Bashing Botulism

Scientists Sleuth World's Most Powerful Toxins

Mom may have warned you not to eat food from a can that's dented, swollen, or leaking. As usual, she was right. Her concern may have been that you'd get botulism.

This illness is caused by botulinum toxin, which is produced by a soil-dwelling microbe, *Clostridium botulinum,* and several of its *Clostridium* cousins. A damaged can could become contaminated, and, in the dark and warmth of your kitchen cupboard, the microbe could flourish—producing the deadly toxin.

Even though cases of foodborne botulism poisoning are rare in the United States today, they're nonetheless of concern. After all, botulinum toxin is the world's most potent natural toxin—millions of times more poisonous than cyanide.

The toxin and the *Clostridium* microbes that produce it are the focus of studies by ARS food safety experts at the Foodborne Contaminants Research Unit, part of the agency's Western Regional Research Center in Albany, California, near San Francisco. These scientists are developing new and better ways to detect the toxin quickly, easily, and inexpensively. And they're looking for important differences in the potency of the natural toxin—the way it occurs in nature—as compared to purified toxin, the kind most scientists prefer to study.

Portrait of a Killer

Botulinum toxin occurs in seven different forms—serotypes A through G. Serotypes A and B are culprits in about 90 percent of the foodborne botulism cases in the United States. Within this suite of seven serotypes, scientists have identified more than 100 subtypes.

No one knows for certain why *C. botulinum* produces the toxins. "It probably produces toxins to kill the organism that it is invading," says John Mark Carter, chemist and leader of the Albany research team.

Botulinum is a protein that behaves as a neurotoxin, interfering with the neurological system that would otherwise transmit vital signals throughout your body. In particular, the toxin can cut off normal messaging to muscles, causing paralysis. In the worst case, the toxin paralyzes the muscles of the diaphragm, leading to death by suffocation.

There's no federally approved vaccine against botulinum. An injection of horse antiserum can help remove the toxin from a patient's bloodstream, but this treatment may produce serious side effects. Victims who don't die from botulism poisoning require months of treatment.

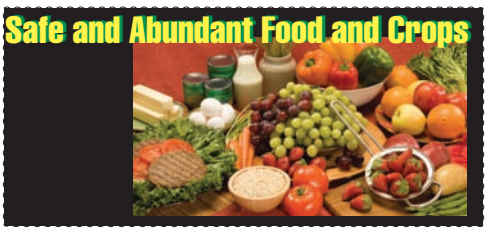
According to the U.S. Centers for Disease Control and Prevention, botulism symptoms include drooping eyelids, slurred speech, blurred or double vision, dry mouth, and, of course, weakening and paralysis of muscles. The good news is that, in most instances, botulinum toxin in food can be inactivated by routine cooking—boiling, baking, or roasting, for example.

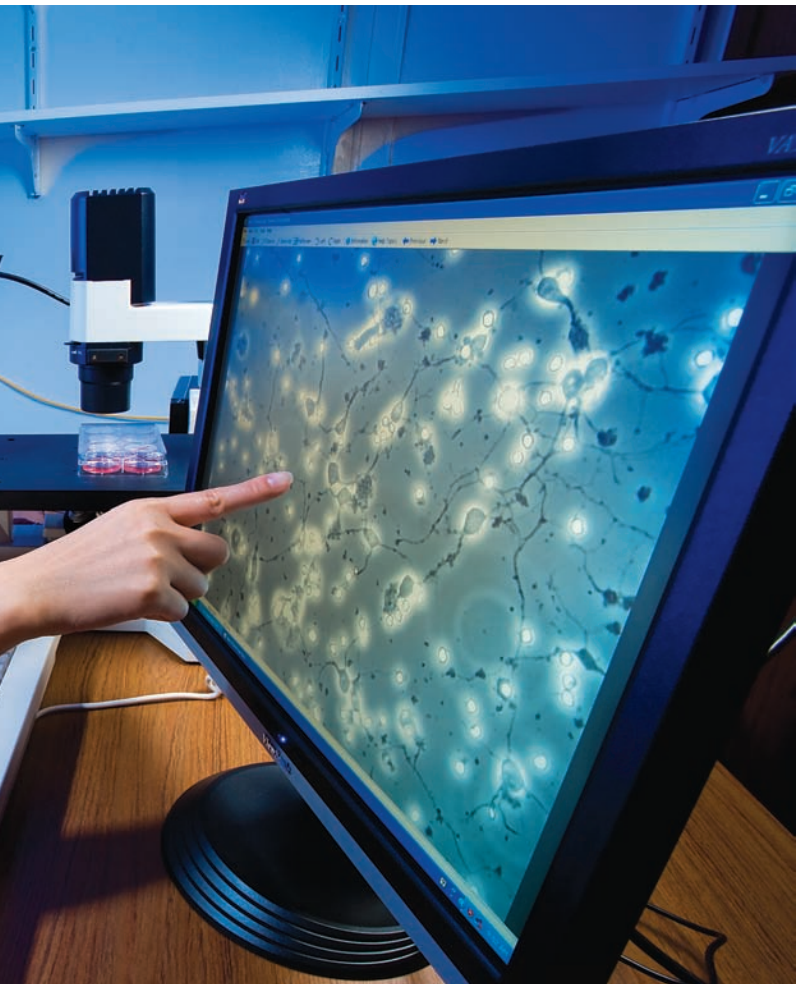
STEPHEN AUSMUS (D1416-2)

Biologist Luisa Cheng and technician Thomas Henderson II use an inverted microscope to view nerve cells grown in the laboratory for use in assays to detect botulinum toxin.

New Antibodies Improve Detection

For decades, the gold standard of tests for detecting botulinum toxin has been an assay that requires the use of laboratory mice. The assay takes at least 4 days, preferably 8, to perform correctly and is neither portable nor economical. That's why biologist Larry Stanker and two Albany colleagues—biologist Luisa Cheng and biochemist Miles Scotcher—developed a new and improved assay for detecting botulinum toxin serotype A.





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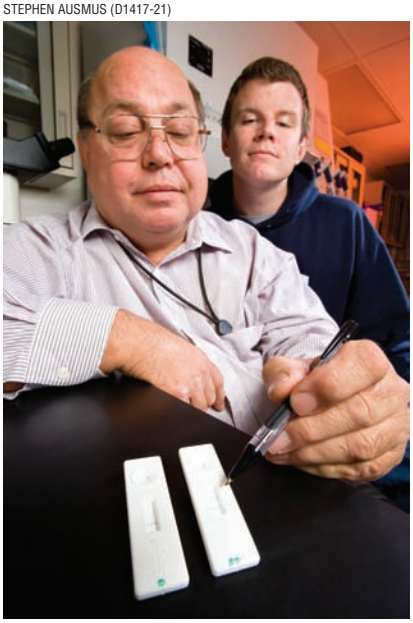

Biologist Larry Stanker (foreground) and biochemist Miles Scotcher evaluate the results of an assay, developed with a corporate partner, designed to detect botulism toxin in as little as 15 minutes from a single drop of sample.

The new assay is based on laboratory-built molecules, known as "monoclonal antibodies," which can bind to the toxin. Monoclonal antibodies that bind to serotype A toxin aren't new, but the ones the Albany team developed may be the most sensitive yet produced, that is, they're capable of detecting the toxin in minuscule amounts.

Stanker has formatted these antibodies into an assay that's 10 times more sensitive than the mouse assay, yet is less expensive and is easier to use.

Stanker expects to complete assays for detecting serotypes B and E sometime this year. Already, he has worked with Safeguard Biosystems, Inc., of San Diego, California, to package two of the serotype A antibodies into a dipstick-style test kit that looks and operates much like a home pregnancy test. The botulinum kit is intended for testing liquids, such as beverages, or clinical specimens such as blood or urine.

Test Tells Toxin's Activity Level

Though it has many advantages over the mouse assay, the dipstick test can't differentiate activated from inactivated toxin. To address this problem, chemist Reuven Rasooly and Albany colleagues incorporated one of the new serotype A antibodies into a test that determines whether the toxin is active or inactive.

Toxin that's been inactivated by heating "won't show up in this test," Rasooly says.

The test sequentially targets two different parts, or subunits—a so-called "heavy chain" and a "light chain"—of the botulinum toxin. For the test, magnetic plastic beads—about 5,000 times smaller than a marble—are coated with one of the new antibodies. Then, the antibody-coated beads are exposed to a sample, such as a fruit juice.

If there are any botulinum toxin molecules in the juice, their heavy-chain subunits will bind to the monoclonal antibody on the magnetic beads. This isolates and concentrates the toxin on the beads, allowing it to be easily extracted from the sample by collecting the beads with a magnet.

Next, these toxin-coated beads are exposed to a liquid containing laboratory-built molecules known as "synthetic peptides." The light-chain subunits of active toxin can cut these peptides neatly in two.

In the human body, this cleaving disrupts neuron function and leads to muscular paralysis. In the laboratory test, this same cleaving causes the peptides to fluoresce. Only active toxin—not heat-inactivated toxin—can cut the peptides. Thus, the fluorescence—which can be measured easily and with high sensitivity—is an excellent indication of the activity level of the toxin.

The idea of measuring the cleaving of peptides isn't new, nor are the magnetic beads. It's Rasooly's particular combination of the magnetic beads, the synthetic fluorescing peptide, and the superior new monoclonal antibody that make his peptide-

cleaving test more successful than others of this sort devised for measuring serotype A activity levels.

The assay is comparatively inexpensive and takes only about 20 minutes to perform, Rasooly says. To develop the test, he worked with research leader Carter and others in the Albany team, including Cheng, Stanker, chemist David Brandon, and molecular biologist Xiaohua He.

Natural Versus Pure Toxin: Differences Probed

Scientists have studied botulinum toxin for years. But most of that research was done with highly purified toxin.

"Purified toxin provides beautiful results in scientific laboratory tests," says Cheng. "But in nature, the toxin is accompanied by a host of nontoxic accessory proteins. The accessory proteins help protect the natural toxin from harsh conditions in your stomach created by enzymes and by gastric acid."

Purification removes those accessory proteins. So, Cheng is leading a series of innovative studies of the potency and availability to the body—or "bioavailability"—of the purified, as compared to natural, toxin.

In a study conducted with Brandon, Stanker, and former Albany chemist Bruce Onisko and colleagues at the University of Wisconsin-Madison and University of California-Davis, Cheng has discovered new properties of these proteins. Onisko's mass spectrometry analyses of the proteins have provided new information about their composition.

Cheng's studies are providing more information about whether accessory proteins, when ingested with food, make natural toxin more—or less—bioavailable than purified toxin.

Meanwhile, in related work, the team completed a study of the damage that the natural and purified serotype A toxins cause to lab-animal organs and tissues. This study is one of the most detailed of its kind for this serotype.

The researchers have documented their findings in *Toxicology,* the *Journal of Immunological Methods,* the *International Journal of Food Microbiology,* and *PLoS ONE*. Each of their discoveries helps science move a step closer to loosening the grip of botulism's seven deadly toxins.—By **Marcia Wood,** ARS.

This research is part of Food Safety, an ARS national program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

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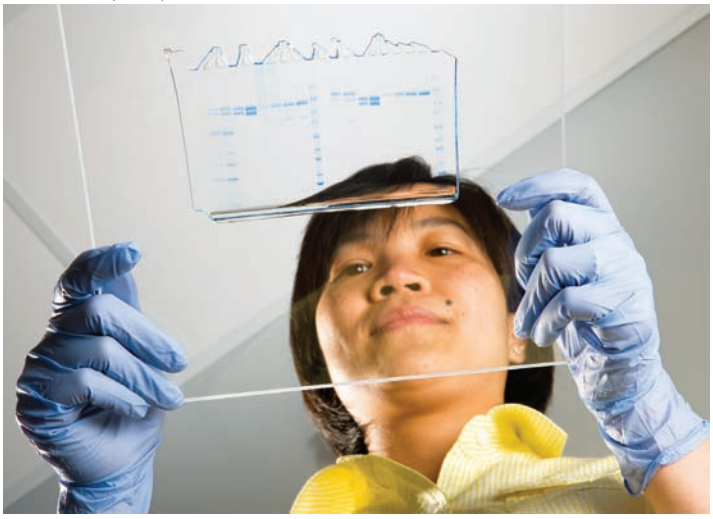

Using protein gel electrophoresis, Luisa Cheng separates crude botulinum toxin into individual protein bands that will help identify which protein component could affect toxicity.

The botulinum toxin assay is comparatively inexpensive and takes only about 20 minutes to perform.

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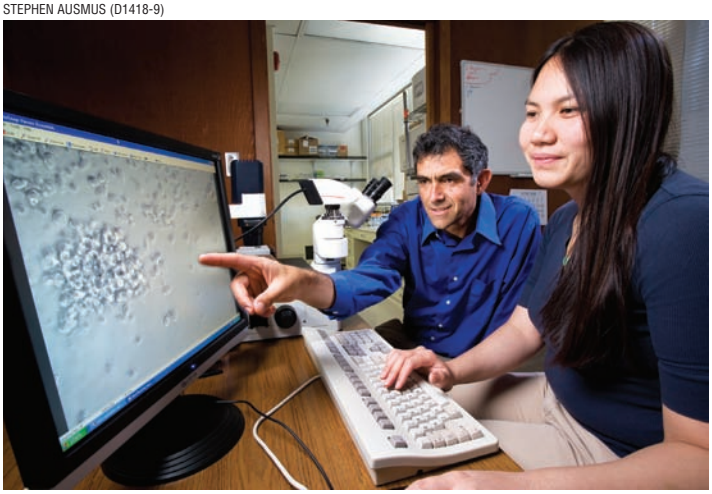

Besides investigating botulinum toxin, chemist Reuven Rasooly is studying toxins produced by *Staphylococcus aureus.* Here, after extracting staph toxin from food, Rasooly and bioscience technician Paula Do look for replication of spleen cells. Replication is a measure of the amount of biologically active toxin.