One evening last week, before heading home, Jay Bolden stopped at an oasis of green in the industrialized neighborhood of Indianapolis where he works. It was drizzling, but he quickly swapped his work shoes for hiking boots, grabbed his binoculars and scope, and headed toward the woods where the rush of spring migration resounded. Listening intently, he soon spied his first Orchard Oriole of the season, and his second Black-throated Green Warbler. (He’d seen the first that morning with his seven-year-old daughter.)

His eyes glued to his binoculars, Bolden, a senior biologist at the pharmaceutical giant Eli Lilly, delighted in every warbler, sparrow, and thrush he found. Confidently reeling off the birds, he’s at the same time a bit bemused that in only one week, he, an admitted introvert, would travel across the country to Cape May, New Jersey, to make a major announcement about the culmination of years of work in his lab—a project with major implications for a bird rarely seen here in Indianapolis.
That bird is the *rufa* Red Knot, one of six subspecies of Red Knot worldwide. A russet-breasted shorebird about the size of a robin, it makes a monumental 9,000-mile migration, each way, between Tierra del Fuego, where most of the birds winter, and the Canadian Arctic, where they breed. As flocks of knots make their way north, sometimes flying for days at a time without stopping, they pause in Delaware Bay, a veritable shorebird Serengeti during spring migration. The spot is also home to the world’s largest remaining concentration of horseshoe crabs. In May and June, when the tides are highest, horseshoe crabs, one of earth’s oldest animals, emerge from deep water to lay their eggs in the sand. The knots, exhausted and famished from their journey north, stop to refuel on the crabs’ tiny, fat-filled eggs to build up enough energy reserves to power their long journey to the Arctic.

Each Red Knot needs to eat about 400,000 pin-size eggs to gain the necessary weight; multiplied by many thousands of birds, the astronomical figure was once easily supplied by Delaware Bay. In 1991, New Jersey’s beaches contained 100,000 eggs per square yard. The mere 5,000 or 6,000 eggs laid per square yard in recent years are hardly enough to feed hungry shorebirds.

What happened? In the early 1990s, fishermen took as many as two million horseshoe crabs each year to use as bait in eel and whelk traps. At the same time, biomedical companies were catching the animals to protect public health. Horseshoe crabs’ blue blood is the key ingredient in an all-important test that detects endotoxin, a potentially life-threatening bacterial contamination that can occur in drugs and medical devices. Approximately 70 million endotoxin tests are performed each year, according to industry. Companies making the assay—known as LAL, or *Limulus* amebocyte lysate, after the horseshoe crab *Limulus polyphemus*—bleed horseshoe crabs, then return them to the water. Academic researchers and industry scientists differ on how many die in the process: estimates range from five percent to 30 percent. And the loss of blood has longer term effects, such as loss of fitness and increased susceptibility to infection, that may also contribute to population declines.
The horseshoe crab plays a vital, if little-known, role in the life of anyone who has received an injectable medication. An extract of the horseshoe crab's blood is used by the pharmaceutical and medical-device industries to ensure that their products, e.g., intravenous drugs, vaccines, and medical devices, are free of bacterial contamination. No other test worked as easily or reliably for this purpose—until now. Photos: Timothy Fadek/Redux

In a recently published paper, shorebird biologist Larry Niles found that when the birds can’t get enough eggs, they take longer to reach their nesting grounds, they may not breed when they finally get there, and may not survive the long flight back. The U.S. Fish and Wildlife Service concluded that a dearth of horseshoe crab eggs in Delaware Bay sent the *rufa* Red Knot population crashing and, in 2014, listed them as threatened under the Endangered Species Act. Adult females, key to the restoration of Delaware Bay horseshoe crabs, haven’t rebounded, and the birds continue to suffer. When they returned to Tierra del Fuego this past winter, the population had dropped to 9,800 birds—a far cry from the 41,700 surveyed there in 1985.
Enter: Jay Bolden, a tall, thin scientist who seems to disappear in his lab coat. He works in a sparkling new lab at Eli Lilly’s sprawling technology development center in Indianapolis. For the last five years, in his lab far from the sea, he’s been steadily working to develop a product that will take biomedical pressure off horseshoe crabs. Building on research carried out in Singapore, and continued in Maryland, he’s been compiling evidence that a synthetic enzyme, recombinant factor C—rFC for short—can replace horseshoe crab blood in endotoxin tests. According to his work, rFC works just as well as LAL, is more efficient and cost-effective, and doesn’t require a live animal. “It will benefit Eli Lilly,” he says.

This synthetic alternative has been on the U.S. market for almost 15 years, but institutional and regulatory barriers have hindered its acceptance. It’s taken Bolden, a birder, to persevere, and he’s collected enough data to convince his employer that it’s time to make the switch. If rFC is taken up by the rest of big pharma, it will be a groundbreaking change, and both horseshoe crabs and shorebirds will likely feel the difference.

Red Knots feeding on horseshoe crab eggs in North Carolina, another important rest stop for shorebirds migrating north in the spring. Photo: Walker Golder

When Bolden came to Eli Lilly in 2000, LAL had been the gold standard for endotoxin testing for two decades. Before U.S. drug regulators approved LAL in the 1980s, pharma companies tested their products on rabbits, waiting to see if they developed a fever after injection. Animal welfare issues, the expense of
housing and feeding them, and their skittishness—they sometimes spiked fevers when strangers walked into the room—made them imperfect test subjects. After LAL proved more sensitive to endotoxin, it largely replaced rabbits.

The problem solved by the crab’s blue blood goes something like this: When gram-negative bacteria like *E. coli* die, they shed endotoxins, which are everywhere—in water, soil, the human intestinal tract. Danger arises when high concentrations of the potent poisons enter a person’s spinal fluid or bloodstream, potentially causing fever, respiratory distress, septic shock, organ failure, and even death. As a result, injected drugs (for people and their pets) or implanted medical devices that come into contact with blood must be tested for endotoxin.

Horseshoe crab blood, exquisitely sensitive to endotoxin, clots in its presence. LAL, the assay made from horseshoe crab blood, ensures that millions of heart stents, pacemakers, joint and cataract replacements, and radioactive tracers in PET scans, along with millions of doses of flu vaccine, insulin, and intravenously delivered antibiotics and chemotherapies, are free of endotoxin. Manufacturers also must test the water and raw ingredients used in their manufacturing. To keep up with demand, companies that make LAL capture and release some 500,000 horseshoe crabs along the eastern seaboard of the United States every year. In Asia, most bled horseshoe crabs are ultimately killed.

When Bolden was still a young boy in elementary school, biochemist Jeak Ling Ding began working on a next-generation endotoxin test—one that didn’t depend on horseshoe crab blood. In the 1980s, Ding and her colleagues at the National University of Singapore, unable to afford LAL, embarked on what turned out to be a 20-year effort to genetically engineer the protein that triggers clotting in the presence of endotoxin. In 1997, they filed a patent, *she published a paper*, and hoped her discovery would usher in a new era of endotoxin testing.
Horseshoe crabs are bled at the Charles River Laboratory in Charleston, South Carolina. Photo: Timothy Fadek/Redux

Ding’s work piqued the interest of scientists at Cambrex Bio Science, now part of the Swiss biotechnology company Lonza. Lonza, which already produced and sold LAL, saw an opening for a cheaper, alternative test. Ding and the National University of Singapore licensed the cloned synthetic rFC to Lonza, which in the early 2000s began manufacturing and selling it to drug companies. Or, at least, it tried to.

“We built it,” says Lonza's Allen Burgenson, then manager for regulatory affairs at the company, “and they didn’t come.”

For 15 years, Burgenson led Lonza's work in making the case for rFC. He petitioned the FDA to regulate it, but the agency declined, he says, on the grounds that rFC isn’t a blood product like LAL, doesn’t diagnose or treat disease, isn’t a medical device, doesn’t emit radiation, and therefore wasn't under its jurisdiction. He also petitioned the U.S. Pharmacopeia—a compendium of drug information—and its Japanese equivalent, which set standards for the formulation, quality, purity, and strength of drugs, to regulate and license its use. They weren't interested, either.

Between 2004 and 2012, biomedical use of horseshoe crabs increased 85 percent.
Meanwhile, LAL production ramped up. Between 2004 and 2012, biomedical use of horseshoe crabs increased 85 percent. Finally, in 2012, the FDA formally announced that the biomedical community could use rFC, provided that companies proved, for each drug or device, that rFC works as well or better than LAL. The European Pharmacopeia followed suit in 2016. Still, drug companies had little incentive to spend time and money testing a newer, less familiar product.

But Bolden had a compelling reason: “I’m a birder,” he says, “so this hit close to home.” He’d seen Red Knots, one in Delaware’s Bombay Hook National Wildlife Refuge, and then another in Ireland’s Ballycotton Marsh. And a few years ago, at an endotoxin summit in Delaware hosted by Lonza, Bolden witnessed the arrival of horseshoe crabs. “We were walking along Pickering Beach, in Delaware,” he recalls, “and the beach, seemingly empty, suddenly filled with horseshoe crabs coming in from the sea. It was a religious experience for me.”

The longtime birder didn’t need to be told how badly shorebirds along the Atlantic Flyway need horseshoe crab eggs. Further, he had learned about the plight of horseshoe crabs worldwide in 2013, during a Lonza webinar with Glenn Gauvry, president of Ecological Research and Development Group, an organization dedicated to the conservation of horseshoe crabs across the globe. While population data on the animals in Asia isn’t fully known, “horseshoe crab populations in Japan, Taiwan, China, Hong Kong, and Singapore, once vibrant, are now endangered,” Gauvry has written. It is questionable, he writes, whether current takes of horseshoe crabs for endotoxin testing “can be sustained, much less meet the projected future demands of this rapidly growing market.”
Bolden, aware that Asian horseshoe crabs taken for biomedical use are often bled to death, became concerned about “supply problems down the road” when he learned that Eli Lilly was planning to build a second manufacturing plant in China, one that would make insulin, which requires endotoxin testing.

“Here,” he recalls thinking, “I can have an impact. I can make a difference. I can be part of conservation.” His vocation and avocation came together.

If Ding in Singapore had started this relay to end the practice of bleeding horseshoe crabs, and passed the baton to Lonza's Burgenson, then Bolden was ready for his turn at the track. But this lap, like the others, would take time. He pitched an Eli Lilly vice president on using rFC, and with his support, then sought approval from two of the company's governance committees: the specifications committee, dealing with quality control, including tests for endotoxin, sterility and pH, and the water committee. Tremendous quantities of pharmaceutical-grade water—some of Eli Lilly's water tanks are 12 feet wide and two stories tall—are required to manufacture injectable drugs and vaccines.
“When we got the green light,” he says, “we were off and running.”

In 2016, Bolden and a colleague, Kelly Smith, began testing rFC in earnest. By then another manufacturer, Hyglos (now owned by bioMérieux) had entered the market, so the duo tested both compounds to compare their efficacy to LAL. The effort required their undivided attention and a painstaking level of precision. “Instead of one test, we did three,” Bolden says. People’s lives are at stake, after all, and they needed to be certain that rFC detects 100 percent of the endotoxin in a sample.

They published a paper in 2017 demonstrating that it does. The same year, a third rFC manufacturer, Seikagaku in Japan, was making a similar recombinant and leading similar comparative studies.

Decades after Ding began her work to create a better, more humane endotoxin test, a growing body of evidence points to rFC’s equivalency, and perhaps even its superiority, to LAL. But the race is not over: Who among the other major drug manufacturers will pick up the baton, and how soon?

Jay Bolden holds two vials of rFC—one from each of two manufacturers. Thanks to his perseverance, Eli Lilly will transition 90 percent of its endotoxin tests from LAL, which relies on bleeding horseshoe crabs, to rFC. Photo: Timothy Fadek/Redux
Early on May 10, armed with his binoculars and scope, which he’d hauled from Indianapolis, Bolden headed to Reeds Beach in Cape May. Thousands of gulls—Laughing, Herring, and Ring-billed—practically carpeted the beach before him, their calls near-deafening. Just beyond, at the water’s edge, were hundreds of horseshoe crabs: large females had two, three, and up to 11 males attached as they spawned in the surf.

“Just being here—it’s the culmination of five years of effort,” he says. “By controlling what I can control, I effected conservation of these animals.”

At 1:30 p.m., a crowd gathered at New Jersey Audubon's Center for Research and Education to hear Bolden make his big announcement, on behalf of Eli Lilly, that points to a more fortuitous future for the horseshoe crab. The event was organized by Revive and Restore, a conservation biotech organization, whose goal for horseshoe crabs is “to overcome corporate and government inertia to the synthetic alternative.” They convened representatives from conservation organizations and the pharmaceutical industry to make a splash about Bolden's announcement—one they hope will be big enough to pressure other companies to follow Eli Lilly's lead. Among the 50 people in the audience were Lonza's Burgenson, as well as Dina Laoni from Hyglos, Ned Mozier, a senior director at Pfizer who's published work comparing rFC and LAL, and representatives from local conservation groups.

After opening remarks from New Jersey First Lady Tammy Murphy, followed by Eric Stiles, president and CEO of New Jersey Audubon, and Ryan Phelan, president and co-founder of Revive and Restore, Bolden had his turn at the podium. Interrupted by bouts of applause, he announced that Eli Lilly plans to make a major transition to rFC. His work found that not only does rFC detect endotoxin as well as LAL, it yields fewer time-consuming false positives. Further, it’s less wasteful, and therefore less expensive. Once a vial of LAL is reconstituted, Bolden had told me in Indianapolis, what isn’t used is often thrown away. With rFC, “we make what we need with a small excess, and then we save what’s left over for the next run,” he says. “And using rFC complies with European Union and U.S. government directives to reduce and replace animals in medical testing and products.”
Jay Bolden, an avid birder and Eli Lilly biologist, looks through his scope at Reeds Beach in Cape May, New Jersey to watch for migratory birds along the New Jersey coast. The months of May and June are mating and spawning season for Horseshoe Crabs and the Delaware Bay area sees a high level of activity. Photo: Timothy Fadek/Redux

The FDA is now reviewing Eli Lilly's application for a new migraine-prevention drug, galcanezumab. According Bolden, if the application is successful, it will be the first FDA-approved drug to be released to the market with rFC as its endotoxin test.

More important to horseshoe crabs, though, is a less glamorous transition already underway at Eli Lilly's facilities. Two of its biggest manufacturing plants now test their pharmaceutical water with rFC, and a third, in Branchburg, New Jersey, only a few hours drive north of Delaware Bay, will begin within the month. By the end of 2019, the company aims for all eight of its manufacturing sites to test water with rFC. When this transition is complete, the company will have reduced its LAL use by approximately 90 percent. After 14 years of trying to find a market for rFC, Lonza's Burgenson says he's “over the moon.”

“We hope that companies will act swiftly,” says Stiles of New Jersey Audubon. “There's a time factor here.” Shorebird populations are crashing. According to Niles, the shorebird biologist, Red Knots are leaving Delaware Bay hungry. Last year, only 20 percent gained the weight necessary to make it to the
Arctic and successfully breed. Stiles and Niles are both hoping that other large pharmaceutical companies, many of which are in New Jersey, will switch to rFC. “It’s a win-win,” Stiles says. “These companies can do well by doing good.”

The good could be substantial. Every year, the biomedical industry takes some 500,000 to 600,000 horseshoe crabs from the sea. If the rest of the pharmaceutical industry follows Eli Lilly’s lead—tests its manufacturing water with rFC, and reduces its use of LAL by about 90 percent—that’d potentially leave an extra 450,000 to 540,000 horseshoe crabs untouched every year. That would benefit not only Red Knots, but the Ruddy Turnstones and Semipalmated Sandpipers that also desperately need those eggs.

Bolden is hopeful. After the press conference, he headed back out again, binoculars in hand, to admire flocks of shorebirds feeding at Delaware Bay. As he gazed at the Red Knots, he could take great satisfaction knowing that in running his leg of the relay, and running it well, he’d set an enormous and important precedent. Now, though, it’s up to other pharmaceutical giants, many located in New Jersey, to switch to rFC, so Delaware Bay can once again be awash in horseshoe crabs and shorebirds.

Deborah Cramer is the author of The Narrow Edge: A Tiny Bird, An Ancient Crab, and An Epic Journey, in which she accompanied Red Knots on their extraordinary migration from Tierra del Fuego to the Arctic. The book received the Rachel Carson Book Award from the Society of Environmental Journalists and the Best Book Award from the National Academy of Sciences.

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