



Tea made with broccoli sprouts holds promise as an antidote to a toxin linked to liver cancer.

WORLDWIDE, BILLIONS EAT AFLATOXIN,
A FUNGAL POISON THAT KILLS.
SO HOW TO KEEP IT AT BAY?
BY REID R. FRAZIER

THE OTHER GREEN TEA

TURKEY X DISEASE

In the spring of 1960, a plague descended on the turkeys of Southeastern England. More than 100,000 turkey poults, partridges, and pheasants fell prey to an ailment that rendered them lethargic, unable or unwilling to eat, and dead within a week of the first symptoms. When they died, they assumed a characteristic arched position with their neck, legs, and head all drawn backward. Autopsies showed tissue death and lesions on the liver, as well as swollen kidneys. At about the same time, a similar liver-affecting illness broke out in trout farms in the Western United States.

There were no indications of a viral or bacterial vector—all signs pointed to some type of poisoning. At first, scientists could find no toxin—at least none that was known. They suspected the poison was a plant-produced alkaloid, or perhaps something from a fungus. The condition was given a name befitting its mysterious etiology: “Turkey X Disease.”

ILLUSTRATION | JULIETTE BORDA

Veterinary epidemiologists soon located a commonality among the affected turkey farms. They all bought feed processed at a pair of mills in and around London. Both processed Brazilian peanut meal brought over on the freighter *Rosetti*. After analyzing the *Rosetti* peanuts with paper chromatography, scientists found a high prevalence of an unknown material that appeared bright blue under UV light.

An analysis of the toxic nuts later showed high levels of hyphae, the filament-like branches of a fungus. The suspect was a mold that liked heat and humidity and often grew in the tropics: *Aspergillus flavus*. The fungus gave science a name for the strange poison that killed 100,000 English turkeys—aflatoxin.

Since Biblical times, people had long suspected that eating moldy foods could make them and their animals sick. Then, with the discovery of aflatoxin, there was definitive proof. Scientists scrambled to identify the mechanisms behind this new class of fungal poisons, or mycotoxins.

Samples of the bright blue material ended up in the lab of Gerald Wogan, an MIT toxicologist. Wogan regrew the fungus responsible for the poisoning, and, along with MIT chemist George Büchi, characterized the chemical's structure. After feeding the chemical to ducks and rats, Wogan found the substance was not only toxic, but highly carcinogenic. In ducks and rats, it could induce liver cancer with a mere parts per billion dose, a level most of the world's food supply would likely exceed.

The bright blue substance, it soon became clear, posed a major public health problem. If tiny doses of this poison could kill turkeys and trout, what would it do to the billions of people who were probably eating it every day? At the time, UNICEF was considering using peanut meal as a high-protein component of infant formula.

One of Wogan's PhD students in the early 1970s was a young scientist named Thomas

Kensler. He remembers those times. "We were really just beginning to understand that carcinogens interact with DNA," says Kensler, who recently joined the Pitt faculty as professor of pharmacology and chemical biology. Kensler researched the compound's mechanisms of action—why it was such a biological hand grenade. By the end of the decade, one of Kensler's MIT colleagues, John Essigmann, found that an aflatoxin metabolite formed an irreversible bond with DNA inside the liver, keeping the cell's genetic message from correctly transcribing. By the time Kensler earned his PhD, the question of how aflatoxin worked was largely answered. Now it was time to address the problems aflatoxin created. This, as it turned out, would be the hard part.

CAST OUT MOLDY STONES

The earliest recorded public health advice concerning fungus is set down in Leviticus, in a dictum from God to Aaron and Moses. "On the seventh day, the priest shall return. If he sees that the mold has spread on the walls of the house, the priest shall order the stones with the affection in them to be pulled out and cast outside the city into an unclean place."

During the Middle Ages, a mycotoxin was responsible for the disease known as St. Anthony's fire, a common ailment that caused convulsions and hallucinations and led to gangrenous blisters on victims' hands and feet. The poison was ergot, a product of *Claviceps purpurea*, a mold that grows on rye grass.

Ergotism made people more susceptible to the Black Plague and has been linked to events like the Salem Witch Trials and the religious movement known as the Great Awakening of 1741, during which "thousands experienced fits, trances, and visions," according to the historian Mary Matossian. Other mycotoxins have proved dangerous, even lethal—fumonisin, ochratoxin, the aptly named vomitoxin (it's linked with gastrointestinal disorders, in case you were wondering), to name a few.

It isn't entirely clear why fungi produce such powerful chemicals. Mycotoxins aren't necessary for the lifecycle or growth of a fungus, but scientists believe they may give the mold a competitive advantage against other microorganisms. "Is it part of a chemical warfare battle that the mold fights with bacteria or other organisms? Almost certainly," Kensler says. "I suspect it

was the acute toxicity of aflatoxin that led to its selection and elaboration by the fungi."

(Their potent antibacterial qualities, of course, led scientists to harness fungi for antibiotics like erythromycin and penicillin.)

The sharpest sword in the fungal arsenal is aflatoxin, so powerful it's been produced as a biological weapon. (Saddam Hussein reportedly produced 2,200 liters of the stuff in the early 1990s.) When ingested in high doses, it causes aflatoxicosis, a severe abdominal ailment that carries a 40 percent mortality rate. In low doses, chronic exposure can cause liver cancer, especially in those with hepatitis B virus, of which 350 million people are carriers.

The aspergillus fungus that produces aflatoxin was first described in 1729 by the Italian priest and biologist Pier Antonio Micheli. (Under a microscope, the mold looked like a holy water sprinkler, or aspergillum.) It's a widespread mold that eats organic matter, especially carbon-rich plant sugars. *A. flavus* and its other toxin-producing relatives, like *A. parasiticus*, are prevalent in the tropics, growing best in humid, warm temperatures, between 25 and 40 degrees Centigrade. It thrives on several important crops—corn, peanuts, and cottonseed. It tends to infect plants already weakened by drought or insect damage, and damp storage conditions accelerate the spread of its colonies. Aflatoxin is heat stable—you can't destroy it in cooking. All of this suits it perfectly to thrive in the fields and huts where much of the world's food originates.

Although feed and food are subjected to other contaminants here, aflatoxin is not a major problem in the United States. Part of the reason is climate—most corn grown in the United States falls outside the fungus' preferred habitat. Southern-grown cotton and peanuts, however, are susceptible to aspergillus. Yet irrigation, insecticides, and other modern agricultural practices keep mold away from many crops in the United States. The FDA also limits the amount of aflatoxin allowed in the food supply.

But none of these methods are feasible in large swaths of the developing world like Africa and East Asia, says Felicia Wu, Pitt assistant professor of environmental and occupational health in the Graduate School of Public Health who also holds an appointment in the School of Medicine.

"When we run across these problems in the United States, we throw out infected crops," Wu says. "You can't really afford to do that in Africa or Southeast Asia, where their choice is to either eat moldy foods or go hungry."



MIKE DRAZINSKI / UDOE

Thomas Kensler notes that aflatoxin is "perfectly engineered as a carcinogen."

Wu is studying ways to get aflatoxin out of the world's food supply. She codirects a pilot study on dampening the effects of aflatoxin in Africa that is supported by a \$2.7 million Bill & Melinda Gates Foundation grant.

As a doctoral student in engineering and public policy at Carnegie Mellon University in the early 2000s, Wu found that genetically modified corn harbored very little mycotoxin. Corn engineered with a gene from the insecticidal bacterium *Bacillus thuringiensis* (Bt) was remarkably resistant to aspergillus infection because the mold capitalizes on plants compromised by pest damage. "Insect damage exposes the sugars of the plant to the environment, and the fungi just love that," Wu says.

Wu is an affable and energetic young scholar (a finalist in the 1994 *Jeopardy!* Teen Tournament). Last fall, she spent time in Kenya, learning how farmers grow, process, and store their corn. She hopes that her team will soon conduct bioassays to determine how much aflatoxin the farmers have in their blood.

"We're looking at where the maize is being planted in the field, when it's being harvested, the conditions in which it's being stored, the conditions in which it's being sold," Wu says. Her team is searching for the most promising and cost-effective point to control for aflatoxin.

One of the interventions she has studied is administering the hepatitis B vaccine, which greatly reduces cancer risk in individuals exposed to aflatoxin. Another measure is biocontrol, in which farmers intentionally spread nontoxicogenic (friendly) strains of *A. flavus* to keep poisonous strains out. If done properly, biocontrol can reduce aflatoxin exposure by 50 to 90 percent. (Using transgenic crops like Bt corn would not work well in Africa, Wu says. There are laws against genetically modified organisms there. Also, African farmers save their seeds, and transgenic crops lose their efficacy after a generation or two.)

Wu and Kensler have taken on an ambitious task. More than 4.5 billion are exposed to aflatoxin through their food, the vast majority of whom live in the developing world. In portions of sub-Saharan Africa, almost everyone is exposed to aflatoxin. Where liver cancer is common, as many as 50 percent of the tumors carry a mutation linked to aflatoxin exposure. Liver cancer, the third-leading cause of cancer death worldwide, kills 600,000 people a year. Its five-year survival rate is 2 percent, and the average age of diagnosis is 47.

But cancer might be just part of the problem. Studies have found that aflatoxin stunts childhood growth and suppresses the immune system.

"We've seen a striking association between exposure to aflatoxin early in life—post-weaning—and impaired growth in the child in West Africa," says Chris Wild, director of the World Health Organization's International Agency for Research on Cancer. Though he cautions these findings are preliminary, Wild says the trends are alarming, especially if exposed children are more susceptible to the bacterial and viral vectors that contribute to the developing world's staggering childhood mortality rates.

Could aflatoxin have an effect on other major public health issues, like the HIV pandemic? It appears so. One study found HIV carriers with high exposure to aflatoxin have a more-severe disease course than those with lower exposure. Another found HIV patients with high aflatoxin exposure in Ghana had lower levels of immunoprotective T and B cells, critical to the body's reaction to HIV.

A RIDDLE

There are no obvious solutions to the worldwide aflatoxin problem. Getting rid of aspergillus in the field as we do in the West costs more than many farmers can afford. Hepatitis B vaccine is a priority, yet wouldn't protect the 350 million already infected by hepatitis. And hardly anyone has heard of the poison you're trying to eradicate.

Solving the riddle means finding solutions that could work anywhere and cost almost nothing. Wu is looking at a variety of interventions, each of which centers on educating farmers. Once they learn about the poison, Wu says, "the farmers ask to be tested for aflatoxin. They say, 'We'd like to know if we are exposed to this toxin.'"

One solution could be an approach Wild and his collaborators tested on farmers in Guinea. The scientists educated the farmers about aflatoxin, then taught them simple ways to dry and store their peanuts properly—making sure they dried harvested crops in the sun, for instance, and stored the peanuts in natural fiber bags, as opposed to plastic ones. Aflatoxin exposure dropped by 60 percent.

Wu says solutions like these are probably going to have the greatest impact.



Felicia Wu is looking for aflatoxin solutions that cost almost nothing.

"What I like so much about this package is that it's low technology," she says.

Even with these types of interventions, it's unlikely aflatoxin will be eliminated from the world's food supply anytime soon, Kensler says. "In these high-risk regions, we have no short-term prospect of getting aflatoxin out of foods. Our fundamental thought is, 'Well, given this unavoidable exposure to aflatoxin, can we make the host more resistant to that unavoidable dose?'" Kensler says.

He believes that one solution may lie in the bottom of your refrigerator.

QIDONG

It is twilight in Qidong, an agricultural peninsula along the northern lip of the Yangtze River Delta. A group of about a dozen factory workers and farmers mill around a concrete-floored kitchen. Tom Kensler stands, binder in hand, making sure everyone drinks the plastic bottle full of brownish liquid he's brought for them.

The brown beverage is a tea made from broccoli sprouts. This cruciferous vegetable is full of a chemical that seems to detoxify aflatoxin. (It appears your mother was right: Broccoli is good for you.)

The next day, at about 5 or 6 a.m., the dozen or so participants will return the liquid favor, in the form of urine samples taken overnight. The samples will be waiting for Kensler when he gets to his lab at 8. He'll then have the unenviable job of measuring them. "It's a great way to start your day," Kensler says. In a few months, Kensler will fill several rolling suitcases with bags of frozen urine and cart them back to the United States as checked baggage. ("It makes for some interesting conversations with customs agents," Kensler says.)

Qidong is relatively close to the mega-city of Shanghai, 50 kilometers to the south, across

the yawning mouth of the Yangtze. Though it's growing rapidly, Qidong remains largely rural, and many there still rely on locally grown crops for food, especially maize, served as a filling porridge. The hot and humid climate makes the peninsula a haven for the aspergillus mold. In the 1970s, the Chinese government mapped the prevalence of liver cancer around Shanghai. On a gradient map the government produced—with dark brown being the highest prevalence, and white being the lowest—Qidong's district looks like a piece of chocolate dropped into the froth of a cappuccino. The people of Qidong were 50 times more likely to contract liver cancer than their counterparts 150 miles away.

This map eventually made it into the hands of Kensler, who by then was a professor of toxicology at Johns Hopkins University. With John Groopman, a collaborator at both MIT and Hopkins, Kensler had isolated a key biomarker for aflatoxin and was using the biomarker to test ways to keep aflatoxin from binding to DNA in animals. He'd made a breakthrough with the drug oltipraz, which cut the carcinogenic effect of aflatoxin by 55 percent.

"After 10 years of working in animals, I could protect any number of rats and mice from liver cancer," Kensler says. "John Groopman came up to me one day and said, 'What do you think about putting this in people?'"

It wasn't long before Kensler and Groopman were on a flight to China.

Among the first projects the Hopkins group undertook was an epidemiological study of liver cancer in Shanghai. The group collected urine and blood from 18,244 men, then waited five years to see how many of them developed liver cancer. Men with aflatoxin exposure were 3.5 times more likely than nonexposed participants to contract liver cancer. Those with hepatitis B virus were seven times more likely. If they had both hep B and aflatoxin exposure, the men were 59 times more likely.

This was astounding—"an explosion of risk," Kensler says.

The two factors formed "a perfect storm" for carcinogenesis, says Groopman, the study's lead author. "There's an infection with hepatitis B, so there's a lot of cell proliferation, and at the same time there's a lot of DNA damage caused by the aflatoxin. The effect is on the order of magnitude of, say, asbestos exposure and cigarette smoking as risk factors for developing lung cancer."

BERMUDA TRIANGLES

Kensler came to broccoli sprout tea through the aflatoxin molecule's potentially troublesome chemical structure. When metabolized by a liver enzyme, aflatoxin forms an epoxide—a flat molecule with a short dogleg off one end. The dogleg consists of a three-sided chemical bond between a single oxygen atom and two carbons. Triangular shapes in chemistry are generally suspect—the acute angles apply more pressure on chemical bonds. (With their obtuse angles, hexagons are much more stable shapes, chemically speaking.) Those acute angles create what Kensler calls the "Bermuda Triangle" of toxicology. Add to that instability the fact that the aflatoxin epoxide is an electrophile—a positively charged substance seeking a negative charge. "It's a lovelorn molecule that's looking for an electron," Kensler says.

In the liver, the epoxide finds an electron on a negatively charged nitrogen in a specific location within the DNA. The epoxide slides snugly inside the double helix structure and forms an irreversible bond with the nucleotide. It binds 10 times better to DNA than any other bulky carcinogen.

"It's perfectly engineered as a carcinogen—and that's why it's so potent," Kensler says. (He, by the way, is married to another recent Hopkins recruit, Nancy Davidson, who directs the University of Pittsburgh Cancer Institute.)

Once the epoxide binds, it cannot unbind. This is where the cancer starts. "Perhaps that stretch of DNA can no longer be copied or transcribed, or it gets falsely transcribed," Kensler says.

"Mistakes are made, and you have errors in the progeny. It's a subtle chemical change that can have a profound impact in that cell or, more importantly, its daughter cells."

Since the 1970s, researchers had been looking at an enzyme group that could modify this chain of events. These enzymes, GSTs (glutathione-S transferases), produce the antioxidant glutathione, which acts like a sponge. Rather than binding to DNA, the aflatoxin epoxide bonds to a negatively charged sulfur atom in the glutathione molecule. The newly conjugated aflatoxin-glutathione is excreted, with no harm to the body. It's the body's natural way to rid itself of toxins.

Kensler wanted to amplify this effect, to shuttle aflatoxin off with an alternate "dance partner" before it latched on to the DNA. So he started looking for chemicals that could elevate GST expression, like oltipraz. It reduced DNA damage caused by aflatoxin, but there

was a drawback. Oltipraz was expensive and in limited supply. To boot, Chinese are skeptical of Western-style pills. So Kensler began looking for another approach.

Around that time, a colleague, Hopkins pharmacologist Paul Talalay, had found that sulforaphane, a metabolite of broccoli, present in even greater quantities in broccoli sprouts, was 100 times better than oltipraz at upregulating GST. The key to its success, Kensler and others believe, is sulfur. Many of the target molecules Kensler used to amp up glutathione and GST production in the liver are "decorated" with sulfur-based cysteine amino acids. Kensler thinks these amino acids act like antennae, looking for distress signals. If they "hear" a call for help, they send out a clean-up crew of transcription factors and other proteins to mop up the insulter. (Not everyone is convinced of the safety of this pathway. Kensler's convinced it's safe to target with foods like broccoli for disease prevention.)

Because tea drinking is part of Chinese culture, Kensler decided to see whether a broccoli sprout tea would lessen aflatoxin binding to the DNA. His team built a sprout-growing lab in Qidong, brewed tea in cauldrons, and filled hundreds of plastic bottles with the brew and a control liquid. (A little mango juice cuts the bitterness.) The results so far are encouraging—the aflatoxin biomarker is lower in those who drank the sprouts tea.

Kensler says he's had no problem finding volunteers for the study—everyone in Qidong knows someone who has died of liver cancer.

If the GST pathway works on aflatoxin in the liver, what about other organs or diseases?

"We think this is a very central protective mechanism of a cell," Kensler says. "We're working in a little sidebar relevant to a half-million people, but in fact, this pathway is going to touch the way we think about prevention and even treatment [for many diseases]." Kensler and others are testing broccoli sprouts on breast cancer, asthma, and degenerative diseases like chronic obstructive pulmonary disease.

This spring, Kensler moves from Johns Hopkins to a space in Pitt's Biomedical Science Tower. On his first day at Pitt in December, he surveyed the soon-to-be lab as a crew installed wiring in the room's bare aluminum studs. Kensler ordered a single architectural flourish to the layout—a glass splash guard next to a sink, which will hold a laminated satellite image. The photo shows a green peninsula, Qidong, above the cyan-tinted Yangtze. Aflatoxin country. The people of Qidong will remain in Kensler's thoughts here at Pitt. ■