Behavioral Measures OF Neurotoxicity

Report of a Symposium

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and Andrew M. Pope, editors

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Are Neurotoxins Driving Us Crazy? Planetary Observations on the Causes of Neurodegenerative Diseases of Old Age

Peter S. Spencer

Health planners in developed countries are increasingly concerned with their burgeoning populations of elderly subjects and the consequent rising prevalence of age-associated disorders, notably those involving the nervous system. By the *year* 2050, current projections for the United States indicate that the proportion of the population aged 65 or over will be almost double (22%) the 1986 level, whereas the prevalence of senile dementia of the Alzheimer type will triple. It is thus entirely appropriate for the elderly of developed countries to be the subjects of intense scientific scrutiny aimed at understanding the causes and methods of prevention of the major neurodegenerative diseases that all too often accompany the second half of life. There are certain other parts of the world, however, notably in the western Pacific region, where such disorders are far more commonly encountered and where prospecting for etiology is more likely to be profitable. Indeed, one would posit that a knowledgeable extraterrestrial investigator, charged with the task of identifying causes of the great neurodegenerative diseases of *Homo sapiens* on planet Earth, would be unlikely to begin by researching elderly populations in Canberra, London, or New York; rather, the hunt for causation would probably commence in places such as Guam or Irian Jaya where, in certain spots, incidence rates for such diseases have exceeded worldwide statistics by more than one to three orders of magnitude.

If the etiologic search can be likened to the proverbial hunt for a needle in a haystack, why not maximize chances of success by focusing

investigation on haystacks that contain a hundred such needles? Critics of this view charge that the western Pacific combination of presenile dementia, parkinsonism, and motor neuron disease found in the Marian islands (Guam and Rota), Irian Jaya (west New Guinea, Indonesia), and the Kii peninsula of Honshu island (Japan), is little more than a medical oddity and distinct from the neurodegenerative disorders that plague the aged in the West. Far from being a curiosity, others observe that the high-incidence foci of neurodegenerative disease in the western Pacific may actually hold the keys that will unlock the door to lookalike disorders worldwide, if not to the secrets of aging itself.

This is a story about a remarkable and terrible affliction that sometimes presents at onset as motor neuron disease (amyotrophic lateral sclerosis) and, in other instances, as parkinsonism, presenile dementia, or various combinations of all three. The disease is known as western Pacific amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia complex (P-D), or ALS/P-D. Each clinical component in isolation closely resembles its namesake worldwide. Intensive study of this disease on Guam has demonstrated that although ALS or P-D may strike several family members and affect several generations of individual families, the disease is neither inherited nor associated with a transmissible (viral-like) agent, and seems instead to be associated with an environmental factor that is disappearing hand in hand with the declining incidence of ALS.

Armed with this information and knowing of an early suggestion linking Guam ALS to neurolathyrism, I began in 1981 to investigate in India, and later in Bangladesh, China, and Ethiopia, a form of motor neuron disease for which a neurotoxic etiology was clearly established, namely, the spastic paraparesis associated with excessive and continuous ingestions of legume Lathyrus *sativus L.* Lathyrism was of special interest because a detailed understanding of this poorly studied condition seemed likely to provide a base of knowledge and experience on which to examine the unfashionable and largely forgotten suggestion that consumption of a potentially neurotoxic food plant (Cycas circinalis L.) was also etiologically linked to Guam ALS. My conjecture was that detailed study of the neurotoxic factors in these plants might lead to a precise understanding of the chemical triggers of lathyrism and western Pacific ALS/P-D, information that could then be applied to search for the etiology of related neurodegenerative diseases worldwide. This research has led to the conclusion that chemical factors in the environment are involved in triggering neurodegenerative diseases which remain silent for years or decades before their dramatic clinical consequences are expressed. Intensive

research is now underway both to identify the novel molecular and cellular mechanisms that likely underly these newly recognized long-latency neurotoxic diseases, and to determine whether xenobiotics also play a role in triggering neurodegenerative disorders such as Alzheimer's disease and ALS in developing countries.

CLINICAL FEATURES OF WESTERN PACIFIC ALS/P-D

Sporadic ALS, a disorder of middle-aged and elderly people, is more common in males than females (1.6:1), with a mean annual incidence rate in the United States of 1-2/100,000. Much higher *prevalence* ratios for ALS, and much younger ages (18+) of onset, are reported among the indigenous population (Chamorro) of Guam. Surveys conducted in the early 1950s demonstrated that about 10 percent of deaths among adult Chamorros resulted from ALS, frequencies about 100 times those recorded for the population of the continental United States. Males were more susceptible than females to ALS (2:1) and to P-D (3:1). However, during the past 30 years, the prevalence of Guam ALS has dropped steadily, the sex ratio now approaches unity, and the mean age at onset has risen from the 40s to the 50s.

Those who have witnessed the progressive clinical course of ALS-a disease associated with baseball star Lou Gehrig in the United States and with actor David Niven in Britain and its Commonwealth-can attest to the dramatic consequences of the underlying motor neuron degeneration. Patients usually present with signs of lower-motor-neuron deficits, with weakness, atrophy, and fasciculation of limb muscles, or with involvement of bulbar musculature. Upper-motor-neuron changes signaled by spasticity, hyperactive deep tendon reflexes, and extensor plantar reflexes (Babinski sign) are often present early in the course of the disease. Muscle weakness progresses steadily and becomes more widespread and symmetrical. Eventually, the victim expires from respiratory failure or related causes, usually within three to five years of diagnosis. The underlying damage to the nervous system consists of progressive degenerative changes and loss of nerve cells in the motor cortex (upper motor neurons) and of lower motor neurons in spinal cord (anterior horn cells) and brain stem nuclei. Neuronal compromise is accompanied by loss of axons in corticospinal tracts and in motor nerves, the latter leading to atrophy of denervated muscles.

Parkinsonism with progressive dementia is being recognized increasingly as a variant of Alzheimer's disease both in Europe and in the United States. In Guam, where P-D is phenomenally frequent,

patients characteristically show eventful premorbid conditions such as obesity, essential hypertension, late-onset diabetes, hyperuricemia, and significant trauma. One to five years prior to the onset of extrapyramidal dysfunction, psychoneurotic complaints such as dizziness, nervousness, *easy* fatiguability, loss of appetite or libido, and excessive sleepiness, may mask the insidious onset of organic brain syndrome or parkinsonism. Memory loss, disorientation, and personality changes follow. Early deterioration of fine movements, decreased blinking, bradykinesia, and eventually increasing rigidity with impaired postural reflexes occur. Tremors are said to be less common and sometimes different from those classically associated with Parkinson's disease. As the disease progresses, incontinence of urine and feces, osteoporosis and fractures from falls, anemia, and extensive bedsores develop, and patients finally succumb to intercurrent infections. Upper and lower motor neurons are affected as a rule, and quadriplegia in flexion, irreversible contractures of all joints, extension of head, blepharospasm, and a total mutistic and demented state, develop in the advanced stage. Patients may lie in this tragic and pathetic condition for many years prior to death.

Both ALS and its P-D clinical variant have been phenomenally common among three population groups in the western Pacific: (a) Chamorros of the Mariana islands of Guam and Rota and others who have adopted their life-style, (b) Auyu and Jaqai linguistic groups of the southern lowlands of Irian Jaya, and (c) Japanese residents of northern Kii peninsula of Honshu island. In the 1950s the indigenous Chamorro residents of Guam showed incidence rates for ALS more than 100 times that for the population of the continental United States. Subsequent surveys on Guam revealed that P-D was also encountered with remarkable frequency. Moreover, overlapping forms could be demonstrated, suggesting that the two disorders were closely related. Some patients with slowly evolving motor neuron disease later developed parkinsonism or P-D; in others, the appearance of P-D was followed by amyotrophy and spasticity, whereas in yet others, there was a more or less simultaneous onset of both ALS and P-D. Similarly, among Japanese residents of the Hobara region in the Kii peninsula focus of ALS/P-D, ALS incidence rates for the period 1946 to 1965 exceeded the average for Japan by more than 100 times. In the 1960s, a third focus of ALS and parkinsonism (with or without dementia) was discovered among Auyu and Jaqai linguistic groups residing in the southern lowlands of Irian Jaya (then New Guinea), Indonesia. The crude incidence rate for ALS among these seminomadic people was even greater than that for either the Chamorros of Guam or the Japanese of the Kii peninsula.

Neuropathological studies of Guam ALS and P-D demonstrated in both diseases the presence of Alzheimer-type neurofibrillary tangles with few senile plaques, a critically important observation that unified the two conditions. Comparable neuropathological changes were observed in Kii peninsula ALS/P-D. Additionally, a similar pattern of neuropathology was found in 70 percent of 302 brains of Chamorros who had shown no neurological deficit at the time of death, suggesting that subclinical forms of the disorder pervaded the indigenous population of Guam. According to Dr. Leonard Kurland, the extent of neurofibrillary change that one would expect to see at age 75 in the population of the U.S. mainland would be found at age 45 or 50 in Chamorros who had no overt neurological disease. Recent postmortem studies of Guam ALS/P-D have shown plentiful senile plaques of Alzheimer type; the Davies Alz-50 protein of Alzheimer's disease is also found in Guam ALS/P-D; and the amino acid sequence of the neurofibrillary tangles in both diseases is identical. Taken in concert, therefore, remarkable homologies seem to exist between western Pacific ALS/P-D and Alzheimer's disease.

WHAT IS THE CAUSE OF WESTERN PACIFIC ALS/P-D?

The phenomenally frequent and often familial occurrence of ALS/P-D in three western Pacific pockets has encouraged the search for a common etiology. Because the incidence of motor neuron disease is declining in all three zones, and inherited and viral factors have been virtually ruled out, the search for etiology has focused on nontransmissible environmental factors that are disappearing as the susceptible population groups acculturate to modern ways.

Two major etiologic hypothesis have been advanced, neither of which is mutually exclusive: one invokes metal intoxication promoted by a dietary deficiency of calcium, whereas the other implicates the neurotoxic cycad plant used for food and medicine. Examined here are the evidence supporting these proposals and some of the implications that arise if the favored cycad hypothesis is correct.

The hypothesis of defective mineral metabolism proposes that chronic nutritional deficiency of calcium and magnesium provokes a secondary hyperparathyroidism, leading to increased absorption of potentially toxic metals and the deposition of calcium, aluminum, and other elements in neurons of subjects who then develop ALS/P-D. Intraneuronal accumulation of metal elements is proposed to interfere with slow axonal transport by altering neurofilament production, resulting in excessive neurofilament accumulation and formation of neurofibrillary

tangles. One of the major lines of evidence supporting this novel proposal has come from the results of analyses of soil and drinking water in the high-incidence foci of ALS/P-D, where calcium concentrations have been reported to be very low. These data have been challenged recently by an extensive and authoritative study of the rivers of Guam, which found high concentrations of calcium characteristic of borderline hard waters and mean aluminum concentrations similar to those in rivers of the southeastern U.S. mainland. Other recent investigations have revealed adequate calcium concentrations in traditional foodstuffs.

Although these data appear to have dislodged the cornerstone (deficient dietary calcium) of the mineral dysmetabolism hypothesis, there is nonetheless concrete evidence of heavy intraneuronal deposition of calcium and aluminum in Guam ALS/P-D. However, it must be recalled that although aluminum has neurotoxic potential, the associated neuropathologic change of neurofilament accumulation is nonspecific and distinct from the paired helical filaments that characterize the Alzheimer neurofibrillary tangle. Aluminum deposition also occurs in Alzheimer's disease and dialysis dementia, two disparate dementing disorders with distinctive neuropathologic changes. Taken in concert, therefore, the intraneuronal accumulation of aluminum and other metals in ALS/P-D is likely to be a secondary pathological feature of these neurodegenerative disorders. This view is in keeping with the results of experimental studies that have been unable to induce clinical signs of motor-system disease in primates fed a low calcium-magnesium diet, with aluminum lactate added to the drinking water.

Presently favored as the key trigger of western Pacific ALS/P-D is the seed of the cycad *(Cycas spp.),* an established cause of locomotor dysfunction in animals grazing on the plant. Many species contain chemicals with neurotoxic, carcinogenic, and teratogenic properties.

CYCADS: POISONOUS PLANTS USED FOR MEDICINE AND FOOD

The living cycads belong to an ancient line of nonflowering, gymnosperm-like seed plants that arose from the cycad ferns in late Paleozoic times and flourished in the Mesozoic era (200 million years ago). Thus, they are "living fossils" distinct from all other contemporary plants except the equally ancient Ginkgoaceae. Once distributed over the entire planet, cycads became restricted during the last Ice Age to tropical and subtropical climes of both hemispheres. The nine genera in the single surviving order of the Cycadales include the Australian *Macrozamia* (east, central, and west Australia) and *Bowenia* (northeastern

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Queensland); Zamia of the Americas (southern Florida, West Indies, southern Mexico to the Amazon, and Peru); and Cycas, the genus of current interest in relation to the etiology of ALS/P-D. Cycas circinalis has the widest distribution, extending over an enormous range from the Mariana islands (including Guam) southeast to Fiji, Tonga, Samoa, and west to New Caledonia, Queensland, New Guinea, the Philippines, Indonesia, coastal Indochina, Bangladesh, Sri Lanka, Madagascar and the Mascarenes, Zanzibar, and part of the eastern African coast. Other relevant Cycas species are C. *revoluta* (Japan to Taiwan), C. *media* (northern Australia and Queensland), and C. cairnsiana (northern Queensland). Cycads often grow in poor, rocky soils under exposed conditions where other plants are unable to compete, but they also survive in wet forest environs. Hardy by nature, they are resistant to typhoon, flood, and drought, and are among the first plants to regrow after fire has destroyed the vegetation. They are commonly grouped in stands (e.g., Groote Eylandt, Australia), although individual examples survive in relative isolation in the midst of forests of other plants (e.g., southeastern Irian Jaya).

Mistakenly considered by nonbotanists as palmlike, cycads are mostly terrestrial and arborescent, with an unbranched aerial stem (trunk-like) covered with persistent leaf bases as in Cycas, or shrubby with a subterranean, tuberous stem as in some Zamia species. Cycad leaves are borne terminally on the stem in the form of a large crown; when young, the leaves are soft, succulent, and fernlike, but they acquire a stiff, plastic-like quality upon maturation. Reproductively, cycads are either male or female, modified leaves (sporophylls) bearing either pollen or ovules in conspicuous conelike structures (strobiles) resembling pineapples. In Cycas spp., only the male develops a strobilus, whereas the female sports elongate, seed-bearing megasporophylls that together form a crown when young, but later droop individually around the stem displaying the mature seed. Cycad seed is often brightly colored (red, brown, green) and has the appearance of an edible fruit, features that have undoubtedly encouraged human contact. The mature seed (commonly mislabeled, nut) has a fleshy outer husk (sarcotesta) covering a thin, stony shell that houses the starchy kernel (female gametophyte and embryo).

Despite the physical beauty of many cycad species, they are all exceedingly poisonous plants. This statement applies especially to the young leaves and immature seed kernel, the pith of the stem, and the roots. The fleshy seed cover is said to lack poisonous properties, whereas the gum that exudes from the seed micropyle or from the broken megasporophyll or leaf stem of Cycas spp. appears not to have been assessed for toxic potential. The noxious property of cycads

has not deterred many animal species, including *Homo sapiens,* from using them as food. Not only have humans in times past used all parts of cycad plants for their nourishment, they have also employed various components for medicine, recreation (toys such as whistles), and other purposes. Moreover, as if to encourage human contact, some cycads have acquired popular names such as wild pineapple *(Macrozomia)* and wild date *(Encephalartos).* Other names that conceal the toxic potential of these plants stem from the use of their pith for sago: wild sago (Zamia *floridana);* false sago palm (C. circinalis); bead palm (C. rumphii); sago palm (C. *revoluta).* Not only is the popular English name of the latter identical to the true sago palm *(Metroxylon* sagu), but the Japanese name sotestsu is the subject of a well-known love song, a point that emphasizes the degree to which these highly poisonous plants have been incorporated into human culture and life-style. In general, food, medicinal, and recreational uses of cycads are associated with poverty (and, therefore, denied), ignorance, or lack of modern cultural development, whereas their indoor and outdoor ornamental application in developed countries is primarily a hallmark of the (equally ignorant) affluent. Uses of cycads in the high-risk foci of ALS/P-D in the western Pacific include medicinal purposes (Guam, Irian Jaya, Kii peninsula); food, beverage, and as a confection or chew (Guam); and recreational purposes such as childrens' playthings (Kii peninsula). Individual cases of ALS in these regions have been linked with heavy exposure to Cycas seed kernel used as a topical medicine (Irian Jaya, Guam), an oral medicine (Kii peninsula), or a foodstuff (Guam). Obviously, the cardinal principle of the cycad hypothesis is that only certain individuals sustain exposure to cycad chemicals in a sufficient amount to exceed the dose threshold for clear-cut clinical expression of disease. As illustrated below, certain human groups have discovered how to remove the poisonous elements from cycads to a sufficient degree that they are usable as a valuable source of food without recognized adverse health effects. Prominent among these groups is the Australian aboriginal.

Although the practice is now restricted, if not rare, the Australian aboriginal has used cycads as food for at least an estimated 4,500 years. Fire was probably used to clear vegetation and to synchronize cycad growth and seed production. Prior to 1900, the fleshy seed coat of *Macrozamia* spp. was eaten by aboriginals; its flavor was found by some Europeans to resemble that of chestnut. Seed of C. *media* was a significant component of the aboriginal diet in the Northern Territories, including Groote Eylandt where the practice seems to have disappeared. The Tiwi of Melville Island reportedly consumed

cycad seeds at least as recently as the 1950s. At least two Australian doctoral theses have discussed in detail the various methods (burial, water leaching, roasting) used by aboriginals to "detoxify" the seed.

The acute toxic effects of cycads-variously exploited in the past to execute criminals (Honduras), poison rats, and kill unwanted children (Celebes) or enemies (Costa Rica)-were discovered unwittingly by some early European explorers of Australia. Although credit for the first bad experience has been given to Cook's party, Beaton argues that he was preceded in his innocent discovery of the poisonous effects of cycad (and of Australia!) by other explorers.

On December 29,1696, Willem de Vlamingh, in charge of an expedition mounted by the Dutch East India Corporation, anchored his frigate Geelvinck on the east side of Rottnest Island just a few miles from what is now called Perth. Some days later, a party of 86 men went ashore on the mainland and broke up into three reconnaissance parties. In one was an officer who conducted the first known European experiment with cycads:

> The 6th in the morning we split into three Parties, each taking a different route, to try if we could find some Men. After two or three hours we rejoined the company near the River without discovering anything but a few huts and footsteps; so we took a rest. In the meantime they brought me the nut of a certain Fruit [Macrozamial resembling the form of the Drioens, and having the taste of the Dutch great Beans, and those which were younger were like a hazelnut. I ate five or six of them, drunk the water from one of the already mentioned pits; but after about three hours I and five others who had eaten of these Fruits, began to vomit so violently that there was hardly any difference between us and death; so it was with the greatest difficulty that I with the Crew reached the shore...
>
> (Robert, 1972, p. 63).

Some years later, in August 1770, on the east coast of Cape York the present site of Cooktown, Joseph Banks records what Beaton (1977) calls the second Australian experiment in action ethnobotany:

> Palms here were of three different sorts.... The third... was low, seldom ten feet in hight, with small pennated leaves resembling those of some kinds of fern; Cabbage it had none but generaly bore a plentifull crop of nuts about the size of a large chestnut or rounder. By the hulls of these which we found plentifully near the Indian fires we were assurd that these people eat them, and some of our gentlemen tried to do the same, but were deterrd from a second experiment by a hearty fit of vomiting and purging which was the consequence of the first. The hogs however who were still shorter on provisions than we were eat-

> ing them heartily and we concluded their constitutions stronger than ours, till after about a week they were all taken extremely ill of indigestions; two died and the rest were savd with difficulty.. .

(Beaglehole, 1962).

Later, reflecting on the episode, Banks wrote that some of the crew who only ate one or two of the nuts were "... violently affected by them, both upwards and downwards, and our hogs whose constitutions we thought might be as strong as those of the Indians [aboriginals] literally died after having eat them. It is probable however that these people have some method of Preparing them by which their poisonous quality is destroyed... " (Beaglehole, 1962).

Many other examples of acute cycad poisoning, sometimes with fatal outcome are recorded among early settlers in Australia and elsewhere. Eventually, however, the migrant Europeans learned how to "detoxify" the cycad. In the 1920s, settlers in western Australia exploited the stem starch for human consumption, as laundry starch, and as a commercial adhesive. Even as recently as the 1950s, cycad starch was found suitable for conversion into glucose. *Macrozamia* starch has also been recommended as food for poultry, pigs, and calves. By contrast, consumption of raw cycad leaves by grazing cattle has been the cause of locomotor dysfunction and death of huge numbers of animals in Australia and elsewhere. Here is an interesting topic for study by the behavioral toxicologist, because the problem of neurocycadism in cattle (zamia staggers) continues to this day.

Lest the American reader be tempted to scoff at the seemingly extraordinary Australian practice of eating patently poisonous plants, read on! Zamia in Florida was eaten by an extinct group of Florida aboriginals (sixteenth century), thereafter by Seminole Indians who relied upon it during the long wars with the United States, and later by slaves and by white settlers. In 1898, Cuzner notes authoritatively in the *Journal of the American Medical Association:*

> When the poor whites on the east coast are greatly in need of money they go to the woods to dig koonti [Z. *floridanal,* finding a ready market for the roots. Indeed, it is the sole occupation of many people. The roots are not cultivated, as they grow wild in great abundance. A very fine quality of starch and tapioca is manufactured from them, which may be found at all times in the Key West market.... The starch is said to equal the best Bermuda arrowroot and lately its worth as an article of commerce has been fully recognized in Florida. There are a number of factories for its preparation in Southern Florida. A correspondent of the United States Agricultural Department writes: "I ate of koonti pudding, at Miami, and can say that, as it was there prepared, and served, with milk and guava jelly, it was delicious."

At their peak, mills along the Miami river processed 10-15 tons of the tuberous underground stem of *Z. floridana* and Z. pumila, most of which was marketed under the name Florida arrowroot for use in infant foods, biscuits, chocolate, and spaghetti. Gifford noted that water used in washing the starch produced "slow poisoning" when drunk by animals. By 1950, nearly 6 million pounds of sago (prepared from C. *revoluta)* was imported into the United States from the Dutch East Indies for use in the preparation of food, syrup, beer, and adhesives, as well as sizing for paper and textiles.

Although the exposure of seemingly large numbers of people in certain developed countries to small amounts of "detoxified" cycad products may hold more than passing interest, the focus here is on populations that have sustained heavy exposure to patently nondetoxified cycad seed. This takes place when the raw seed is used as a topical or an oral medicine, or when time is too short to complete the lengthy procedure of detoxication by drying, water leaching, or fermentation. Such situations occur during famine periods associated with war or following typhoon, when sources of food other than the hardy cycad are destroyed. In 1696, Cleyer emphasized the importance of sotetsu sago (obtained from the stem of C. *revoluta)* as a famine food and, in 1802, Smith reported "the plant is jealously preserved for use by the Japanese army [because] the pith of its stem is [after careful preparation!] sufficient to sustain life for a long time." The nineteenth and early twentieth century literature is replete with examples of the use of cycads for famine food in parts of Asia and Africa. Preparation ("detoxication") methods differ widely not only between societies but also between families. For example, opinions expressed by Chamorro women of Guam and Rota demonstrate a remarkable range of time (2-30 days) considered appropriate to leach out the poisonous principles of C. circinalis seed prior to consumption. In the past, Chamorro children were said to fall sick after eating cycad products, a few died if preparation was poor, whereas the majority ingested the material without noticeable adverse health effects.

Few societies would be expected to be able to connect an acute illness with a chronic disease that appeared many years later. Nevertheless, in 1905, Safford wrote that Guamanians were well aware that cycad starch was injurious to health if consumed for any length of time, and Whiting recorded in the 1960s that some residents attributed a variety of ailments, including an incapacitating paralysis (lytico)-many cases of which are diagnosed as ALS-to the handling and consumption of cycad plant material.

Gaudichaud first recorded the use of *Cycas* for food among the aboriginal inhabitants of Guam. By 1900, cycad consumption was much

less common, except when maize was scarce or in times of famine after typhoon. However, during the Japanese occupation of Guam (1941-1944), when rice was hard to obtain, cycad seed represented a major source of food for the indigenous residents of Guam. Cycad flour, used to make tortillas (bread), atole (beverage), and soup, is prepared from the kernel; this is removed from the husk, halved, quartered, sliced or crushed, soaked for days or weeks in fresh water that is changed periodically (usually daily) to remove poisonous principles, dried in the sun, and ground into flour. During the wartime occupation, however, the beleaguered Chamorros were sometimes forced through hunger to forego the niceties of cycad preparation and consume products prepared from only briefly washed seed. Additionally, the fresh seed integument was used as a chew "to relieve thirst" and, after drying, as a confection. Cycad seed also enjoyed common use as a Chamorro medicine during World War II: freshly grated cycad seed kernel, or the juice therefrom, was applied to open wounds, leg ulcers, abscesses and boils, and left in place as a poultice for several days. Food and medicine shortages continued after the Americans displaced the Japanese from Guam, and there is a strong possibility that a number of U.S. (and Australian) servicemen active in Guam (and other parts of the Pacific theater) consumed inadequately prepared cycad material during this time. However, as Guamanians have progressively acculturated to the contemporary food and medical practices of the continental United States, the use of cycad for these purposes has declined along with the incidence of ALS. Whereas cycad was the famine food of the Chamorro, today the material is considered a traditional delicacy! Preference for traditional Chamorro food was recently reported to be the only one of 23 risk factors tested that showed a significant relationship with P-D. In the face of this and other data linking cycad to neurodegenerative disease, the governor of Guam strongly discouraged further use of this poisonous plant for anything but decoration.

The link between cycad and ALS was first proposed more than 25 years ago in the writings of Dr. Marjorie Whiting and Dr. F. Raymond Fosberg who were consulted by Dr. Donald Mulder and Dr. Leonard Kurland when they first suspected a toxic nutritional factor operating in Guam ALS. Between 1962 and 1972, six international conferences instigated by the National Institutes of Health in the United States were held to consider the possible relationship between Guam ALS/P-D and the traditional Chamorro practice of employing cycad seed for food. Only the third (Lyon Arboretum, 1964) and sixth (Lyon Arboretum, 1972) conferences were published, but the previously forgotten gems of the other four have become available recently (Lyon Arboretum, 1988). Although the initial focus of these conferences was to eluci-

date why neurodegenerative disorders were so common in certain of the Mariana islands (a possible link with lathyrism was entertained in the opening remarks), the important discovery of cycasin-a potent, single-shot experimental carcinogen in this and other cycad plants-progressively distracted attention away from the central issue toward the more popular concern of the mechanisms underlying carcinogenesis. By 1972, the cycad hypothesis for western Pacific ALS had fallen into disfavor for two principal reasons: cycads were reported *not* to be used for food in either the Kii peninsula or the Irian Jaya foci of ALS, and nonprimate laboratory animals repeatedly exposed to cycad products failed to develop a paralytic disorder. However, what must remain as a most puzzling oversight was the publication in the third conference of an experimental feeding study in *Macaca mulatta* which resulted in one of the three tested animals developing unilateral arm weakness, with neuropathological evidence of nonreactive degeneration of motor neurons (possibly the first primate model of ALS, this important experiment was neither confirmed nor refuted). Because cycad flour lacked cycasin by the detection methods available at the time, the presence of an unidentified noncycasin neurotoxin in *Cycas spp.* was predicted.

FROM LATHYRISM TO CYCADISM

During the initial cycad conferences, debate on the possible relationship between lathyrism and Guam ALS revolved around the observation of skin and bone abnormalities in Chamorro subjects that recalled those of osteolathyrism. This is a purely experimental disease of skin, bone, and blood vessels caused by the disruption of collagen and induced in animals by a gamma-glutamyl derivative of beta-aminopropionitrile isolated *hum Lathyrus odoratus* (sweet pea), a species etiologically unassociated with human (neuro)lathyrism. The neurotoxic species of *Lathyrus (sativus, clymenum, cicera)* contain a nonprotein convulsant amino acid, beta-N-oxalylamino-L-alanine (BOAA), which was isolated in the early 1960s; BOAA was assumed to cause human lathyrism, a nonconvulsant spastic disorder of upper motor neurons induced by heavy consumption of neurotoxic *Lathyrus spp.* By drawing an analogy between the superficially similar paralytic effects of lathyrism and cycadism in domestic animals, a search was begun by E. A. Bell for a BOAA-like compound in *Cycas* spp. Bell failed to find BOAA but, at the fifth cycad conference, described the identification of a related convulsant amino acid, beta-N-methylamino-L-alanine (BMAA). However, at the sixth conference in 1972, Bell and his colleagues reported the disappointing news that prolonged (78 days) feeding of rats with subconvulsive doses of BMAA failed to elicit paralysis. His team concluded that BMAA was unlikely

to be linked with the etiology of Guam ALS. Thereafter, there was little interest in the possible association between exposure to cycad and the development of human neurological disease despite the existence of clear-cut evidence of the paralytic properties of cycad poisoning in animals (see below).

Almost a decade later, in June 1981, I restated the possible relationship between lathyrism and Guam ALS, and called for an evaluation of the effects of prolonged administration of subconvulsive doses of BOAA and BMAA in a suitable laboratory animal (macaque). The first step, however, was to define the neurology of human lathyrism and produce a satisfactory primate model in which the action of BOAA could be examined. Thus, in succeeding years, the socioeconomic setting as well as the clinical and neurophysiological features of this disease were subjected to detailed scrutiny by colleagues from Europe, Africa, Asia, and the United States.

Lathyrism is a major cause of motor-system disease in endemic regions of Bangladesh, India, and Ethiopia, where prevalence estimates range up to an extraordinary 2.5 percent. In these regions, L. sativus is consumed as a component of the staple diet and, after flood or drought when other crops are destroyed, as an insurance crop and famine food. The neurotoxic effects of the chickling pea develop when it constitutes a major part of the diet for a period of weeks or months. Presenting symptoms typically consist of muscle cramping (especially calf muscles), and uncommonly include myoclonus, urgency and frequency of micturition, and nocturnal erection and ejaculation. One or more of these clinical manifestations of central nervous system (CNS) excitation may precede development of leg weakness and then wane or disappear once intake of the toxic diet is reduced or abandoned. The individual seems either to recover or to be left with varying degrees of spastic paraparesis, indicative of permanent dysfunction of selected regions of the corticomotoneuronal system. Those least affected run with difficulty, due to thigh adductor spasm, and walk with a stiff-legged gait; more severely compromised subjects have gastrocnemius spasm and walk on the balls of their feet with a scissoring gait; the most severely disabled have total spastic paraplegia and severe leg weakness, and are forced to crawl on their knees or buttocks (because wheelchairs are unavailable). The few neuropathological studies conducted decades after onset of spastic paraparesis have revealed degeneration of long spinal tracts, notably the corticospinal pathways. Severe loss of Betz cells (upper motor neurons) may occur, most noticeably in the upper part of the precentral sulcus and in the paracentral lobule. Anterior horn cells are not lost, and in studying

Spanish peasants who developed lathyrism during World War II, we were unable to confirm reports from Israel that long-standing subjects with the upper-motor-neuron deficits of lathyrism eventually develop additional disease of anterior horn cells, a picture that would resemble ALS. Thus, the clinical picture of lathyrism is dominated by spastic paraparesis; the disease is distinct from ALS, and neither parkinsonism nor dementia is a recorded feature.

The next challenge was to develop a model of lathyrism in well-nourished primates and compare the effects of repeated, subconvulsive doses of BOAA. These studies demonstrated that myoclonus, hindlimb extensor posturing, and neurophysiological evidence of corticomotoneuronal dysfunction appeared in cynomolgus monkeys fed for 3-10 months with an exhaustively analyzed diet of chickling pea that had been supplemented beyond the minimum nutritional requirements for that species. Similar changes were brought on more rapidly (weeks) when animals were fed either L. sativus plus BOAA or BOAA alone. Cessation of dosing led to disappearance of characteristic signs of BOAA-induced neurobehavioral dysfunction, indicating the successful modeling in the primate of the early reversible stage of human lathyrism. International efforts are now underway to remove BOAA selectively from L. sativus so that this hardy crop may be used safely by human and animal populations in Asia and Africa.

Once the primate studies had confirmed BOAA as the culpable agent of lathyrism, my attention turned to the relationship between the acute neurotoxic properties of BOAA and BMAA, and then to the primate toxicity of the latter. Initial studies were performed with mouse spinal cord explants. Application of micromolar concentrations of BOAA resulted in the appearance of discrete, postsynaptic CNS edematous vacuolation in the circumventricular organs of postnatal rodents administered convulsive doses of BOAA. Armed with these data, in September 1983 I approached Dr. Peter Nunn, a member of Bell's original team, which had discontinued work on the Cycas amino acid more than a decade earlier. Nunn kindly provided samples of BMAA which, at higher concentrations than BOAA, proved to induce comparable stereospecific neuronal pathology in mouse CNS explants. Thus, by 1984 it was predictable from the results of these tissue culture experiments that BMAA would likely produce some type of motor-system abnormality when fed to primates. These studies with Nunn and other colleagues began in the summer of 1985. Our predictions were proved correct within a few weeks of dosing the pilot animal, although the motor-system disorder that developed was markedly different from lathyrism and clinically recalled the manifestations

seen in humans with ALS/P-D. In the majority of animals, the forelimbs were affected first, with wrist drop, clumsiness, and difficulty in picking up small objects. Muscle weakness and loss of muscle bulk followed. Many animals displayed unilateral or bilateral extensor hindlimb posturing, with or without leg crossing (a feature of spasticity seen in primate lathyrism), stooped posture, unkempt coat, and tremor and weakness of the extremities. Both resting and action tremor were noted in the same animal. More prolonged intoxication led to periods of immobility with an expressionless face and blank stare, crouched posture, and a bradykinetic, shuffling, bipedal gait performed with legs flexed and rump close to the ground. Two of these animals treated with an oral antiparkinsonian drug showed selective recovery of marked facial movement and spontaneous activity. Additional features of BMAA intoxication included brief "wet-dog" shaking and limb/torso scratching, reduction or loss of aggressive behavior, disinterest in the environment, changes in normal diurnal patterns of vigilance, urinary incontinence, altered vocalization, slowed mastication, and whole-body tremor.

Electrophysiological studies demonstrated changes in the entire motor pathway, and neuropathological examination showed a hierarchy of regional susceptibility: motor cortex (most affected), spinal cord (less affected), and substantia nigra (mostly unaffected). Striking changes were found in giant Betz cells which, with smaller pyramidal cells in the cortex, underwent central chromatolysis, neurofilament accumulation, and chronic cell degeneration similar to that seen in ALS. Similar, though less marked, abnormalities were found in motor neurons of Rexed laminae VI-IX of the spinal cord. Clusters of glial cells suggestive of neuronophagia were present in one animal that had received BMAA for 17 weeks. This animal also displayed abnormal neuritic swellings, containing twisted filamentous structures embedded in an amylaceous core, in the pars compacta of the substantia nigra. Otherwise the basal ganglia, hippocampus, and cerebellum of BMAA-treated animals were similar to controls.

These data, reported for the first time in the summer of 1987, demonstrated an intriguing parallelism between chronic BMAA intoxication and human ALS/P-D. However, because the experimental disorder lacked certain important features of the human disease, notably nigral degeneration and plentiful paired helical filaments, clear-cut loss of motor neurons, and muscle denervation, it was inappropriate to refer to the primate disorder as a model of ALS/P-D. However, it is noteworthy that the disorder appeared rapidly and that the neuropathology was studied weeks to months (rather than years to decades) after initial exposure to BMAA and commencement of the

pathological process. Furthermore, primates were not exposed to whole cycad seed, to other environmental factors (discussed earlier) proposed as causally related to ALS/P-D, or to the combined effects of toxic damage and age-related attrition of vulnerable neurons (see below).

Although BMAA is able to induce convulsions in rodents and a motor-system degenerative disease in primates, the role of this compound in the production of seizures in humans and paralytic disease in grazing animals has yet to be established. In the past, these effects have been associated with hepatotoxic and neurotoxic properties of methylazoxymethanol (MAM), the aglycone of the cycad seed glycoside cycasin. The effects of acute cycad poisoning in humans and grazing animals are strikingly similar. Signs of acute toxicity in humans commence 12-40 hours after ingestion of incompletely detoxified cycad components. Nausea and vomiting develop suddenly, hepatomegaly and convulsions then appear, and the subject loses consciousness and usually dies. The disease in grazing animals (cycadism) is also marked by hepatotoxicity, enterotoxicity, and death. Cycadism has been responsible for the loss of thousands of head of cattle in Australia alone. Rapid twitching of the eyelids, nostrils, lips, and jaw muscles, with periodic tremors of the body, are reported in sheep, and muscle fasciculation has been noted in poisoned heifers. Animals that survive acute cycad poisoning develop some weeks later a locomotor disorder associated with weakness and wasting of hindlimbs. Initially, there is a staggering, weaving gait, with crossing of the legs, incoordination, and "ataxia." More severe forms are characterized by posterior motor weakness, dragging of extended hindlegs and, occasionally, a stringhalt-like action of the hocks. Function of bladder, anus, and tail is said to be unimpaired. The few pathological reports of this condition in cattle described degeneration of long, presumably motor tracts in the lumbar region, with changes in the fasciculus gracilis and dorsal spinocerebellar tracts in the cervical area. Information on the status of motor neurons that innervate the weakened and atrophied limbs of animals with cycadism is unavailable. Studies of the brain, spinal cord, peripheral nerves, and muscles of animals with long-standing cycadism (available in Australia and Japan) are urgently needed to determine whether changes are related to those seen in humans with western Pacific ALS/P-D.

LINKING CYCADS TO ALS IN IRIAN JAYA AND JAPAN

The second major reason for discarding the cycad *hypothesis* in the early 1970s was the reported absence of cycad use for food by the

Japanese of the Kii peninsula of Honshu island, or by the Auyu and Jaqai of southeastern Irian Jaya, both of whom suffered from a high incidence of ALS/P-D. Thus, in 1987, after familiarizing myself with the literature on the distribution and usage of cycads in Asia and Oceania, I set out with Valerie Palmer to determine whether the reported absence of cycad use for food in these regions was correct. Indeed it was, but other facts came to light which demonstrated heavy exposure to cycad seed of individuals who subsequently developed ALS.

Nobel Laureate D. Carleton Gajdusek had shown that cases of ALS and parkinsonism occur with remarkable frequency among Auyu and Jagai people of the remote southern inland plain of Irian Jaya where head hunting was practiced as recently as the 1940s. The Auyu and the Jaqai (their former head-hunting neighbors) now appear to live peaceably in organized riverine villages. These were constructed by Dutch missionaries who brought the Stone Age hunter-gatherers out of their forest dwellings in the first half of this century. The villages are reachable only by helicopter or canoe. My transportation was restricted to the latter because my budget also had to cover the expenses of two Indonesian physicians, a nurse, a translator, and a policeman equipped with a machine gun for protection in the event of civil unrest! Although the physical environs were not conducive to the comfortable pursuit of research, and no cycad trees were sighted in the long hours spent canoeing through the forest rivers, it turned out to be a relatively simple matter to discover the sought-after link between ALS and cycad seed. Questioning revealed that *Cycas* seed, obtained from solitary trees whose exact location in the forest was known to the Auyu, was considered an ideal medicine for topical treatments (in individuals of all ages) of various skin lesions, including open sores. For this purpose, scrapings of the kernel of a raw seed are crushed, the resulting pulp is immersed in the poisonous milky exudate, and the sodden mass is applied directly to the lesion on a leaf, which is then strapped into position. The poultice is replaced daily with freshly prepared pulp until the skin is healed. Although this medical use of cycad seed kernel was acknowledged by many, all vigorously denied employing any part of the plant for food because it was considered poisonous. Instead, the people relied for food on sago obtained from the stem of the true sago palm *(Metroxylon spp.).* Thus, in the absence of shops, these people obtained their food and medicine from the forest. On a single occasion at age 15, one 29-year-old male with ALS of recent onset told how he had applied the preparation for a month to an open sore (5-10 cm diameter) on the ankle. An elder brother with no clinical disease said he had used a cycad poultice for only two weeks to promote healing of a deep cut

of one foot. Their mother, who had taught them the procedure, was "paralyzed" before death at age 50 and, according to the ALS patient, suffered from the same disease. Immediately, the association between cycad exposure and familial ALS became clear.

Establishing a link between cycad and ALS in the Kii peninsula focus of neurodegenerative disease was more difficult. My initial suggestion of the need to study this question was greeted with surprise by a prestigious group of Japanese neuropathologists that had honored me with a guest lectureship in Osaka. Nevertheless, with the help and interest of Dr. Masayuki Ohta and Valerie Palmer, it was possible between 1987 and 1988 to establish the link. Intensive questioning of residents of Kii peninsula confirmed earlier reports that neither the seed nor the stem of C. *revoluta* had ever been used for food in this region of Japan. Indeed, sotetsu trees in Kii are used only as ornamentals for temples, schools, parks, public buildings, and adorning the gardens of the affluent. However, continued study revealed that pharmacies in the high-risk ALS area stocked untreated sotetsu seed and occasionally filled prescriptions written by practitioners of folk medicine (kitoshi). The seed was imported selectively into the region from one of the southern Ryukyu islands. Japanese tests recommend daily use of an oral aqueous steep prepared from 3-10 g of the potentially poisonous seed for the treatment of ailments such as diarrhea, dysmenorrhea, tuberculosis, neuralgia, and to "strengthen the body." Subsequently, the latter was discovered to be the reported reasoning of a concerned grandmother who administered the cycad potion repeatedly to a young girl five years of age. Additionally, the grandmother periodically provided her with a basket of young, brightly colored, and highly poisonous orange sotetsu seeds which were used by the girl as marbles, to make necklaces, and to fashion whistles. She developed into an apparently healthy teenager and did well in long-distance running races until, at the age of 18, she began to complain of backache and spasm of the calf muscles. She was diagnosed with ALS at age 20 and died some years later. Thus, in this case, as in the Auyu man from Irian Jaya, a pathological process that was not to become clinically evident for many years had silently begun at the time of the toxicologically uneventful cycad treatment. We appeared to be dealing with a previously unrecognized type of long-latency neurotoxicity involving a slow-acting toxin.

These challenging but fruitful field investigations focused on the youngest available ALS cases; if cycad exposure had occurred, it seemed likely that it would have been greatest in subjects who developed the disease at an early age. Recent events would be more memorable, and parents (who would probably still be living) would be able to

record events in the earliest years of the victim's life. Using this approach, therefore, I was able to establish unequivocally that human exposure to untreated cycad occurred in all three high-risk regions for ALS/P-D. However, the common thread was not food use of "detoxified" cycad components-a practice that some cultures (e.g., Australian aboriginals) apparently had perfected-rather, it was exposure to the *untreated* cycad seed kernel as medicine. In Guam and in Irian Jaya, this took the form of topical exposure to open wounds (tropical ulcers), whereas in the Kii peninsula, oral medicinal use had been incorporated into the local culture. This is not denying the possible additional role of improperly prepared cycad food, especially since a recent epidemiological study linked traditional Chamorro food with P-D.

These studies also revealed that cycad use was declining in all three regions, thus accounting for the declining incidence of ALS. In the Kii peninsula, cycad prescriptions are now issued by a few elderly kitoshi and the practice is dying out. In Irian Jaya, the recent introduction of Dutch and Indonesian education, coupled with the limited availability of relatively modem methods of medical treatment, is reducing Auyu dependence on cycad seed for medicine. Finally, in Guam, the medical practices of the Chamorro folk doctor (surahana) have been curtailed by law, and food products prepared from cycad seed are viewed as delicacies. Thus, the declining patterns of utilization of cycad seed in all three communities at risk for ALS/P-D fulfill the criterion of a disappearing environmental factor required as an etiological link to ALS/P-D.

TIMING OF CYCAD EXPOSURE

If cycad is the principal trigger for western Pacific ALS/P-D, when does the critical exposure occur, and do the timing and degree of intoxication influence the nature of the resulting clinical compromise? Although the answers to these important questions are unknown, some clues link motor neuron disease in the western Pacific loci with cycad exposure at a remarkably early age. One is the presence of multinucleated and misplaced neurons in the cerebellum and vestibular nuclei of some Japanese and Guamanian subjects with ALS/P-D. This suggests exposure during the later phases of brain development (up to the age of 1 year) to an agent that arrests the developmental mitotic and migratory responses of neurons; one such substance known from experimental rodent studies is the neuroteratogen MAM. Administration of MAM to newborn rats results in cerebellar microplasia associated with misplaced and multinucleated neurons, an experimen-

tal observation not previously connected with the human disease. A second, more direct link, is the appearance of ALS in Japanese and Irian Jaya subjects within 10-15 years after exposure to cycad seed in the first or second decade of life, as discussed earlier. This is consistent with the observation that some Chamorro subjects developed ALS 1-34 years after leaving Guam; based on age of migration, the minimum duration of exposure to the Guam environment to have acquired disease susceptibility was the first 18 years of life. Additionally, according to longitudinal data for the incidence of neurodegenerative disease in the Chamorro, the peak for ALS among males in 1955 followed approximately 10 years after the period estimated for maximum reliance on cycad for food and medicine, whereas the peak for P-D occurred in 5-10 years. Taken in concert, therefore, these data indicate that ALS/P-D is a long-latency disorder that may be acquired years or decades prior to clinical expression.

Changes in the demographics of ALS and P-D that have occurred as cycad use has declined provide clues as to why some patients develop ALS, and others-who tend to be older-develop P-D. The incidence of ALS among male Chamorros was twice that of P-D in the early 1950s, whereas 20 years later the relative proportion of such cases was inverted. Although comparable data are unavailable for Kii peninsula ALS/P-D, a similar proportional decrease of ALS (relative to parkinsonism) appears to be occurring in the epicenter of the Irian Jaya disease focus. Additionally, teenage cases of ALS are no longer seen in any of the high-incidence disease areas and, on Guam, the mean ages for onset of ALS and P-D (seen in older subjects) have increased over the past 30 years. These several pieces of data are consonant with the proposal that degree of intoxication is a critical factor dictating both the age of onset and the clinical characteristics of the resultant disease. Specifically, heavy exposure may precipitate ALS by lethally damaging motor neurons. The heavily exposed subjects also sustain some damage to the apparently less susceptible nigrostriatal pathway-a silent change demonstrable neuropathologically and by fluorodopa positron emission tomography, but because the lesion is insufficient to overcome the large functional reserve of this pathway (thereby permitting the clinical expression of parkinsonism), the victim seemingly suffers only from motor neuron disease (ALS). Other subjects who are less heavily intoxicated (or have a lower degree of susceptibility) may survive for many years with motor neuron compromise and eventually develop parkinsonism as a consequence of the additive effects of toxic damage and attrition of the age-susceptible nigrostriatal neurons. A further possibility is that dementia represents the late effects of the lowest clinically significant level of cycad exposure,

whereas the majority of elderly Chamorro subjects with subclinical neurofibrillary tangles represent those with extremely low exposure or a relatively high tolerance. In summary, therefore, this working hypothesis states that the various forms of western Pacific neurodegenerative disease represent individual points on a three-dimensional dose-response curve for plant toxicity in which time represents the third dimension.

RELEVANCE FOR VETERANS OF THE PACIFIC WAR

If our working hypothesis is correct, individuals who receive small doses of cycad toxins develop a dementing illness in old age that could be readily mistaken for Alzheimer's disease. Because many U.S., Australian, and Japanese veterans were subjected to shortages of food and medicine in the Pacific theater of World War II, it is possible that some resorted to cycads during this period. This point is of special relevance for U.S. personnel serving on Guam during the immediate postwar period. However, surveys conducted in the 1970s revealed that neither the 2 million U.S. Armed Forces veterans who passed through Guam or the northern Marianas since 1945, nor some 10,000 U.S. construction workers in Guam from 1945 to 1954, had a measurable increased risk for ALS/P-D. Unfortunately, these studies were conducted on many subjects who had yet to reach the present mean age for onset of (male) Guam ALS (52 years) or P-D (60 years). In view of this consideration and several anecdotal reports of U.S. veterans who developed ALS or Alzheimer's disease after serving on Guam or New Guinea, it seems important to reexamine this issue.

TOXIC COMPONENTS OF CYCADS

Two toxic agents have been identified in Cycas seed: cycasin(s) (the active form of which is MAM) and BMAA: MAM is a potent hepatoxin, carcinogen, and teratogen, which is also able to interrupt cerebellar development in mice; BMAA is an excitant neurotoxin that produces a motor-system disorder in primates after repeated subconvulsive dosing. Although BMAA is present in low concentrations relative to that of cycasin in cycad seed kernel, what constitutes a significant dose of BMAA has yet to be established. Dr. Glen Kisby has recently developed a method to quantify BMAA in plant and animal tissue after derivitization of amino acids with fluorenylmethyl chloroformate (FMOC), separation by high-performance liquid chromatography, and detection of FMOC-BMAA by fluorescence. Recent studies from our

laboratory show that BMAA (1) increases in concentration with time after the seed kernel of C. circinalis has been crushed, (2) appears when serum is incubated in vitro with serum alanine, and (3) is found in serum of primates after intraarterial injection of MAM. The potent methylating action of MAM therefore has the potential to increase the concentration of BMAA in both plant and animal tissue. Other studies show that cycasin in cycad leachate diminishes in concentration prior to BMAA. Thus, individuals ingesting seed kernel that has been soaked in water for only a few days would receive doses of both MAM and BMAA. However, larger doses of both compounds would probably be absorbed/generated in subjects with an open wound treated topically with a cycad poultice; this practice provides 24-hour/day subcutaneous exposure for weeks, with daily replacement of fresh plant material.

The precise locus of initial action of BMAA in the nervous system has been determined with a combination of tissue culture and animal studies in which synthesized L. sativus neurotoxin (BOAA) has served as a comparison compound. These studies showed that micromolar concentrations of both compounds rapidly induced postsynaptic dendritic neuronal changes comparable to those seen with potent glutamate analogues (excitotoxins) which cause sustained depolarization of neuronal membranes and thereby trigger convulsions; BMAA was less potent and slower acting than equimolar concentrations of BOAA.

The differential neuronotoxic (excitotoxic) action of these compounds was delineated by pretreatment of the tissue explant with drugs acting selectively at glutamate receptor subtypes. Glutamate receptors, presumably located on the surfaces of dendrites and neurons, have been classified according to their responses to three potent agonists: N-methyl-o-aspartate (NMDA), quisqualate, and kainate. Whereas the neuronotoxic action of BMAA in cortical explants is dose dependently attenuated by 2-amino-7-phosphonoheptanoic acid, a selective antagonist for the NMDA receptor, BOAA neuronotoxicity was similarly impaired by pretreatment with piperidine dicarboxylic acid (PDA), a nonspecific antagonist that blocks the action of the plant-derived excitotoxic amino acids kainate and quisqualate. These data, collected by Dr. Stephen Ross, suggest that the neurotoxic action of BMAA is mediated by the NMDA receptor complex.

Dr. Ross made comparable observations in young CD-1 mice by measuring the duration of hyperexcitability induced by BMAA or BOAA administered by intracerebroventricular injection (i.c.v.). The BMAA induced a transient hyperexcitable state followed by long-lasting whole-body shaking and wobbling. Pretreatment of mice i.c.v. with the NMDA antagonist AP7 provided complete protection against

this BMAA-induced behavioral change; AP7 also showed a nonsignificant trend for protection against the early, transient hyperexcitable state caused by injection of BMAA. By contrast, PDA had no effect on BMAA-induced hyperexcitability, although this drug was dose dependently active in attenuating the seizuregenic responses triggered by administration of BOAA i.c.v. These results confirmed the differential acute neurotoxic actions of BOAA and BMAA, as well as suggesting again that the neurotoxic action of BMAA is mediated via the NMDA receptor complex.

These neuropharmacological studies have been supplemented and extended by measuring electrophysiologically the time course and patterns of BOAA- and BMAA-induced depolarization of synaptically interconnected mouse hippocampal cells grown in primary cell culture. Voltage-clamp recordings made using the whole-cell configuration of the patch-clamp technique have shown that similar waveforms of currents activated by BOAA also displace specific ligands for the quisqualate site in mouse brain tissue. In comparable electrophysiological studies, pressure injection onto the cell surface of either NMDA or the BMAA elicited similar patterns of depolarization. Under the same conditions, both the NMDA and the BMAA currents were blocked by AP7. Taken in concert, therefore, these studies suggest that whereas BOAA preferentially (and reversibly) binds to the quisqualate receptor, BMAA acts in its parent form at a site associated with the NMDA complex.

Although the different neuronal receptors targeted by BOAA and BMAA may be etiologically linked to the distinct patterns of neuronal vulnerability in human lathyrism (cortical motor neuron) and western Pacific ALS (upper and lower motor neurons, substantia nigra, and hippocampus), the motor-system disorders show another clinical distinction that must be cardinally important to an understanding of their pathogenesis. Whereas lathyrism is a largely self-limiting disorder that typically appears subacutely in individuals who consume excessive amounts of BOAA-containing chickling pea for several weeks or months, ALS/P-D appears to be a long-latency disease that is triggered years or decades prior to the clinical appearance of a *progressive* disease. Because the onset of western Pacific ALS in teenagers cannot be explained by concurrence of toxic damage and age-related attrition of the same cellular population, there must be another explanation for this tardive phenomenon. Thus, it seems likely that cycads contain factors ("slow toxins") which are able to penetrate neurons and establish irreversible changes that trigger their progressive downfall. Because changes of this type are unlikely to be mediated via the cell surface, it is important to determine whether BMAA or other cycad toxins are able to

enter selected neurons (perhaps *by way* of the structural entity that is critical for cell survival). One important question under investigation is whether this event occurs at the level of the cell nucleus.

SUMMARY

Three of the most devastating degenerative disorders of the human nervous system-ALS, parkinsonism, and progressive presenile dementia-have been present in combined form and remarkably high incidence at three foci in the western Pacific region. In all three areas, ALS is associated with oral or percutaneous exposure to the untreated seed kernel of *Cycas* plants. Consumption of Cycas or other cycads precipitates a poorly defined locomotor disorder in grazing animals (cycadism). Although the culpable neurotoxic agent has yet to be identified, at least two compounds with differential neurotoxic properties have been previously isolated from Cycas seed: BMAA and MAM, the latter being capable of generating BMAA in serum. Methylazoxymethanol is an experimental hepatotoxin, carcinogen, and neuroteratogen: it arrests rodent cerebellar neuronal migration and mitosis during development, and multinucleated and displaced neurons in some cases of ALS/P-D may represent a biological marker of early exposure to this compound. Beta-N-Methylamino-L-alanine is an excitant neurotoxin in rats and a neurotoxin in mouse cord and cortex explants; neuropharmacological and neurophysiological studies indicate that the depolarizing, excitotoxic, and neuronotoxic actions of BMAA are mediated by the NMDA-receptor complex. Although BMAA produces an interesting and potentially important constellation of motor neuron, extrapyramidal, and behavioral dysfunction in cynomolgus monkeys repeatedly fed subconvulsive doses of the pure compound, insufficient neuropathological changes have been generated to merit description of the primate response as an animal model of western Pacific ALS/P-D. The role of BMAA, MAM, and other factors in the etiology of this disease is being studied.

Epidemiology and other data strongly suggest that human exposure to cycad toxins may precede by years or decades the onset of clinical ALS or P-D, the nature of the clinical disease and the age at which it appears possibly being linked with cycad doses and host susceptibility. The mechanism underlying the proposed long-latency adverse effects of cycad exposure is believed to represent the single most important question in elucidating the pathogenesis of this neurodegenerative disorder.

An understanding of the chemical factors that trigger western Pacific ALS/P-D is expected to lead to the identification of comparable

chemical factors in other environments. Research of this type should not be restricted to chemical components in plants used for medicine or food but should also take into account the wider potential for chemical factors that might have a role in triggering some forms of motor neuron disease, parkinsonism, and senile dementia of the Alzheimer type. By intensive exploration of the chemical exposure cupboards of young-onset patients with neurodegenerative diseases, it may be possible to obtain pertinent leads that can be tested in the laboratory. However, because exposure and disease onset may be separated by prolonged periods of time, and the nature of the putative factors is unknown, such an effort will require every weapon in the combined armamentaria of the epidemiologist and the neurotoxicologist. Experience in the western Pacific loci teaches that individual patients and their families, rather than large statistically selected populations, are the preferred targets of initial study, and intensely probing interviews are more likely to generate clues than prescribed questionnaires which miss the key questions and fail to record important responses. This approach, combined with intensive laboratory studies, is surely the most rapid way to find out if neurotoxins are driving us crazy in developed countries, just as they are tragically the apparent cause of dementia in certain communities of the western Pacific.

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