PLANTS THAT HARM

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Hours after the raid on Pearl Harbor, Japanese aircraft from Saipan attacked the U.S. Marine barracks on Guam and bombed the USS *Penguin*. Two days later, 400 Japanese troops defeated the small U.S. Marine contingent there, taking over the island. Two and a half years later in the war, the United States retook the island, securing Guam on August 10, 1944.

The Japanese occupation of Guam was extremely difficult for the indigenous Chamorro villagers. Deprived of imported food, the Chamorros relied on traditional wild plants for sustenance, making flour from the seeds of the cycad trees (*Cycas micronesica* [Cycadaceae]), which are common in the island's forests.

Soon after the American recapture of the island, Harry M. Zimmerman, an associate professor of pathology at Yale, was assigned to the U.S. Naval Medical Research Unit on Guam. He reported a puzzling paralytic disease among the Chamorros, which he initially identified as amyotrophic lateral sclerosis (ALS):

> During the past few months, there were admitted to the medical wards of the Civilian Hospital 7 or 8 patients with a full blown clinical picture of Amyotrophic Lateral Sclerosis. This was quite surprising in view of the infrequency of which this neurologic disorder is encountered in the States ... . It is now planned to investigate the hereditary background of all these patients in the effort to throw some light on the factors concerned in the etiology of this obscure malady.

Following that report, National Institutes of Health (NIH) researcher Leonard Kurland and his assistant Donald Mulder found many Chamorros with tremors and cogwheel rigidity—a stop-and-go, ratchet-like motion—characteristic of Parkinson's disease, as well as cognitive impairment similar to that of Alzheimer's patients. Examining hundreds of death certificates dating back to the turn of the century, they linked their findings with the puzzling paralytic disease described by Zimmerman into what is now called amyotrophic lateral sclerosis/Parkinsonism dementia complex (ALS/PDC). The epicenter of disease was the remote southern village of Umatac: "One-third to one-fourth of all adult deaths in this village are due to amyotrophic lateral sclerosis." Kurland later added that "one-half of the adult population in this village dies either of ALS or of the Parkinsonism dementia complex." Studying autopsy tissue from the villagers, Zimmerman's collaborator Asao Hirano found that up to 20% of their brain mass was missing, replaced with dense neurofibrillary tangles formed from misfolded tau protein, a neuropathological hallmark of Alzheimer's disease. ALS/PDC was soon deemed a "neurological Rosetta stone."

Pedigree charts showed no clear hereditary relationship between afflicted villagers in Umatac, thus ruling out a genetic cause. Careful experiments by microbiologist Clarence Gibbs showed that an infectious agent was not involved. In 1967, nutritionist Marjorie Whiting sent several cycad seeds from

Figure 3.1 Cycad trees in Guam contain a toxin linked to a puzzling paralytic illness.

Figure 3.2 Female cycad trees produce seeds used to manufacture flour by the Chamorro villagers.

Guam to E. Arthur Bell, director of the Royal Botanic Gardens, Kew, for analysis. Could consumption of flour made from cycad seeds cause the puzzling paralytic disease suffered by the indigenous Chamorro people? He and his postdoctoral student Armando Vega isolated a new neurotoxic amino acid, β-N-methylamino-L-alanine (BMAA), from the seeds. Soon thereafter, Peter Nunn of King's College, London, discovered that chicks fed BMAA could not stand up and instead became paralyzed. Interest in BMAA increased in 1987 when Peter Spencer, Peter Nunn, and other investigators at the Albert Einstein School of Medicine reported that macaques dosed with BMAA had neurological deficits similar to those of ALS/PDC patients. This was consistent with NIH epidemiological studies, which showed the only significant risk factor associated with Guamanian ALS/PDC was the consumption of a traditional Chamorro diet. However, this second revival of the BMAA hypothesis was dashed when Mark Duncan at NIH argued that Chamorro villagers would have to consume hundreds of kilograms of cycad seed flour to receive an equivalent dose.

A key piece of the Guam puzzle was still missing: NIH investigators did not know that flying foxes—large megabats of the genus *Pteropus*—are considered a local delicacy by the Chamorro people. This disconnect illustrates the cultural asymmetry that is often present between scientists and indigenous people. Interviews conducted in the English language in a clinical setting can be intimidating to indigenous people and can inadvertently serve as a deterrent to disclosure of culturally sensitive information. Chamorro villagers knew that Westerners are often fearful of bats and would likely regard local consumption of these volant mammals to be distasteful. Ethnobotanists, in contrast, are trained to learn local languages, interview villagers in their own homes, and adapt their own behavior to the dictates of the indigenous cultures. Such empathetic steps can reduce the cultural asymmetry that would otherwise decrease the flow of information.

Figure 3.3 The kernels or gametophytes of cycad seeds are washed in water and then ground into flour for tortillas or dumplings.

β-N-methylamino-L-alanine (BMAA)

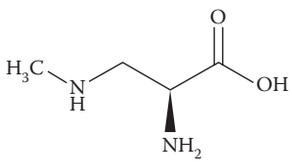

Figure 3.4 BMAA is a neurotoxic amino acid linked to Guam ALS/PDC.

Figure 3.5 Flying foxes, a delicacy among the Chamorro people of Guam, accumulate the neurotoxin BMAA when they forage on cycad seeds.

In the distant islands of Samoa, Paul Cox monitored the decline of flying foxes. He knew that the greatest threat to the diminishing megabat populations of Samoa was a result of commercial hunting and export to Guam, where the carcasses could command prices upward of $35 each. With colleagues Thomas Elmqvist, Dixie Pierson, and Bill Raney, he traveled to Lausanne, Switzerland, to ask the Convention on International Trade in Endangered Species (CITES) to place Samoan flying foxes on the endangered species list, thus banning international export. In Samoa, he read an article entitled "This Obscure Malady" in the October 29, 1990, *New Yorker* magazine by Terence Monmaney, which vividly portrayed the puzzle of the mysterious disease in Guam.

Sitting on the beach that evening, Cox saw a large flight of flying foxes pass overhead on their way to nighttime foraging in the rain forest. He suddenly realized that cycad toxins could potentially be biomagnified in the flesh of these large bats, as they feed on the juicy covering of cycad seeds. Later he shared his idea with his former graduate student, ethnobotanist Sandra Banack.

Several subsequent expeditions to Guam revealed flying foxes to be the most culturally salient food item among the Chamorro people. Many Chamorro men identified themselves as hunters of flying foxes. Prior to the war, flying fox hunters needed to climb trees at night, carrying woven nets. However, after the American military recaptured Guam, Chamorros obtained semi-automatic shotguns, greatly accelerating hunting success. Some hunters reported eating five flying foxes per week. One flying fox species in Guam became extinct, and the other became critically endangered. How could the hypothesized link between flying fox consumption and the obscure malady of Guam be confirmed?

Twenty-one flying fox specimens obtained from the Museum of Vertebrate Zoology at the University of California, Berkeley, and the Smithsonian Institution were analyzed for BMAA using high-performance liquid chromatography.

During the laboratory work, Sandra Banack, then a professor at California State University, called Cox at 2:00 a.m. He answered on the first ring. "We've got it," she said with excitement, referring to the BMAA peak on the chromatogram of flying fox tissue. BMAA concentrations in flying fox flesh ranged between 29 and 1202 μg/g, with consumption of a single flying fox yielding a BMAA dose equivalent similar to that of consuming 1,014 kg of cycad flour.

The research team discovered that BMAA is produced by cyanobacteria of the genus *Nostoc* harbored in specialized roots of the cycad tree. Accumulation of BMAA in the flying fox flesh increased 10,000-fold over microbial production. Previous examples of biomagnification, including DDT, were based on compounds that are soluble only in fat and thus accumulate as they

Figure 3.6 Baked or boiled in coconut cream, flying foxes are the most culturally important food items for the Chamorros.

BOX 3.1 LIQUID CHROMATOGRAPHY ON A NAPKIN

If you have ever spilled wine on a napkin, you likely have observed different shades of color appear as the stain enlarges. In essence, the different pigments in the wine separate based on their ability to move through the fabric. High-performance liquid chromatography (HPLC) utilizes the same principle by separating different chemical entities moving through a column of solid adsorbent material based on their mobility in a solvent. At the end of the separation, an ultraviolet or fluorescence detector records the light reflected from the molecules. The resultant reflectance or absorbance is plotted against time of movement on what is known as a chromatogram, with each peak showing a different molecular entity. In an advanced version called ultra performance liquid chromatography, high-pressure pumps produce a similar result with smaller quantities and greater precision.

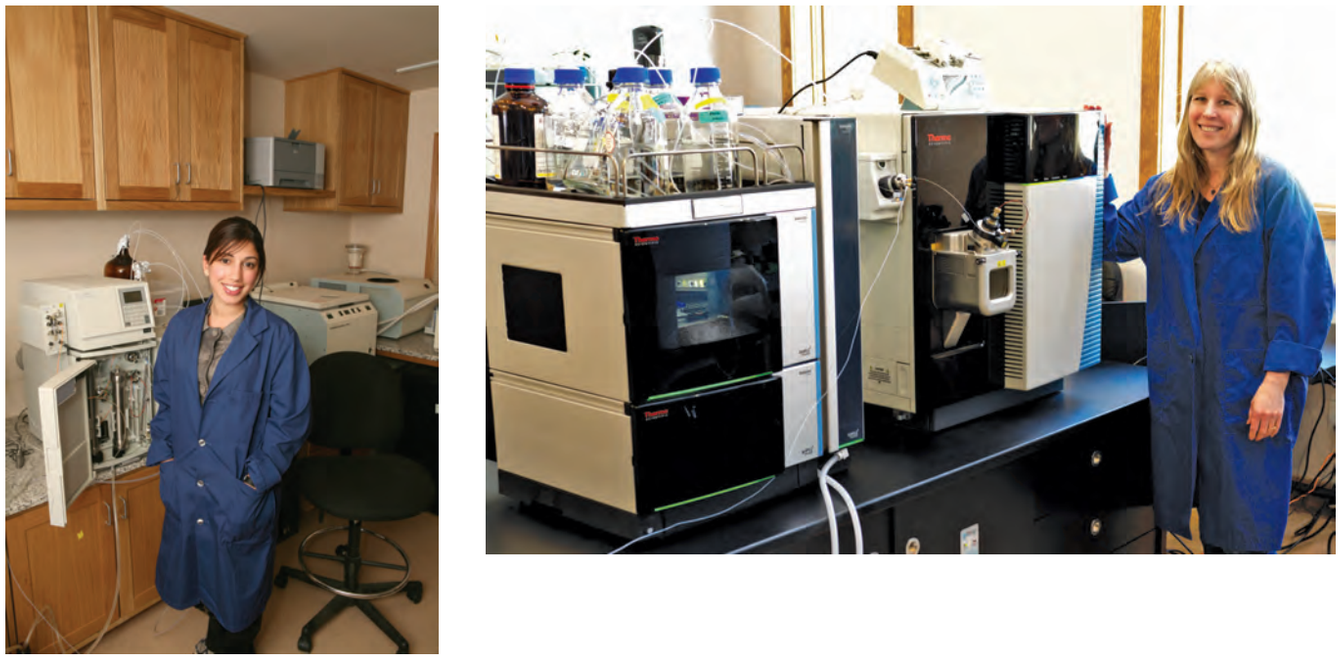

Figure 3.7 (Left) HPLC technology is used to identify different chemicals found in plants by passing the molecules through a large column (cylinder in opened instrument), separating them by size. The instrument is operated by technician Farnaz Bakhshi, Brain Chemistry Labs, Jackson Hole. (Right) Molecules from brain tissues in small vials in the sample manager (instrument at upper left) are separated by the liquid chromatograph (instrument at lower left) and then injected into the triple quadrupole mass spectrometer (instrument at right) to provide highly accurate analysis of toxins based on their mass and fragmentation patterns at the Brain Chemistry Labs. Dr. Sandra Banack operates the instrument.

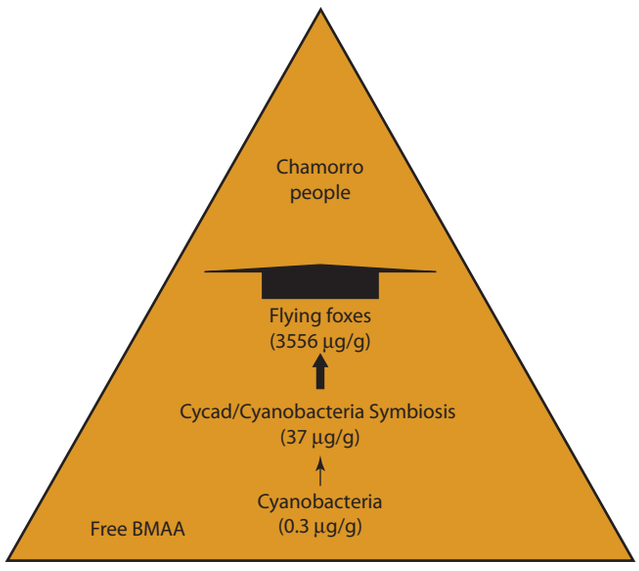

Figure 3.8 Biomagnification of cyanobacterial BMAA in Guam. The widths of the arrows are proportional to the concentration of free BMAA delivered to the next higher trophic level.

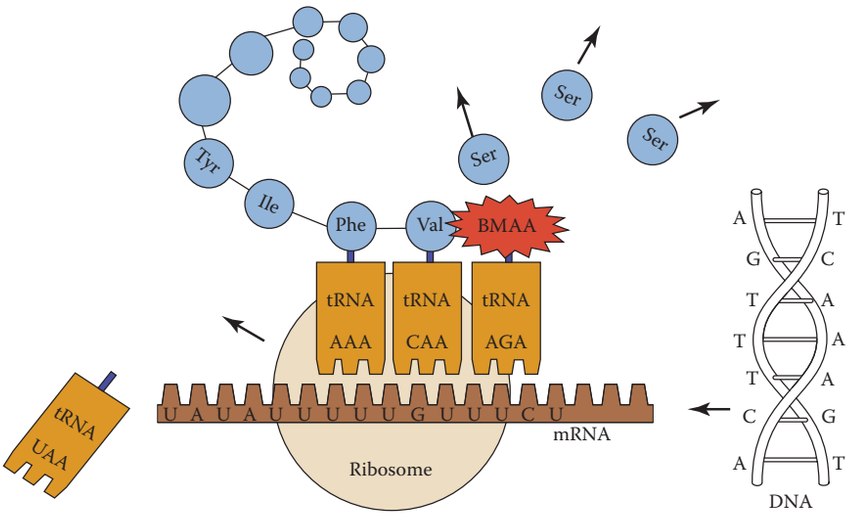

Figure 3.9 The neurotoxic amino acid BMAA can substitute for L-serine in proteins, causing protein misfolding and neuron death. In this drawing, a DNA molecule in the cell nucleus codes for a messenger RNA (mRNA) template in the ribosome. Each amino acid is coded on the mRNA with a three letter codon. Within the ribosome, amino acids are linked together with peptide bonds to form the protein specified by the mRNA template. Each of the 20 amino acids normally used to make up human proteins (shown as blue circles) have a specific transfer RNA (tRNA) (depicted as orange rectangles) that they attach to. Each of these tRNA molecules, in turn, have a specific three letter anticodon that matches a specific codon on the mRNA template for the protein. In this drawing, the amino acid phenylalanine (Phe) is attached to its tRNA bearing the anticodon AAA. The amino acid Valine (Val) is attached to its tRNA with the anticodon CAA. Serine (Ser) is attached to its specific tRNA with the anticodon AGA. As each of the amino acids are brought in sequence as dictated by the mRNA sequence, peptide bonds are formed between the adjacent amino acids, with the growing protein being shown spiraling off to the upper left. After the peptide bond is formed, the tRNA is detached and recycled for further use.

If the non-protein amino acid BMAA is present, it can bind to the tRNA for Serine. If BMAA is incorrectly inserted instead of Serine, the protein can misfold or break. Misfolded and broken proteins are sticky, and can aggregate within the brain, forming neurofibrillary tangles from misfolded tau proteins or amyloid plaques from amyloid precursor protein fragments.

move up the food chain. However, since BMAA is a polar amino acid and thus water soluble—not accumulating in fat—how could biomagnification possibly occur?

Since acid hydrolysis of Chamorro brain tissues showed an excess of BMAA, Cox decided to test all 20 of the protein amino acids to see if BMAA could be substituting for one of them. Using radioactive BMAA, his collaborators Ken Rodgers and Rachael Dunlop in Sydney, Australia, performed 20 different experiments on human cell cultures, each deficient in a different amino acid, to see if BMAA might be misincorporated. Using this technique, Rodgers and

Figure 3.10 Neuropathologist David Davis, a research professor at the Department of Neurology, University of Miami, examines an image from the brain of a vervet on the Caribbean island of St. Kitts which received a chronic dose of the cyanobacterial toxin BMAA for 140 days. At its peak, ALS/PDC killed 25% of the adults in two villages in Guam. Produced by cyanobacteria resident in specialized roots of cycad trees, BMAA contaminates flour which villagers manufacture from cycad seeds as well as the flesh of animals that forage on cycad seeds. By telescoping the lifetime BMAA exposure of Chamorro villagers into a 140-day period, researchers were able to reproduce in vervets the same neuropathological features that occur in the brains of the villagers with the progressive neurodegenerative illness now known as Guamanian ALS/PDC. The researchers also discovered that adding the amino acid L-serine to the diet of vervets dosed with BMAA reduces the density of this neuropathology up to 85%.

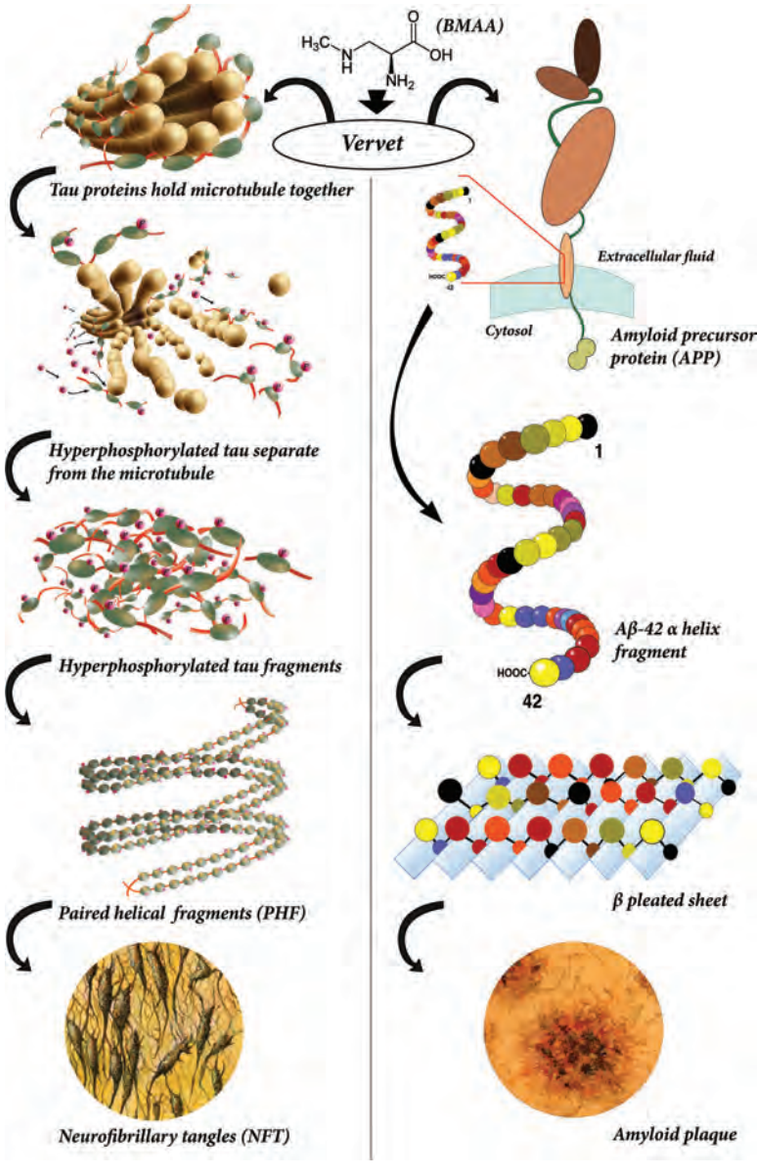

Figure 3.11 Theoretical pathways of development of Guamanian ALS/PDC and Alzheimer's neuropathology from chronic dietary BMAA exposure. (Left) Tau proteins that bind microtubules become hyperphosphorylated, leading to dissociation of hyperphosphorylated tau fragments. These form paired helical filaments, leading to the formation of neurofibrillary tangles. (Right) The amyloid precursor protein is cleaved, producing β-amyloid (Aβ-42) fragments that are in an α-helix conformation. These change to a β-pleated sheet conformation and oligomerize, forming amyloid plaques.

Dunlop found that our cellular machinery can mistake BMAA for L-serine. They also found that adding extra L-serine to the culture media effectively stopped protein misfolding and aggregation in the cells. To confirm that chronic exposure to BMAA-laden food items in the traditional Chamorro diet was the cause of ALS/PDC, an experiment was designed in which vervets, nonhuman primates in a research colony on the Caribbean island of St. Kitts, were daily offered as part of their regular meal a piece of fruit containing a test substance. One test group received rice flour as a placebo. A second group received L-serine as a positive control. A third group received BMAA, while a fourth group received equal amounts of BMAA and L-serine. After 140 days of dosing, the placebo and positive control animals showed no unusual neuropathology. The BMAA-dosed animals showed dense neurofibrillary tangles formed from misfolded tau protein with sparse amyloid plaques: the precise neuropathology that occurs in the brains of Chamorro villagers with ALS/PDC. Equally important, vervets dosed with both BMAA and L-serine showed a 50%–85% reduction in the density of brain tangles. Dr. David Davis, at the University of Miami Neurology Department, and the Brain Chemistry Labs in Jackson Hole have found that BMAA also triggers early signs of ALS in vervets but that L-serine can significantly slow disease progression. As a result of these investigations, the FDA approved clinical trials of L-serine initially for ALS and subsequently for Alzheimer's patients.