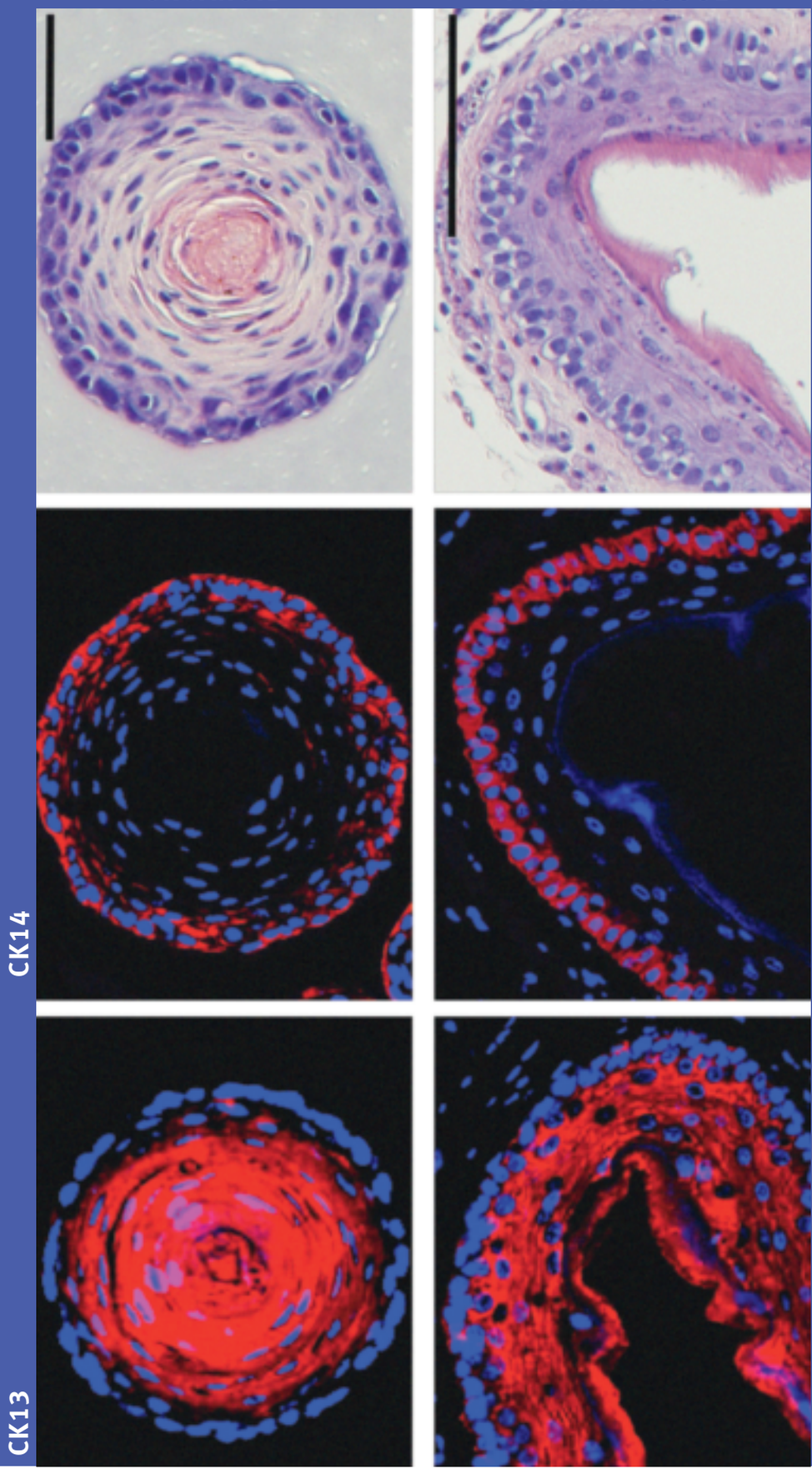


CELLULAR SURPRISE: Eric Lagasse has discovered stem cells in an unexpected location, the esophagus. These images compare esophageal tissue from mice to an "organoid," a piece of esophageal tissue grown from the stem cells of a mouse sample. In the middle and lower rows, the red staining shows the presence of stem and progenitor cells (CK14, cytokeratin 14) and differentiated cells (CK13, cytokeratin 13), respectively. The nuclei of the cells are stained blue.

DIGGING FOR CELLS

TURNS OUT, THE ESOPHAGUS HAS STEM CELLS | BY DANA YATES

Researchers once thought the esophagus was devoid of tissue-derived stem cells, the undifferentiated cells that can renew themselves indefinitely and generate highly specialized cell types. That's because stem cells from elsewhere in the body typically remain at rest until activated by injury or disease; yet all the cells in the lining of the muscular esophagus are in an active state.

The University of Pittsburgh's Eric Lagasse found it hard to believe that the esophagus lacked stem cells. "If the intestine had them [which it does], it made sense that the esophagus would, too," he says.

His research team grew pieces of esophageal tissue from mouse samples, and then conducted experiments to identify and monitor the different cells in the deepest layer of the tissue. As it turns out, the lining of the esophagus does indeed have its own stem cells; they just divide rather slowly. Lagasse, a PharmD and PhD, is an associate professor of pathology and of clinical and translational science. He also directs the Cancer Stem Cell Center at the McGowan Institute for Regenerative Medicine. He coauthored a paper on these findings with postdoctoral researcher Aaron DeWard, a PhD, and Julie Cramer (PhD '14); it was published online last year in *Cell Reports*. Financial support for the studies came from the National Institutes of Health and the Commonwealth of Pennsylvania.

The knowledge of this new source of stem cells could have applications in regenerative medicine.

One appealing thought is to use such cells to grow new esophagi for people afflicted with esophageal cancer.

These cells might also be a source of cancer. Esophageal cancer is an especially virulent disease. It's often diagnosed at an advanced stage when it's challenging to treat and has a low survival rate. According to the American Cancer Society, approximately 15,600 people will die from the cancer in the United States this year.

The disease is associated with Barrett's esophagus, a precancerous condition in which the lining of the esophagus changes to resemble that of the small intestine. The exact cause of Barrett's esophagus is unknown; it may be brought on by the damaging acids associated with gastroesophageal reflux disease. Lagasse and his collaborators will use their findings to investigate the cellular process behind Barrett's esophagus.

"We hypothesize that the stem cells may cause cancer by being reprogrammed to become gut cells, and then mutations happen," he says. "If we can demonstrate how the mechanism works, then hopefully we can stop the mechanism from developing into full-blown cancer." ■

EYE (AND) TEETH

Other stem cell work at Pitt may one day restore vision to millions. Corneal blindness, often caused by injury or infection, can be treated with transplants of donor corneas. But transplant rejection and a worldwide shortage of donor corneas make using a patient's own stem cells an appealing option to explore. James Funderburgh, PhD professor of ophthalmology and of cell biology and associate director of the Louis J. Fox Center for Vision Restoration of UPMC and Pitt, was senior investigator on two recent intriguing studies:

One showed stem cells from human wisdom teeth can be turned into cells of a mouse's cornea. (Other researchers have been able to grow bone, cartilage, and neural tissue from these cells.) This was reported online in February in *Stem Cells Translational Medicine*; the lead author was Fatima Syed-Picard, a PhD, also of the Department of Ophthalmology.

In another study, the team found corneal scarring in mice can be repaired by growing stem cells from tissue extracted from the eye of a human cadaver (specifically the transparent connective tissue known as the corneal stroma) and then placing those cells on the injury site. *Science Translational Medicine* published these findings in December. The lead author was Sayan Basu, a corneal surgeon from the L.V. Prasad Eye Institute in Hyderabad, India. Today, the method developed by Funderburgh's team is being tested in a small clinical trial in Hyderabad. Although Pitt is not directly involved in the pilot study in which a few patients will receive their own corneal stem cells as a treatment, Funderburgh's team trained the Indian researchers on how to isolate stem cells. —*DY

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A detailed look at the body's response to thrush tells us that the immune system's innate and unlearned powers are wider than previously believed.

THE CASE FOR CANDIDA

THE BODY HAS AN INNATE RESPONSE TO THE FUNGUS

BY HEATHER BOERNER

Our immune system has a long memory. Exposed to a pathogen, it takes a week or two to learn that the bug is an invader—but then is never caught off guard again. Future exposure triggers a swift response. But you don't always need immune memory to fight off a microbial invasion. Consider what the University of Pittsburgh's Sarah Gaffen is learning about thrush, an overgrowth of a fungus, *Candida albicans*, that can cause painful, white lesions on the tongue or can manifest as diaper rash or a vaginal yeast infection. Common in the immunocompromised, thrush is a hallmark of HIV infection.

Apparently, in the case of *Candida*, the body's defense starts with the tongue. Yet, for a long time, "no one was looking at immunity in the mouth," says Gaffen, PhD professor of medicine in the Division of Rheumatology. But that's exactly where Gaffen and Heather Conti, a PhD and postdoc in Gaffen's lab, found a key immune protein, called interleukin-17 (IL-17), being made in response to the fungus.

IL-17, it turns out, was being made immediately—not after a learned response—by a special kind of cell, called a natural T helper 17 (nTh17) cell. Buried deep in a bed of sticky collagen on the tongue, nTh17 cells go straight from exposure to *Candida* to making IL-17 and marshaling an immune response.

Instead of taking weeks for the mice in Gaffen's lab to gather forces against *Candida*, they showed markers of immune activation within 24 hours—which indicates a natural, or innate, response.

"This finding blurs the [line] between innate or early responses and immune memory," says Gaffen.

"T cells learn and then respond to pathogens—that's what they do. We don't think of the T cell response as being innate, but the natural Th17 cell response is."

Their finding was published in *The Journal of Experimental Medicine* last fall.

FOLLOW THE YELLOW-LIT PATHWAY

We humans have a natural colony of *Candida* in our mouths, acquired at birth. But mice do not. So Conti, Gaffen, and the team used mice to test the innate immune response to the fungus, to see how an immune system responds the first time it's exposed to *Candida*. Then, by using mice genetically engineered with cells that light up in yellow every time a cell makes IL-17, they were able to trace the immune system activity from response back to its origin.

Finding the nTh17 cells—rather than the typical Th17 cells (which make IL-17 in response to previous exposure to a pathogen)—was a surprise. "It was a combination of process of elimination, plus perseverance, plus technical talent on Heather's part," says Gaffen. "We were finally able to see the cells, and they didn't look the way we'd expected."

Conti created a protocol to pull the cells from their bed of sticky collagen to study their response in a test tube, using a technique called flow cytometry. And sure enough, when exposed to *Candida*, the nTh17s secreted IL-17, with no adaptive response required.

A POSSIBLE TURN FOR HIV RESEARCH

Gaffen says these nTh17 cells, whose very existence is controversial, have rarely been studied. "But our data help prove not only their existence, but also a key biological function [of nTh17]."

Conti would like to explore next how cancer therapy might affect susceptibility to fungal infections.

And there's another area they believe is ripe for exploration. The fact that nTh17 cells responded immediately to *Candida* may indicate that HIV's impact on the immune system is broader than previously thought.

HIV hobbles CD4 T cells and CD4 receptors, notes Gaffen. "So the implication—and this is not proven—is that you may lose your innate cells, too, which [could explain] why HIV patients are so exquisitely sensitive to oral thrush." ■



COURTESY PAULO FONTES

A new perfusion system, developed by Paulo Fontes, includes a novel recipe for the all-important fluid used and rethinks the temperature and the oxygen environment. (In the cart shown here, the organ would get a chilled bath in the sink while the machine pumps fluid into the organ.)

NEW APPROACH LEARNS FROM BEAR HIBERNATION

BY NANCY AVERETT

HOW TO PRESERVE AN ORGAN

It was 1 a.m. when Paulo Fontes's (Fel '93) phone rang. The surgeon had just returned home after spending 18 hours performing liver transplants on pigs in a study at Pitt.

"The animals are walking around like they've never been operated on. They're trying to jump out of their cages," a staff member told Fontes, who quickly headed back to the lab. There, he saw that some of the pigs were livelier than many human transplant patients were immediately after surgery. "I thought to myself, *I've been putting bad livers in my patients my whole life,*" Fontes says.

It was a bittersweet realization. In the course of two decades he had assisted in and performed more than 1,000 liver transplant procedures and had recently dedicated several years to finding a better way to preserve donated livers. The lively pigs meant maybe he had found the answer.

Fontes is a Pitt associate professor of surgery and director of the machine perfusion program, a collaboration of the Starzl Transplantation Institute, the Department of Surgery, and the McGowan Institute for Regenerative Medicine. He has long been frustrated by the fact that some 20 to 40 percent of the time, donated livers cannot be used. The current technique for storing organs, cold static preservation, or CSP, relies on low temperature, 4 degrees Celsius, and no oxygenation to preserve tissue. Yet, the organs always decay some before they are placed in the recipient—some are rendered unusable.

In 2010, Fontes set out to find a better method to preserve organs. Researchers were beginning to experiment with "machine perfusion," or MP, in which an organ is placed into a device that pumps specialized fluid around tissue to ward off deterioration. He found a promising-looking apparatus made by the Dutch company Organ Assist. Using a $1 million donation from a patient, he brought the new technology to Pittsburgh.

The next step: Find the best fluid. The human body uses hemoglobin, a molecule in red blood cells that transports oxygen from the lungs to the organs; yet red blood cells often break when perfused ex vivo by artificial pumps below the body's normal temperature.

So he explored synthetic hemoglobin products. A company in Cambridge, Mass., had created one by isolating hemoglobin molecules from red blood cells, purifying them, and then combining them chemically to create a bigger, chain-like molecule (a polymer). Their polymer could carry oxygen and would not break at lower temperatures. The FDA, however, had deemed it unsafe for in vivo use as artificial blood.

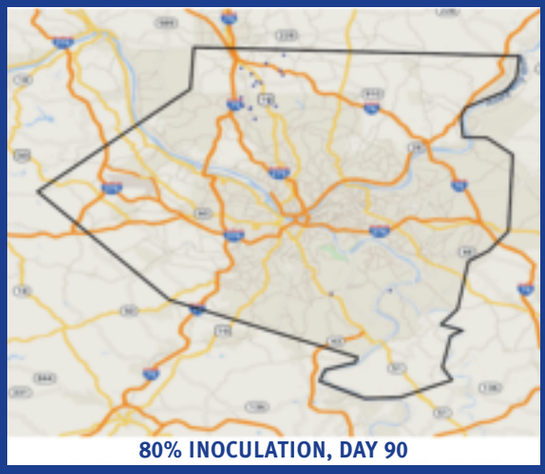
Fontes had an idea for how to fix the problem. He called the company's lead investigator and told him he wanted to alter his formula by reducing the amount of hemoglobin, changing the temperature, adding a buffer to control pH, and adding nutrients for the cells.

Another puzzle was getting the perfusion temperature right. Fontes wanted one that was warm enough to allow partial tissue function but cold enough to create a barrier for microbial growth. After performing some analyses and looking at studies of mitochondrial function at different temperatures (including data on the livers of hibernating bears), he settled on 21 degrees Celsius (about 70 degrees Fahrenheit).

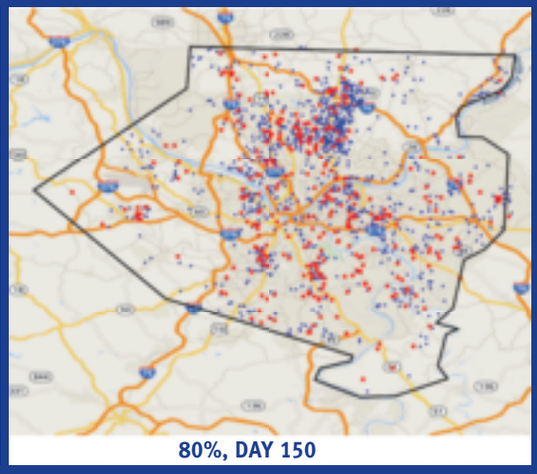
He then took 12 pigs and transplanted into them livers that had been kept for nine hours outside their donors. Six of the livers were preserved with the MP system, the other six with the conventional system, CSP.

Then came the 1 a.m. call. Five days after the surgery, only two of the CSP pigs were still alive compared to all six of the MP pigs. Further tests revealed the MP organs were functioning better than when they were in their donors: measurements of the 100 most affected genes showed increased expression of those related to metabolic, anti-inflammatory, and regenerative functions, as well as protective mechanisms against free radicals. "If you provide oxygen," Fontes says, "you can actually improve the quality of the organ."

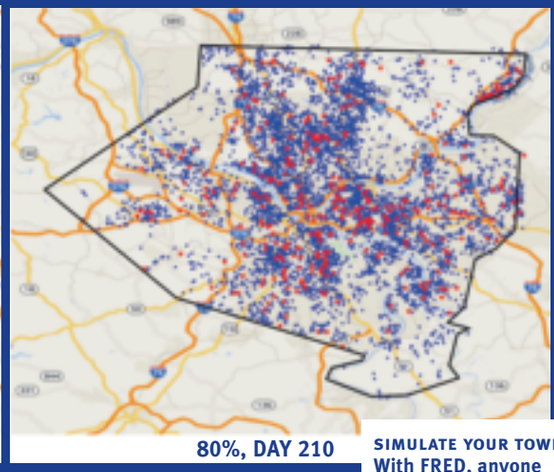
The results were published in the *American Journal of Transplantation* in January. Fontes hopes to start clinical trials later this year. He has founded a spinoff company, Virtech Bio LLC, which will develop MP systems for transplanting other organs, and even limbs. ■



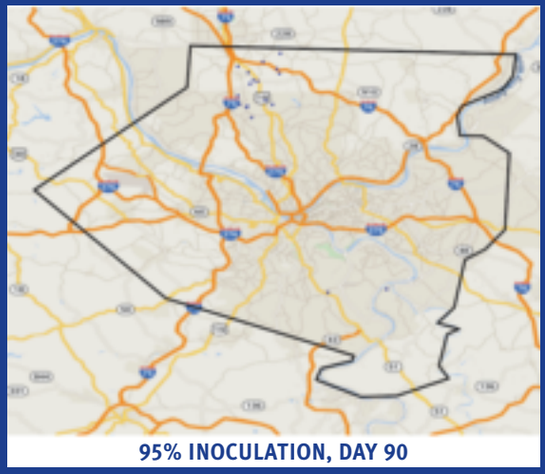
80% INOCULATION, DAY 90



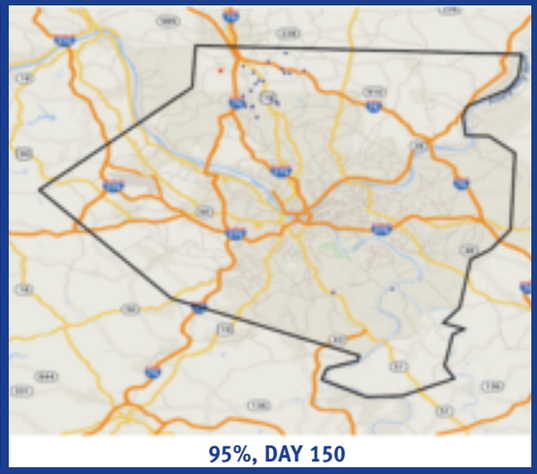
80%, DAY 150



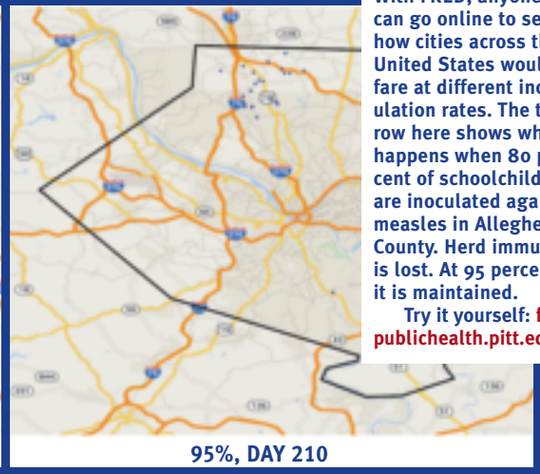
80%, DAY 210



95% INOCULATION, DAY 90



95%, DAY 150



95%, DAY 210

SIMULATE YOUR TOWN: With FRED, anyone can go online to see how cities across the United States would fare at different inoculation rates. The top row here shows what happens when 80 percent of schoolchildren are inoculated against measles in Allegheny County. Herd immunity is lost. At 95 percent, it is maintained.

Try it yourself: fred.publichealth.pitt.edu

RED DOT = INFECTIOUS CASE BLUE DOT = RECOVERED CASE

THIS PAGE: COURTESY PHDL. OPPOSITE PAGE: COURTESY PROJECT TYCHO.

GONE VIRAL

PITT TOOLS MAKE ANOTHER CASE FOR VACCINATIONS | BY BRETT MURPHY

A simulation tool from the University of Pittsburgh's Public Health Dynamics Laboratory called FRED—Framework for Reconstructing Epidemiological Dynamics—shows what a measles outbreak might look like in your hometown. And the platform has gone viral.

FRED illustrates the strength-in-numbers law of nature: When one bison loses a step to an injury or age, the collective strength and speed of its herd protects it from predators. People benefit from their packs, too. In what's known as herd immunity, infants and others who are vulnerable likely won't contract or spread an infectious disease because so many around them have a defense against it—vaccination.

The inoculated are the swift bison.

But if a population's vaccination rate drops below a certain threshold, herd immunity is lost, and a disease can blanket a city.

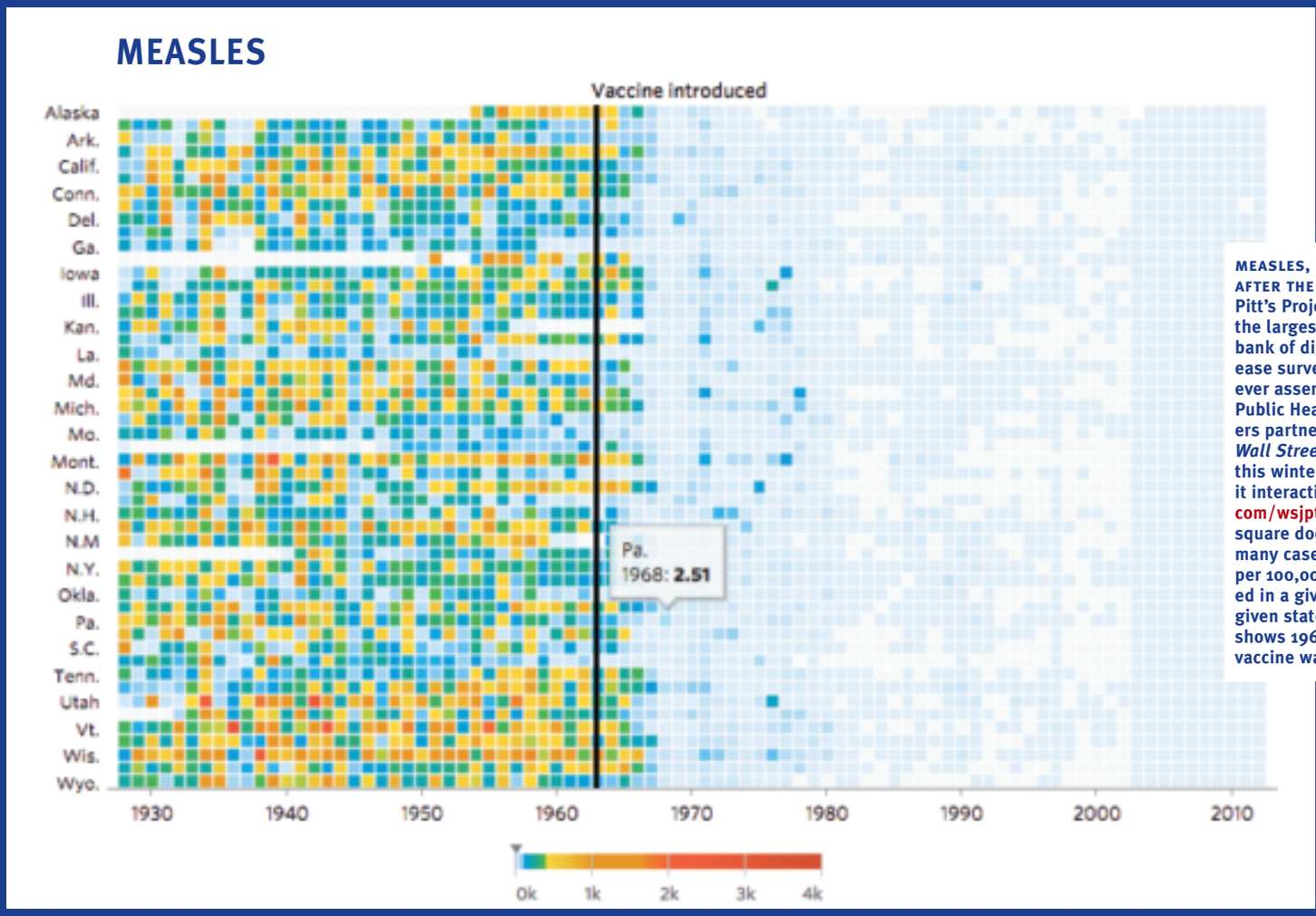
An outgrowth of years of research into infectious disease epidemics, FRED was produced in collaboration with Carnegie Mellon University and the Pittsburgh Supercomputing Center. John Grefenstette, a PhD professor of health policy management in the Graduate School of Public Health, is the lead software designer and lead programmer on the project.

FRED can be adjusted to model an outbreak in any U.S. city, says epidemiologist and Pitt Public Health dean Donald Burke, an MD and Pitt Distinguished University Professor of Health Science and Policy, UPMC Jonas Salk Professor of Global Health, as well as professor of medicine.

The beauty of the tool is in the simplicity of its presentation. Pitt's David Galloway, a research programmer for the platform, says FRED's user-friendly interface has miles of code behind it—all applying "natural history parameters," or how people tend to act when they're sick.

Do people stay home from work or trudge in? How sick does your daughter have to be before you keep her home from school? How close is the nearest hospital?

These are just some of the considerations churning in FRED's measles epidemic simulator. Click on the play buttons, and two simulations will unfold in the city of your choice: one where



MEASLES, BEFORE AND AFTER THE VACCINE: Pitt's Project Tycho is the largest centralized bank of digitized disease surveillance data ever assembled. Pitt Public Health researchers partnered with *The Wall Street Journal* this winter to make it interactive (tinyurl.com/wsjpt). **LEFT:** Each square documents how many cases of measles per 100,000 were recorded in a given year in a given state. The line shows 1963, when the vaccine was introduced.

95 percent of schoolchildren are vaccinated and another where only 80 percent are. At 80 percent, you are likely to see the map enflame in a sea of red dots, cases spreading as the days tick on. At that point, herd immunity has been lost. About 92 percent of children across the United States have been vaccinated against measles. Pennsylvania's vaccination rate is slightly above average. In both West Virginia and Ohio, the inoculation rate is lower—86 percent.

In a recent interview with *Pitt Med*, Carl Zimmer, a science columnist for *The New York Times*, said that describing how an outbreak works can be difficult. "Visual illustrations online can help a lot," he says of the FRED simulator's accessibility for folks outside of scientific circles. "And those simulations do an elegant, simple job" of showing why herd immunity is important.

Zimmer was one of hundreds of thousands to recently come across FRED shortly after it went viral across social media this February. Pulitzer Prize–winning journalists and national media correspondents praised (in 140 characters or less) the tool's value.

"Want to see what #measles cld do in your town if vax rate = 95%? Or only 80%?" Laurie Garrett of the Council on Foreign Relations tweeted with a link. "Cool interactive frm @PittPublicHlth."

FRED isn't the only Pitt Public Health interactive tool hitting the mainstream lately. Pitt's Project Tycho, a digital database that provides open access to U.S. disease surveillance data, teamed up with *The Wall Street Journal* this winter to create interactive graphs illustrating a history of infectious diseases. The headline of the article—"Battling Infectious Disease in the 20th Century: The Impact of Vaccines."

At 90 million case files chronicling reports of 58 infectious diseases in every state before, during, and after vaccination licensure from 1888 to recent times, Project Tycho is the largest centralized bank of digitized disease surveillance data ever assembled. Wilbert van Panhuis, an MD/PhD assistant professor of epidemiology at Pitt Public Health and lead investigator for the project, says Tycho has very real applications for researchers, policymakers, health officials, and parents. "There's a lot of misinformation out there, and people don't realize the consequences of not vaccinating," he says. "Our data help put things in perspective, historically and state by state."

This magazine (see our Spring 2014 cover story) and other publications wrote about Project Tycho as well as FRED last year, as the tools became publicly available. And now many more people know about them.

Bill Gates, who helps fund both FRED and Project Tycho through the Bill and Melinda Gates Foundation, tweeted about *The Wall Street Journal* article to his 21 million-plus followers.

Burke says these painstakingly researched platforms can help people make better choices. "We, as a society, don't do a great job thinking through complex systems," he says of problems like the current measles outbreak.

"Having these tools literally allows you to turn the knobs and think about the consequences of certain actions." ■