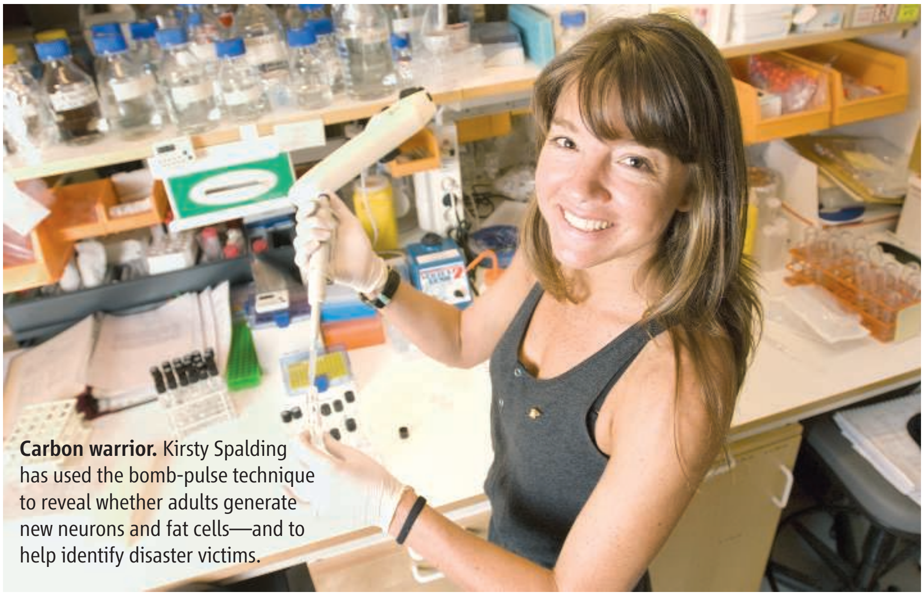
Spalding began working with Arner, and by 2006 she had developed a regimen for isolating fat cells from the vast array of other cells found in human flab. Analyzing fat biopsies and liposuction leftovers from people of various ages, Spalding showed that people born a few years before atomic bomb testing began had fat cells with high levels of ^{14}C, which only made sense if these cells were generated after the fallout had spiked the isotope's levels. When Spalding looked at people born after the bomb tests, she saw fat cells with different amounts of ^{14}C, levels corresponding to various dates on the bomb-pulse curve. In all, the data indicate that people replace half of their fat cells about every 8 years, Spalding reported this summer in *Nature*.

"It's a landmark paper … and a phenomenal advance on a number of fronts," says Prins. "You've got this technique out of *Star Trek*, and now everybody thinks that fat is a dynamic organ." No drug company would have looked into fat turnover before, he says, "but now people will start to consider therapeutic perspectives."

Loving the bomb

As the years go by, the ^{14}C level in the atmosphere is slowly returning to its prewar levels. Rising carbon dioxide emissions, chock-full of ^{12}C, have only hastened the isotope's demise. And yet the bomb-pulse technique is just taking off.

Both Spalding, who left Frisén's lab in



Carbon warrior. Kirsty Spalding has used the bomb-pulse technique to reveal whether adults generate new neurons and fat cells—and to help identify disaster victims.

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FORGERS FACE THE NUCLEAR OPTION

Graham Jones knows a good wine when he tastes one. For nearly 2 decades, the enologist has been teaching students and winemakers at the University of Adelaide in Australia how to become better connoisseurs of the beverage—and how to spot a fake. So when the Australian wine industry became concerned about its vintage wines being disputed in the wake of surging exports to Europe in the late 1990s, it turned to Jones.

"We wanted to develop a technique that, if our wines were challenged, we had the ability to authenticate them ourselves," says Jones. A colleague suggested he take a look at the bomb-pulse technique (see main text). Applied to wines, the method should allow researchers to verify the year a wine was made. That's because, when grapes grow, vine leaves take up ^{14}C-containing carbon dioxide from the atmosphere and convert it to sugar, which eventually becomes the alcohol in wine. Jones's lab developed a procedure to separate the alcohol from other components of wine, but he needed a way to measure its ^{14}C content. So he turned to scientists at Australia's only nuclear research accelerator, based at the Australian Nuclear Science and Technology Organisation (ANSTO) in New South Wales.

A good year. Graham Jones has used the bomb-pulse technique to authenticate wine vintages.

The team was able to accurately calculate the vintage, within 1 year, of a variety of South Australian Cabernet Sauvignons bottled between 1958 and 1997. Although the technique is too expensive to be used regularly, Jones thinks the study warded off European regulators. "The fact that they haven't challenged any of our wines yet is a definite plus for the work," he says.

The ANSTO team has since moved from wine to illicit drugs. When sold illegally, narcotics like morphine tend to be produced and shipped quickly, says Jones, whereas legal morphine can sit around for a while after it's made. ANSTO researchers have shown that it's possible to date these drugs—via the ^{14}C content of the poppy plants they come from—as a way of gauging their legality.

Scientists elsewhere have targeted another type of illicit activity: poaching. Bruce Buchholz, who runs an isotope detector at Lawrence Livermore National Laboratory in California, is collaborating with researchers to date ivory tusks and lion teeth. Because tusks grow throughout an elephant's life, scientists can determine if one has the ^{14}C signature of a time after an ivory ban went into effect. Similarly, ^{14}C in teeth could ostensibly reveal whether hunters are killing off too many young male lions. Buchholz has also heard about groups using the technique to gauge whether a painting supposedly made before the 1940s is a recent forgery, based on the ^{14}C content of the canvas. "If it's supposed to be old and it has bomb carbon in it," says Buchholz, "you know something's wrong." **–D.G.**

2006 to become an assistant professor at Karolinska, and Frisén are expanding its applications. Entering debates similar to the ones about neurogenesis and fat turnover, they're looking at whether heart cells and insulin-producing beta cells in the pancreas renew throughout life or whether we're stuck with the ones we're born with. In tissues in which stem cells have been identified, they plan to examine how often these cells divide and how they are made.

"The clinical implications are huge," says Yuval Dor, a cell biologist at The Hebrew University–Hadassah Medical School in Jerusalem, Israel, and an observer of the bomb-pulse technique. "There are hundreds of great biological questions that can be answered. … We're all very much looking forward to how this will turn out."

The weight isn't all on Spalding and Frisén's shoulders. Other groups have begun to experiment with the technique as well. Like Frisén, diabetologists David Harlan and Shira Perl of the U.S. National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Maryland, are using ^{14}C to measure turnover in beta cells. And Lawrence Livermore's Buchholz says he's been approached by a number of labs interested in everything from climate modeling (changing weather patterns are reflected in ^{14}C levels in coral) to dating confiscated ivory tusks and authenticating wine vintages (see sidebar, above).

Still, it's not a technique that most labs have the resources to adopt. "There are no kits you can buy to do this," says Buchholz. And most labs don't have access to the powerful isotope detectors needed to perform the ^{14}C analysis.

Critics also point out that the bomb-pulse technique has limitations. Although Spalding's work supported Rakic's stance on neurogenesis, Rakic notes that when damaged cells repair DNA, that DNA could incorporate new ^{14}C, suggesting new cell formation when there is none. Conversely, fat-turnover expert Prins says that new cells sometimes recycle DNA from dead cells, giving the impression—under ^{14}C analysis—that no new cells have been made.

And Spalding admits that the forensics applications have a shelf life: As ^{14}C levels recede to background in the atmosphere—Buchholz estimates a return to prebomb conditions by 2020—it will become harder and harder to tell a corpse's year of death. But she's optimistic that as isotope detectors become more sensitive—she's working with Wild and Kutschera to help make this happen—police will be solving cases with the technique for years to come. Brain, fat, and other clinical research won't be affected by the dissipation, as scientists can turn to tissue samples banked over the decades after the bomb tests.

Back at Karolinska, Spalding, Frisén, and a few other collaborators have just formed a Center of Excellence to map the regenerative potential of the entire human body. Over the next 10 years, they'll try to gauge the turnover of every cell type they can. "I love this technique," says Frisén. "We're having a lot of fun with it."

Next year, for a sabbatical, Spalding will head off to California, where she will look for new challenges while continuing her brain and fat research. Stay tuned for an upcoming paper on neurogenesis in the hippocampus—and some more surprises with fat turnover.

Meanwhile, at the birthplace of the atomic bomb in New Mexico, retired Los Alamos National Laboratory scientist Donald Barr reflects on what Spalding and the other bomb-pulse researchers are doing. He's been at the lab for more than 50 years, keeping tabs on nuclear fallout in the atmosphere, and he still comes in a couple of days a week to chat isotopes with his former colleagues. The mushroom clouds from nuclear detonations do indeed have a silver lining, he says. "There are questions we can now answer because of that testing that scientists never thought about at the time." **–DAVID GRIMM**

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