

# Of Mad Cows and Englishmen

## Telltale protein betrays disease

By STEVE STERNBERG

**A** decade ago, many cows on farms throughout the United Kingdom began to fall ill. Worried owners watched as the disoriented animals staggered about, lowing pitifully, and eventually died. Veterinarians recognized the symptoms as similar to those of scrapie, a communicable brain disease that attacks sheep.

The cattle had become infected, it was thought, when they ate feed that had been fortified with sheep offal—brains, spinal cords, and other organs. Though the practice of adding offal to feed was banned in 1989, the epidemic of mad cow disease continued into the 1990s and peaked in 1992 at 37,000 new cases.

The story took an alarming new twist this spring, when doctors found that 10 people had died of what looks like a human version of mad cow disease (SN: 4/13/96, p. 228). The cases prompted the 15-nation European Union to ban exports of British beef and demand the slaughter of British cattle. The United Kingdom agreed to destroy 4.5 million head—almost half the nation's herd—that were old enough to have eaten infected offal. Some 170,000 cattle have been killed so far.

The epidemic might have been kept in check if there had been a simple test for mad cow disease. But the only way to detect infected cows was to remove brain tissue from each animal and study it, a virtual impossibility with the millions of cows involved.

Now, teams of researchers in the United States and Germany report that they have independently developed more practical tests for mad cow disease. If the tests prove accurate, the researchers say, they will allow farmers to cull individual infected cows from their herds.

The tests also enable doctors to diagnose Creutzfeldt-Jakob disease (CJD)—a rapidly degenerative disease of the human brain that was first recognized in 1920. Furthermore, the U.S. test appears to detect the strange human variant of mad cow disease that galvanized officials in the United States and Europe into taking steps to avert a new epidemic.

**M**ad cow disease and CJD have proven difficult to diagnose because the protein thought to cause both ailments is tricky to detect in living tissues. Known as Prp, for prion-related protein, it is the Dr. Jekyll and Mr. Hyde of brain biochemistry. In healthy people, Prp acts as an ordinary cell-surface receptor, but its function is not clearly understood. In some humans and animals, however, Prp undergoes a deadly transformation.

This misshapen Prp—a prion—transforms normal Prp molecules into more prions. As prions proliferate, they clump together in tangled masses inside nerve cells, overloading them and perforating their delicate membranes (SN: 9/24/94, p. 202). Some scientists theorize that prions work alone, others that they act in concert with another protein or virus.

Either way, prions riddle the brain with holes until it is as porous as a sponge. These "holes in the cells themselves" prompted Clarence J. Gibbs of the National Institutes of Health in Bethesda, Md., to name this family of diseases transmissible spongiform encephalopathies (TSEs). Besides mad cow disease, scrapie, and CJD, this family includes Kuru, a disease in New Guinea thought to result from cannibalism.

The new tests don't look for prions but for particular proteins in an infected person or animal's spinal fluid. These proteins, members of the family called 14-3-3, normally reside only in brain cells. When found in spinal fluid, these substances, known as marker proteins, signal that something has damaged the brain cell membranes, causing them to leak.

Developed by Michael G. Harrington of the California Institute of Technology in Pasadena and Gibbs' team, the U.S. test detects 14-3-3 proteins. The German test relies on two of these proteins, known as 130 and 131. Inga Zerr of the Georg August University in Göttingen and her coworkers describe their test in the Sept. 28 LANCET.

"We surmise that the reason we find a high concentration [of 14-3-3] in spinal fluid is the pathological destruction of brain tissue," Gibbs says. He and his col-

leagues report their findings in the Sept. 26 NEW ENGLAND JOURNAL OF MEDICINE.

**F**or more than a decade, researchers had been looking for a way of detecting TSEs without taking samples of brain tissue. That costly and risky procedure can go awry, leading to bleeding, infection, or death. "There's also the risk of exposing hospital personnel to an infectious agent," says Martin Zeidler of the National CJD Surveillance Unit in Edinburgh.

To diagnose both CJD and the human version of mad cow disease, Zeidler has relied primarily on observations made without sampling brain tissue. People with either disease spiral rapidly downward into dementia, their limbs jerk involuntarily, and a peculiar brainwave pattern, sharp spikes at 1-second intervals, shows up on an electroencephalogram. The spikes betray damage to nerve circuits throughout the brain.

Quick death also characterizes these diseases—patients typically survive just months after dementia sets in.

If the course of the disease is prolonged or if a person's brain waves vary from what doctors have come to expect, diagnosis becomes far trickier. In such cases, doctors may mistake CJD for Alzheimer's disease.

**I**n their search for a practical diagnostic test, Gibbs and Harrington focused on proteins 130 and 131, which they found in the spinal fluid of the 21 CJD patients they had tested. Healthy people and most people with other brain diseases did not have these proteins in spinal fluid. Of 500 people with Alzheimer's disease, Parkinson's disease, or other neurological ailments, only a handful had 130 or 131 in their spinal fluid.

Although these proteins appeared to be excellent markers for the disease, they were difficult to detect. The only test available was so difficult to perform and so excruciatingly slow to show results—4 or 5 days—that it was virtually useless except for research and for

diagnosing particularly tricky cases.

To produce a simpler test, Gibbs and Harrington needed to identify these proteins in small amounts of spinal fluid. Working with Gibbs, Gary Hsich, now a pediatric resident at the University of Pennsylvania in Philadelphia, developed a way to separate the proteins from spinal fluid. Then he added an antibody that latches onto the telltale proteins. The resulting protein-antibody complex appears as a dark horizontal stripe on a piece of nitrocellulose paper.

Gibbs says the method is simple and reliable. "It can be used by any diagnostic lab in the world." Almost as important, the test is relatively quick. "Now we can do 40 samples per gel in 24 hours," he asserts.

**G**ibbs, Harrington, Hsich, and their colleagues then used the test to demonstrate that proteins 130 and 131 are members of the 14-3-3 family found inside brain cells. The test also indicated that 14-3-3 proteins are not prion-related.

The researchers tried the test on spinal fluid from 71 people with TSEs. It accurately detected 14-3-3 proteins in 96 percent of the specimens. In 66 samples from people with viral encephalitis, stroke, brain hemorrhages, and other non-TSE neurological disorders, the test showed 14-3-3 in only a few cases. This indicates that the test is specific for TSE,

the group reports.

To satisfy himself that the test is accurate, Zeidler sent Gibbs and his colleagues 16 additional specimens from patients with known or suspected CJD and 12 from people with the human version of mad cow disease—including samples from two cases that have not yet been made public. He also sent off specimens from patients with other neurological diseases. Zeidler declines to reveal the results of this comparison because he and his colleagues are preparing to submit them in a letter to LANCET.

Gibbs and his colleagues also tried the test on spinal fluid from cattle, sheep, and chimpanzees. Some of the animals had been infected with prions, some had not. The test detected 14-3-3 in 87 percent of the infected animals. In animals that were not infected, only 3 percent of the tests gave a positive result.

Pharmaceutical companies have no reservations about the value of such a test. Gibbs says they have begun queuing up to license the new technology, which was patented by NIH and Cal Tech. The researchers are preparing to simplify the test even further so that it can be administered as routinely as an HIV test.

**E**ven before the new test becomes available, regulators in the United States have announced that they will take steps to ensure the safety of livestock feed—and ultimately of the

people who dine on beef.

The Food and Drug Administration is likely to ban the use in cattle feed of all protein derived from certain animals or just that from their eyes, brains, spinal cords, and lower intestines. The banned group could include sheep, beef, other ruminants, and perhaps swine, says Bert Mitchell, director of surveillance and compliance in the FDA's Center for Veterinary Medicine.

Since the emergence of mad cow disease in the United Kingdom, the U.S. beef industry has relied on a Department of Agriculture surveillance program that each year culls cattle showing symptoms of neurological disease, slaughters them, and examines their brain tissue.

Although about 5,000 head are tested each year, none has been found to have mad cow disease—proving that the ailment has not yet emerged in the United States, says Gary Weber, executive director of regulatory affairs for the National Cattlemen's Beef Association in Washington, D.C.

Cattle farmers would welcome a simple, accurate test, says Weber. "That's where this new test from NIH could help us."

Not surprisingly, Gibbs is delighted with the outcome of years of painstaking effort. "It's a very great satisfaction," he says, "knowing that you have been able to do something that's going to help in human medicine—and veterinary medicine as well." □

#### lecture assignment #4

"Infectious agent" is a term often applied to a disease-causing substance that is transmissible from one organism to another. Ordinarily, this transmission occurs between members of the same species. Sometimes, members of different species, usually closely related, may also become infected. Bacteria, viruses, fungi, certain protozoans and various worms have long been recognized as infectious agents. Within the last few years a new and rather mysterious infectious agent called a **prion** has been described. Some of the answers to these questions can be found in this article. Others can be found on the Internet by performing a search for the key word PRION. **You may respond by e-mail or in typewritten form (handwritten papers will not be graded) no later than 1:30 p.m. on Friday, 8 November.** Subject line for e-mail is 101#4 Your Name. 15 points.

1. According to this article, what do the letters Prp stand for?
2. According to this article, where are Prps found in normal, uninfected cells?
3. What do the letters PRION stand for?
4. What are PRIONS thought to do to Prps?
5. What effect do prions have on the *plasma (cell) membranes of individual nerve cells*?
6. What is the *cumulative effect* of prions on the brain as a whole?
7. The article mentions a number of diseases caused by prions, including ① **mad cow disease (bovine spongiform encephalopathy = BSE)** in cattle, ② **scrapie** in sheep, and ③ **Kuru** and ④ **Creutzfeldt-Jakob Disease (CJD)** in humans. Search the internet and identify four prion-caused diseases different from those mentioned in the article, two in non-humans and two in humans.
8. Describe how a prion-related disease *progresses in humans* from beginning to end.
9. The article mentions testing spinal fluid for the presence of "14-3-3" proteins as a way to tell whether or not a person is infected with a prion disease. The 14-3-3 proteins are increasingly being found to be very important signaling molecules within cells.
  - a. The article talks about proteins 130 and 131, both members of the 14-3-3 protein family. Where are proteins 130 and 131 normally found in the human body?
  - b. Why might the presence of 14-3-3 proteins in the spinal fluid be an indication of prion infection?
  - c. According to this article, how *reliable* is the test for 14-3-3 proteins as an indicator of prion infection?
  - d. In general, how reliable do you think a test should be in order to warrant taking remedial action? Explain the reasoning behind your answer.

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Even though prions have been known about and extensively researched for a number of years, the actual function of the Prp in the uninfected cell has been a mystery. It appears that the normal function of the Prp is now coming to light. Read the article “Prion protein’s role in body revealed” (below) and answer the following questions:

10. According to this more recent article, what might be the function of the normal form of the prion protein (PrP) in an uninfected cell?
11. In your own words, describe the experiment that researchers did in order to come to this conclusion.

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“This question of the normal function of PrP has become an obsession for me.” —ADRIANO AGUZZI

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## Prion protein’s role in body revealed

Normal form of disease agent is important for nerve function

By Tina Hesman Saey

A molecular Dr. Jekyll finally has a day job — as an electrical lineman. A new study suggests that the normal form of prion protein helps maintain the insulation that speeds electrical signals along nerves.

In its twisted Mr. Hyde form, the prion protein causes fatal brain-wasting diseases, such as mad cow disease in cattle and Creutzfeldt-Jakob disease in people (*SN*: 8/16/08, p. 20). But the normal form of the protein, which is found in neurons in people and other mammals, is also a good guy, a new study shows. The protein appears to direct cells called Schwann cells to wrap around neurons and pro-

duce myelin, a type of insulation that aids electrical communication between the cells. This newly discovered role for the normal form of the prion protein — called PrP(C) or just PrP — could link problems with the protein to nerve disorders called peripheral neuropathies, researchers led by Adriano Aguzzi, a neuropathologist at the University of Zurich, report online January 24 in *Nature Neuroscience*.

Much attention has focused on how the protein’s infectious form leads to disease, but its usual role in the body has been a mystery. “This question of the normal function of PrP has become an obsession for me,” Aguzzi says.

In the new study, researchers studied

mice genetically engineered to lack the prion protein. The mice had abnormal myelin sheaths around their sciatic nerves, long nerve cells that run from the lower back to the foot. Electrical signals traveled more slowly down these nerve fibers in mice lacking prion proteins compared with normal mice, the team found. Restoring prion proteins to nerve cells allowed the cells to keep sheaths healthy.

If the prion protein does play a role in myelin maintenance, that could pose a problem for gene therapy that would remove PrP from animals to prevent prion diseases, says Claudio Soto, a neuroscientist at the University of Texas Health Science Center at Houston. He and colleagues have genetically engineered cattle that lack the prion protein. The question remains whether a lack of PrP can cause peripheral nerve problems, Soto says. 