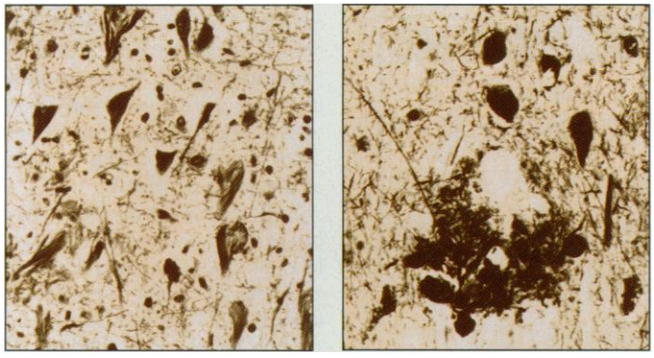
Guam: Deadly Disease Dying Out

A condition once thought to be an excellent model for diseases such as Alzheimer's and ALS may vanish before its causes can be fully understood

On the island of Guam in the western Pacific, a killer that has stalked the local population for more than 100 years, eluding all attempts at capture, is now threatening to escape its pursuers forever—and its modus operandi remains unsolved. Although the vanishing act is good news for the Guamanians, it's bad news for neuroscience. The disappearance of the killer, a disease known as Guam ALS-PDC, could rob science of an important tool for studying three major degenerative diseases: Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (ALS). Guam ALS-PDC "provides a great model for the major neurodegenerative diseases," says Donald Calne, a neurologist at the University of British Columbia. Its loss would be keenly felt because researchers have had great difficulty developing animal models for noninherited forms of these illnesses.



RALPH GARRUTO

Striking similarity. Neurofibrillary tangles from the brain of an Alzheimer's patient resemble those from a patient with ALS-PDC *(left)*.

Indeed, not long ago researchers thought they understood the cause of the Guam disease and hoped that insight would translate into better understanding of the major neurodegenerative disorders. In 1987, Peter Spencer, then at Albert Einstein College of Medicine, and his colleagues reported that huge doses of an amino acid found in seeds on Guam caused ALS-PDC symptoms in macaques by overstimulating and destroying nerve cells. This finding fit in with other suggestive evidence that such "excitotoxins" may have a role in many types of brain disorders. "It was absolutely vital to follow up [Spencer's finding]," says Washington University neurotoxicologist Dennis Choi. "The implication was that an excitotoxin, or some other common factor, might account for several neurodegenerative diseases."

But that picture has changed recently, as several recent studies seriously undercut Spencer's findings, making the link between excitotoxins and ALS-PDC more of a stretch. And since Spencer's Guam work was one of the stronger connections between excitotoxicity and common neurodegenerative disorders, researchers are feeling at a loss. "I think it's disappointing and depressing to see that [Spencer's study] has gone by the wayside," laments clinical neurologist William Langston, director of the California Parkinson's Foundation. "It's gone from light at the end of the tunnel to 'We don't know where we're going.'"

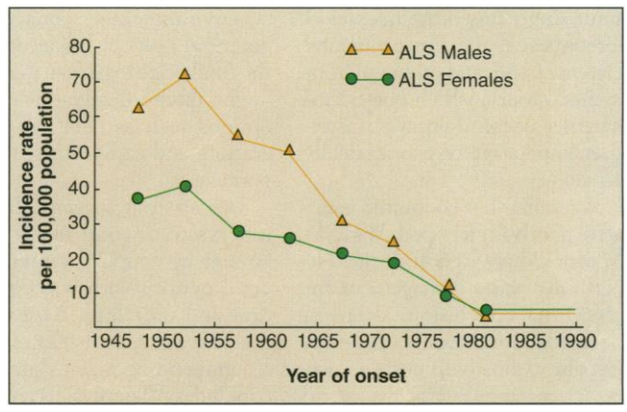
The confusion could not have come at a worse time, since the ALS-like symptoms, once the predominant manifestation of the disease on Guam, seem to be disappearing. In November 1989, the National Institute on Aging (NIA) set up a $5 million, 5-year study of ALS-PDC, as a concerted effort to solve Guam's riddle once and for all. Today, midway through the study, researchers find that new cases with ALS symptoms have dwindled from more than 50 per 100,000 to a mere five per 100,000. Many researchers are, like Calne, worried that "the disease may die out before we get a handle on what causes it."

ALS-PDC takes its name from the diseases it imitates: its full moniker is amyotrophic lateral sclerosis-parkinsonism dementia complex. Though jaw-breaking, the name is apt, since the illness sometimes destroys its victims' motor neurons, leaving them paralyzed in a manner similar to ALS. Other victims of the always-fatal disease shuffle around as if they have Parkinson's. Many also develop an Alzheimer's-like dementia.

This complex of symptoms first drew the attention of science in the late 1940s, when Western physicians noted that Guam natives—the Chamorros—were dying of a malady indistinguishable from ALS, but at a rate 50 times that of the industrial countries. Within a decade, researchers began to notice that some of the ALS patients also had symptoms of parkinsonism, or dementia, or a mix of all three. Given the high concentration of cases, it seemed likely that whatever was causing the disease was prevalent on Guam and would be much easier to find than the cause of the major neurodegenerative diseases in the industrial countries. "If you're looking for a needle in a haystack, you're more likely to find it in a haystack with 100 needles," explains Spencer, now at Oregon Health Sciences University in Portland.

That logic was compelling enough that the National Institutes of Health (NIH) set up a research center on Guam in 1956 specifically to tackle the illness. For the next 30 years, researchers scoured the island and family histories, searching for genetic causes, bacterial or viral agents, or environmental poisons. They found nothing that was fully convincing.

Spencer took on the problem in 1981. He was coming from a different angle: excitotoxicity. He had previously found that an excitotoxin was involved in a disease called lathyrism, marked by spastic leg movements, and he wondered whether a similar compound might be to blame for the Guam disease. Earlier research had identified the excitotoxic amino acid β-*N*-methylamino-L-alanine (BMAA) in the seeds of Guam's cycad trees. Indeed, in the late 1950s Mayo



Disappearing disease. The incidence of ALS-type symptoms on Guam has declined dramatically over the past 40 years.

Clinic epidemiologist Leonard Kurland first proposed cycad as the culprit, after a research team he led found that Chamorros ground cycad seeds into flour for tortillas, a food many had subsisted on during the war.

With the intriguing mix of suggestive data and history, it didn't take Spencer long to launch his macaque feeding studies. After ingesting a synthetic version of BMAA, monkeys grew weak, their muscles trembled, and they shuffled around their cages—all symptoms that were dead ringers for those of ALS-PDC. In a report published in 1987 in *Science* (31 July, p. 517), Spencer proposed that BMAA, or some other cycad component, might act as a "slow toxin" that damages nerve cells over the years. And Spencer offered other evidence: People in New Guinea and Japan who showed symptoms of ALS-PDC used cycad seed as medicine, either as a topical poultice or in a potion.

To many neuroscientists, Spencer's research was just a confirming study or two away from closing the book on the cause of ALS-PDC—and from there the other neurodegenerative diseases seemed within reach. "He seemed to solve the mystery," says Mark Duncan, a neurochemist at Australia's University of New South Wales.

But the book refused to close. The first blow came little more than a year after Spencer published his theory. In a November 1988 letter to the *Lancet*, NIH virologist D. Carleton Gajdusek and NIH biologist Ralph Garruto reported that they were unable to reproduce Spencer's findings. Then Duncan, at the time a postdoc in the lab of NIH neuroscientist Irwin Kopin, launched his own follow-up studies. "We really entered into this wanting to find evidence in support of Spencer's claims," he says. But he didn't.

Instead, in a series of studies described in *Advances in Neurology* in 1991, Duncan found that the doses Spencer used to get toxic effects were unrealistic. After collecting extracts of fresh cycad seeds and flour samples prepared by Chamorros, Duncan reported that the Chamorros soak the seeds for days, leaching out most of the toxin—85% to 99% of it, in fact. Duncan estimated that to get doses similar to those that Spencer had fed macaques, humans would need to consume the absurd amount of 100 kilograms of processed cycad flour per day. Duncan also calculated the maximum possible BMAA dose in a cycad poultice and found it to be orders of magnitude less than the dose in Spencer's study; he reported these results last year in *Neurobiology of Aging*. Another Duncan study, published in 1991 in the *Journal of Pharmacology and Experimental Therapeutics*, even undermined the most basic aspect of the BMAA theory. His data indicated that BMAA is metabolized rapidly and transported poorly into the brain in rats. This, Duncan says, casts serious doubt on BMAA's ability to cause any neurological damage whatever.

"I think Duncan knocked the cycad hypothesis out of the box," says Lewis Rowland, a clinical neurologist at Columbia Presbyterian Medical Center who has worked on ALS for years. Spencer doesn't accept that assessment, but he agrees that his colleague may be on to something. "Duncan's viewpoint is that the concentration of BMAA is too low to be of significance, and he may be right," he concedes.


PETER SPENCER

Exciting epidemiology. Peter Spencer *(at far left)* has found sources of excitotoxins in diets of Pacific islanders.

If BMAA has indeed been knocked out of the box, there are several other relief pitchers ready to step in. Yet none of the substitute theories includes the links between a strong presence on Guam, human consumption, and a symptom-producing response in animals that gave BMAA such a boost. Despite this, all the theories have their champions—and the notion of excitotoxicity at the heart of the disease isn't dead, since one of the alternatives is an excitotoxin.

That one is glutamate. Last year, Johns Hopkins researchers found that specially prepared neural tissue from ALS patients did a poor job of transporting this excitotoxic amino acid across the cell membrane. This, they hypothesized, could lead to a neurotoxic buildup (*Science*, 5 March, p. 1397). Another candidate is a cycad toxin called cycasin, which comprises about 2% of the cycad seed. Cycasin's metabolite methylazoxymethanol (MAM), though not an excitotoxin, damages DNA, and Spencer and toxicologist Glen Kisby have found plenty of MAM in rat brain tissue exposed to cycasin in cell culture. Even BMAA isn't completely out of the picture: It's been suggested that the compound could be inhaled with cycad pollen and end up in the brain's olfactory bulb.

Then there's the notion of aluminum toxicity, which has been around for 30 years. In the late 1960s, Gajdusek and Garruto found high levels (compared to other parts of the world) of aluminum in soil and watersheds of certain regions of Guam and West New Guinea. A short time later, neuropathologist Daniel Perl of the Mt. Sinai Medical Center, working with Gajdusek, reported high levels of aluminum in jumbles of dead nerve cells, called neurofibrillary tangles, that are found in both ALS-PDC and Alzheimer's patients. But to Perl, the "$64,000 question" is how aluminum, a known neurotoxin, gets into the brains of ALS-PDC sufferers. No one has provided a convincing explanation.

Still other findings suggest a combination of causes: Metals in the environment and excitotoxins may act as co-conspirators. Duncan has found that cycad flour made by the Chamorros is contaminated by zinc, and last January, Choi and his colleagues reported in *Neuron* (vol. 10, p. 43) that stimulation of the receptor for BMAA enhances zinc's neurotoxic effects. "Perhaps there's a synergy between BMAA and zinc toxicity," Choi speculates. The findings, says NIH's Kopin, "are intriguing, worth following up."

But the time for follow-ups is running out. NIA's 5-year study of ALS-PDC, run by Kurland, Perl, and others, has confirmed that the incidence of ALS-like illness on Guam has declined dramatically, bringing the rates on the island down nearly to western rates. The decline has been occurring steadily over the last 40 years, from about 40 new cases documented each year in the 1950s to fewer than five a year in the early 1990s.

Furthermore, the pattern of the illness's symptoms is changing in a mysterious way. While ALS symptoms are diminishing, Kurland says, parkinsonism and dementia "are holding their own." But if they are holding their own, they're beginning to do it independently, since Kurland says his team is finding more dementia cases without parkinsonism, a phenomenon they've dubbed "Mariana's dementia" after the island chain that includes Guam.

All this recent epidemiological shapeshifting has scientists scrambling for an explanation. One cause they've ruled out is genetics, since the island's population hasn't changed dramatically.

And that brings this long search more or less back to its starting point: a still-unknown environmental toxin. This idea accompanies the supposition that recent changes in Chamorro lifestyle, including less consumption of cycad seed, have led to changes in exposure to the toxin, and therefore to changes in the symptomatology. "As the

country has acculturated to a more western lifestyle, exposure to the environmental factor has declined," Spencer argues. He concludes that high levels of exposure led to the most serious symptoms, ALS, which are so debilitating they mask any parkinsonism or dementia. A lower level of exposure and the ALS symptoms drop away; still lower levels leave only the dementia.

Of course, that explanation would be more convincing if someone could identify the toxin. And the trend toward a diminishing disease means there may not be many more opportunities. "We may only have 10 or 20 more years at the most to conduct studies and store brain tissue for analysis in the future," Kurland says. So he and others on the NIA grant intend to leave no stone unturned: Aside from compiling the remaining cases of ALS-PDC, they're performing brain scans and blood tests on Chamorros to look at everything from levels of parathyroid hormone (implicated as a player in the aluminum hypothesis) to levels of superoxide dismutase, an enzyme implicated in familial ALS.

This NIA study may be scientists' last, best chance to catch the killer, and no one thinks it's going be easy: "If it was what we expected, we'd have found it already," Kopin asserts. But the fact that the search has been difficult has only helped to raise the stakes. "ALS-PDC is the Rosetta Stone of neurodegenerative diseases," says Perl. "Whoever provides a translation will win the Nobel Prize."

–Richard Stone

PULMONARY PHYSIOLOGY

Helping Premature Lungs Breathe Easier

The next time you're on the basketball court and stop to fill your lungs, you should offer up some silent thanks to a little known benefactor: your lung surfactant. What's that, you ask? Lung surfactant is the foamy material, blended of fats and proteins, that lines the inner surface of the lungs. Why give thanks? Well, without surfactant, catching your breath wouldn't just be hard—it could well be impossible, since this lubricating substance helps your lungs expand to draw in air after exhaling.

Lack of surfactant is just the problem faced by the 65,000 premature infants born each year in the United States with "respiratory distress syndrome." Their immature lungs can't produce the material, and until recently, when researchers learned to synthesize surfactant substitutes or get them from animals, as many as 10% of the babies died. But the substitutes could be improved, which is where a paper in this issue of *Science* comes in. On page 453, biochemist Alan Waring of the University of California, Los Angeles, and his colleagues explore the role of a surfactant protein known as SP-B. Though SP-B is a small part of the surfactant complex, it is crucial to its functioning; it is also expensive and difficult to obtain. Though not all in the research community are convinced by the results, Waring's group hopes their data will lead to improved replacements for SP-B, and hence to better treatments for premature infants.

JIM OLIVE/UNIPHOTO

A better start. Surfactant therapy has revolutionized premature infant care.

Not that current treatments are disastrous. On the contrary, Ivan Frantz, chief of newborn medicine at the New England Medical Center, says surfactant substitutes, which are already cutting the death rate in half, constitute "the most important therapeutic advance in the care of premature infants in 20 years." But investigators feel they can make even more effective replacements once they puzzle out the inner workings of how the surfactant lubricates the lungs. The goal is to simplify the recipe for a replacement surfactant, thereby reducing the cost of treatment, which can be as high as hundreds of dollars per dose.

The current recipe for surfactant starts with large amounts of a phospholipid known as dipalmitoylphosphatidylcholine (DPPC). DPPC is extremely effective at reducing surface tension within the alveoli (the lung's many air sacs), which is the key step in carrying out the surfactant's function. But the substance needs help to do the job. When prepared in solution, DPPC tends to form bilayers or globules, instead of the molecular monolayer needed to coat the alveoli, and thus can't be administered by itself into the lung. Additional ingredients, such as palmitic acid, SP-B, and other proteins found in natural surfactant, must be added. Palmitic acid, which is a negatively charged molecule, apparently acts to "spread" the DPPC into a monolayer, thereby helping it line the alveoli.

Understanding SP-B's role in all this might make it easier to design a better synthetic surfactant, which is one reason Waring and co-workers performed their study. By measuring the surface tension of various solutions of palmitic acid and synthetic peptide mimics of SP-B, they deduce that the positive charges on the protein interact with the negatively charged palmitic acid, locking it into place in the surfactant complex. Without the protein, the palmitic acid would be "squeezed out" of the surfactant monolayer, thus reducing its capacity for decreasing surface tension. "The proteins appear to hold the palmitic acid in a monolayer with low surface tension," says University of California, Santa Barbara, chemical engineer Joseph Zasadzinski, a co-author on the report.

Not everyone in the small surfactant research community agrees, however. "There's just insufficient data. I think they're missing the important function of SP-B," contends Charles Cochrane of the Scripps Research Institute, who published his own theory about the protein's role in *Science* 2 years ago (*Science*, 25 October 1991, p. 566). Cochrane points out that the amount of free palmitic acid in surfactant is quite small. So although SP-B may indeed interact with the fatty acid, Cochrane maintains that the primary role of SP-B is to strengthen the phospholipid monolayer composed of DPPC. In this scenario, he says, rather than locking in the palmitic acid, SP-B provides greater stability to the monolayer, like reinforcements that buttress a bridge.

The Waring group responds that they're confident that SP-B associates predominantly with palmitic acid, although Zasadzinski admits, "it's a complicated affair." He suggests that new experiments they have under way to explore the "squeeze-out" phenomenon may show whose interpretation is correct. But what intrigues Zasadzinski and his colleagues is that their experiments show that simple polymers like polyethylenimine, which bear similar charges to SP-B but are otherwise unrelated in structure, can have the same impact on palmitic acid as the protein. If preventing squeeze-out is truly SP-B's only important function, then such polymers may provide a superior alternative to the synthetic peptides being used now in surfactant replacements. And if that's the case, then even more premature infants, and their parents, might breathe easier.

–John Travis