Guam ALS/Parkinsonism-Dementia: A Long-Latency Neurotoxic Disorder Caused by "Slow Toxin(s)" in Food?

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ABSTRACT: Parkinsonism (P) with progressive dementia (D) of the Alzheimer type is recognized as a clinical variant of a form of amyotrophic lateral sclerosis (ALS) that has occurred in high incidence among the Chamorro people of the islands of Guam and Rota in the Marianas chain of Micronesia. The declining annual incidence, upward shifting of the age of onset, narrowing of the sex ratio, and occurrence of the disease among non-Chamorros, point to a disappearing environmental causation peculiar to the traditional culture of these islands. Evidence is presented in support of the proposal that heavy use of certain toxic plants, notably cycads, a traditional source of food and medicine for the Chamorro people, plays an important etiological role. Clinical and epidemiological approaches are offered to test for a relationship between ALS/P-D and long-latency plant toxicity.

RESUME: Le syndrome SLA-Parkinsonisme-Démence de Guam: une affection neurotoxique avec période de latence prolongée causée par des "toxines alimentaires lentes"? Le parkinsonisme (P) associé à une démence (D) progressive de type Alzheimer est reconnu comme une variante clinique d'une forme de sclérose latérale amyotrophique (SLA) qu'on retrouverait avec une incidence élevée parmi le peuple Chamorro des îles de Guam et de Rota dans la chaîne des îles Mariannes en Micronésie. La baisse de l'incidence annuelle, l'élévation de l'âge de début, une distribution plus égale selon le sexe et la présence de la maladie chez des non-Chamorros suggèrent qu'il existe une cause environnementale à cette maladie, cause qui est en voie de disparition et qui est particulière à la culture traditionnelle de ces îles. Nous présentons des données qui supportent l'hypothèse selon laquelle l'utilisation fréquente de certaines plantes toxiques, particulièrement le cycas, source traditionnelle de nourriture et de médicament pour le peuple Chamorro, joue un rôle étiologique important. Nous proposons certaines approches cliniques et épidémiologiques pour vérifier s'il existe une relation entre la SLA/P-D et la toxicité de cette plante.

Outlined here is a chronology of clinical and experimental research, begun in 1981, that from the outset sought a relationship between Guam ALS/P-D and lathyrism. A form of spastic paraparesis induced by subsistence on the neurotoxic chickling pea (*Lathyrus sativus*), lathyrism has been highly prevalent after periodic famines in parts of Bangladesh, India and Ethiopia. Similarly, it is proposed here, the wartime period of heavy consumption of toxic cycad seed (*Cycas circinalis*), a traditional source of food and medicine for the Guamanian people, and perhaps other toxic plants such as cassava, may be responsible for the former high incidence of ALS/P-D on certain of the Mariana islands.

The cycad hypothesis is not new: indeed, this idea arose 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. After a decade (1962-1972) of vigorous investigation and debate, the cycad hypothesis was thrown overboard where, but for the persistent interest of Kurland, it would have drowned.

The cycad hypothesis has been rescued and revived (in modified form) through systematic study of lathyrism, a poorly known and probably related motoneuron disorder, and through the application of organotypic tissue culture to compare and contrast the properties of chemically related neurotoxic principles in *Lathyrus sativus* (BOAA: β-N-oxalylamino-L-alanine) and *Cycas circinalis* (BMAA: β-N-methylamino-L-alanine). These studies led to the development of primate models of lathyrism, BOAA and BMAA toxicity, the latter possessing features of motor neuron disease, parkinsonism and behavioral...
changes. The primate response to repeated oral administration of synthetic BMAA has reawakened widespread interest in the possible etiological role of cycads in Guam ALS/P-D. Provided here is additional information gleaned from published and unpublished reports which, in concert, implicate toxic plants, notably cycads, in the etiology of neurodegenerative and other, perhaps related, diseases on Guam.

GUAM ALS/PARKINSONISM-DEMENTIA

Epidemiology Parkinsonism (P) attended by progressive presenile dementia (D) of the Alzheimer type is considered a clinical variant of a form of amyotrophic lateral sclerosis (ALS) that has occurred in high incidence in at least three Western Pacific loci, including the islands of Guam and Rota in the Marianas chain of Micronesia. In this locus, these seemingly disparate disorders are essentially restricted to the indigenous Chamorro people and a few Filipinos and other immigrants who have adopted and employed the Chamorro lifestyle for periods of 15-26 years. By contrast, neither the 2 million Armed Forces veterans who have passed through Guam and the northern Marianas since 1945, nor some 10,000 U.S. construction workers stationed in Guam from 1945-1954, have a measurable increased risk for ALS or P-D. However, Guamanian-born Chamorros who, at age 18 or older, migrated to the U.S. mainland or elsewhere, carry an increased risk for ALS that may appear more than three decades later. Additionally, 70% of 302 control brains of Chamorros aged 35 years and above, who died of non-neurological causes, contained significant numbers of neurofibrillary tangles (NFT), and 15% of those with tangles were indistinguishable from definitive P-D cases. While P-D and/or ALS may strike several family members and affect several generations of individual families, the disease lacks a Mendelian pattern of inheritance and is therefore thought not to have a genetic etiology. Moreover, an infectious agent, including that of the slow-virus type, has not been identified. These observations, coupled with the declining age-adjusted annual incidence rates for P-D (males) and ALS, suggest the etiology is to be found in a disappearing environmental factor that is widespread within, and peculiar to, the Chamorro culture.

Discovery Mulder, Kurland and Iriarte were the first to note the combination of parkinsonism (initially attributed to Japanese B virus encephalitis), disturbed sleeping habits and memory defects, in a paper describing the remarkable prevalence of ALS in Guam and Rota, as compared to that observed in the more northerly Mariana islands of Tinian and Saipan. Earlier reports by Zimmerman and Arnold and colleagues provided clinical and pathological demonstration of high-incidence ALS on Guam, while that of Koerner noted additional supranuclear abnormalities in 80% of 35 ALS patients. Subsequent research revealed that an unusual prevalence of "hereditary paralysis" on Guam had been reported as early as 1901 and that, in 1936, a Japanese physician described parkinsonism in an individual whose brother was subsequently found to exhibit P-D and a son with childhood epilepsy and adult-onset schizophrenia. The first detailed clinical description of ALS in a Guamanian is also to be found in the Japanese literature circa 1925 (Kurland, pers. comm.).

Although the pathology of Guam ALS was initially considered to be typical of motor neuron disease, neurofibrillary changes of the Alzheimer type were discovered by Malamud in 1957. Thereafter, the relationship between the clinical and pathological features of Guam "parkinsonism-dementia complex" (known locally as bodig, manman, or rasput) were evinced by Malamud's group and Hirano and colleagues who ruled out Japanese B encephalitis as causal. Lessell and co-workers noted 4 patients with P-D who originally were registered as having ALS, and 11 others who showed amyotrophy and fasciculations. ALS and P-D therefore came to be considered clinical variants of the same disease mechanism.

Clinical Features Chen and Yase have provided a condensed description of P-D (see also 18): "Patients characteristically show eventful premorbid conditions such as obesity (60%), essential hypertension (40%), late-onset diabetes (45%), hyperuricemia (55%), and significant trauma (30%). One to five years prior to the onset of extrapyramidal symptoms, psychoneurotic complaints attributable to autonomic dysfunctions, such as dizziness, nervousness, fatiguability, loss of appetite or libido, excessive sleepiness, etc. may mask the insidious onset of organic brain syndrome or parkinsonism. There is early deterioration of fine movements, hypomimia, decreased blinking, bradykinesia, and eventually increasing rigidity with impaired postural reflexes. Tremors, unlike [their] idiopathic counterpart, are seldom present. They are faster and finer [in] action or static tremors and, only in rare cases, 'pill-rolling' or guitar-picking resting tremors are seen. As the disease progresses, incontinence of urine and feces, osteoporosis and fractures from falls, anemia, and extensive bedsores develop and the patients finally succumb to intercurrent systemic infections caused by immunodeficiency. Upper and lower motor neurons are affected as a rule and quadriplegia in flexion, irreversible contractures of all joints, extension of head and a total mutistic state . . . develop in the advanced stage.

Parkinsonism-dementia with amyotrophy, in addition to typical parkinsonism-dementia features, present unequivocal amyotrophic lateral sclerosis findings, including multisegmental muscle atrophy, spasticity, and hyperactive DTRs, and,
The clinical presentation... into three patterns: 1) simultaneous onset of both features, 2) initial onset of parkinsonism-dementia followed by amyotrophy and spasticity, and 3) onset of amyotrophic lateral sclerosis features followed by dementia or parkinsonism-dementia. Additional "‘borderline’ cases... present two or more of the following features: older age at onset of dementia, dementia without parkinsonism, or ‘senility’ with rather vague neurologic symptoms. Many of these patients are also complicated by hypertension, late-onset diabetes, gout or hyperuricemia, microcytic anemia, or history of minor strokes.”

Etiology The Guamanian disease originally was seen as a purely dominantly inherited disorder peculiar to the Chamorro people (a view that continued to be expressed as late as 1969), but discovery of ALS cases among Carolinians on Saipan forced consideration of the possible role of exogenous agents, including a nutritional-toxic factor. Thereafter, a combination of hereditary or metabolic predisposition, coupled with nutritional-toxic or other exogenous factors, was implicated, As evidence of the decline in incidence of ALS has amassed, there has been a corresponding shift of interest toward the etiologic role of unidentified environmental factors. Yase’s proposal of mineral deficiency resulting in metal intoxication has held the limelight in recent years and has been strongly supported by Gajdusek and colleagues. This group suggests that defects in mineral metabolism and secondary hyperparathyroidism, provoked by chronic deficiency of calcium and magnesium, lead to increased intestinal absorption of toxic metals, such as aluminum and magnesium, the mobilization of calcium from bone, and the deposition of these and other elements in CNS tissues. Intra-neuronal accumulation of aluminum, calcium and silicon, has been demonstrated by cellular analytical techniques, but there has been no confirmation of the reported paucity of calcium in the majority of tested rivers on Guam. Although aluminum is neurotoxic, it is yet to be shown that calcium/magnesium-deficient primates receiving aluminum develop a disorder akin to P-D or ALS. In the absence of an animal model, the Yase/Gajdusek proposal must be considered unproven although, it should be noted, the idea is not incompatible with the cycad hypothesis that follows.

THE CYCAD CONFERENCES

Between 1962 and 1972, six international conferences instigated by N.I.H. were held to consider the possible relationship between Guam ALS/P-D and the traditional Chamorro practice of employing the toxic seed of the false sago palm, Cycas circinalis, for food and medicine. Although the initial focus of these conferences was to elucidate why neurodegenerative disorders were so common in certain of the Marianas islands, the important discovery of cycasin, a potent carcinogen in this and other cycad plants, progressively distracted attention away from the central issue toward mechanisms underlying experimental carcinogenesis. Furthermore, failure to induce experimental animal models of cycad-associated motor neuron disease led to a discontinuation of interest and support for further research on a link between cycad exposure and ALS/P-D. In 1978, Kondo noted the cycad hypothesis was “unique but negative”. “Search for a toxic agent included lathyrogens because Guam ALS showed collagen changes in the skin and exostotic disease was prevalent in the population, but the effort was in vain”.

The first conference on the Identification of Toxic Elements in Cycads was held at N.I.H. on February 28, 1962, with Dr. Kurland presiding. In the opening remarks of this historic and critically important meeting (proceedings unpublished), Kurland notes Fullmer’s discovery of changes in skin collagen in Guam P-D and ALS and Krooth’s demonstration on Guam of the world’s highest incidence of diaphyseal aclasis (multiple exostoses).

Kurland continues: “These findings led me to suggest in our conferences here at N.I.H. in July, September, and November [1961], that there might be an analogy between the neurological conditions in the Marianas and odoratism and lathyrism... The basis for the intense studies now underway is the thought that lathyrism and odoratism — human [neurolathyrism] and experimental [osteolathyrism] — which are due to toxic compounds found in natural products, have in appropriate species at appropriate ages and with appropriate compound neurological involvement, collagen change, and a form of multiple exostosis in common with the problems described above. Consequently, it was suggested that there might be a basis for intensive studies of some of the natural products and known toxic materials to which the population on the island were exposed... In 1954, we [Mulder and Kurland, in consultation with the botanist, Dr. R. Fosberg] encouraged Dr. Marjorie Whiting, who was on the island at the time, to carry out a diet
Following her field studies, Dr. Whiting suggested a relationship between this disease and ingestion of *Cycas circinalis*, an important indigenous source of food for this population. Dr. Whiting, who has been responsible for much of the pioneering work in this area, is going to report to us this morning on the uses of cycad in various populations and the reported toxic effects in animals and humans.”

Whiting: “Thank you. I wish to present some of the findings which led to our choice of the cycad for intensive investigation in connection with medical research on Guam. I should remind you that it is only one of several toxic plants [emphasis added] indigenous to the island and community ingested as a food, a chew, or medicine. It was decided that I should live a month [during 1954] in each of two villages, Umatac and Yigo (Figure 1). Umatac, until recently had been geographically isolated and at that time had the highest incidence of ALS [and P-D] of any Guamanian village. In many ways it was still a rural community with only a few of the residents commuting to other parts of the island for school or employment” . . . whereas “Merizo [with a lower incidence of ALS and P-D] had a road to the central and northern part of the island for several years. The two- or three-mile strip of road between Merizo and Umatac was not completed until recently (1957). “During World War II, the residents of Umatac, because of their isolation, were perhaps better able to resist the demands of the Japanese and to make use of food obtained from fishing and from jungle farming. Yigo, on the other hand, was located close to air bases and hospitals, and to Agaña, the government and business center of the island.

... As a basic premise for the research, I felt that any nutritional factor or toxic substance implicated in the causation of a neurological disease on Guam would have to be associated with some peculiarity of the Chamorro cultural pattern. The substance would either have to be used by Chamorro and not by the native populations of other Pacific islands [she had worked on Ponape for the preceding 2 years], or be commonly found in other areas but prepared or treated by the Guamanians in a manner somewhat different from that of other groups. I was aware that there might be combinations of food (or of chew or medicinal) mixtures which could produce ill effects. With these points in mind, I became an avid collector of recipes for native dishes paying close attention to each step in the preparation and to all possible variations of a recipe. I used such leading questions as “What do you use as a substitute when you have no store flour . . .? How did you manage to survive during World War II? I checked reports of sudden illness and of a few deaths which reputedly followed consumption of a particular food item. Some followed consumption of bitter cassava, others of cycad starch (emphasis added, vide infra). Generally they were recognized as due to inadequate soaking or preparation of a known toxic plant. Some persons can never eat the federico (cycad). They get a headache even though they like it. When I enquired about the cause of ALS (or, in the vernacular “leetiko”), several persons suggested the cycad [emphasis added]. Some people like to eat it and . . . go to considerable lengths to obtain and process it. Because of its peculiar mucilagenous property it is highly desirable for making tortillas and as a thickener for other dishes. On the other hand, “everyone” knows of the

**Figure 2** — Top left: Immature seed (arrow) on seed-bearing megasporophylls of *Cycas circinalis*. Lower left: Two mature seeds (center) of the type used to prepare flour. Upper right: Mature seed (right), split seed showing the starchy white endosperm (center), and the latter removed and soaking in water (left) to leach out water-soluble poisonous principles. Lower right: Traditional method of grinding the washed and sun-dried cycad seed endosperm (arrow) to produce flour used in tortillas and other local foodstuffs.
toxic property of the plant. Dogs and chickens reputedly die if they drink the wash water. Preparation is laborious. Directions vary but soaking is required for “several” days with “frequent” changes of water. During the process of beating off the husks, some persons become dizzy [probably from liberated HCN] and have to leave their work for a time to recover. Children are not allowed to participate in this stage of the processing. Only small amounts are given to children because many become ill when they first eat a dish made with cycad starch [emphasis added].

During the [Second World] war, many women [of Umatac] have told me that their chief occupation throughout the day, day after day, was preparation of cycad starch for their family, breaking the nuts, soaking them, grinding them” (Figure 2). “During this period, which lasted two or three years, there was practically no import of rice. The Japanese controlled much of the shore line. The people were in the hills and had to depend pretty much on native food, on what they could get through night fishing expeditions, or through some trading with the Japanese.”

Kurland: “The rate [of ALS/P-D] is as high on Rota as in Umatac. Rota is quite isolated and is almost entirely independent — in fact it supplies agricultural products for Guam! But this isolation would not explain the low frequency in Saipan . . .”

Whiting: “In Saipan, early in the century, the Germans cut down the cycad trees to make room for sugar plantations. Since new cycads have not been available until quite recently, Guam provided cycad starch for the Chamorros who live there. I know that Guamanians did send cycad starch to Saipan for their relatives, but perhaps Chamorros on Saipan did not have as much contact with the nuts [cycad seeds] as those living on Guam. I am sure not as much as in Umatac, for I believe they probably make a large amount of starch for the island there. The local residents would have more contact with it as well as more use of it. . . . Guamanians enjoy atole, a hot beverage made with coconut cream and starch . . . from corn, cycad, or some other grain or root. . . . Besides a starch resource [from washed seed], other portions of the plant are eaten. The green husks of the seeds, [allegedly non-toxic] are chewed in the jungle “to relieve thirst”. These outer husks are also dried and eaten as a confection in Guam and by aborigines of Australia. Animals grazing in areas where the cycad plant grows eat the sprouts and young leaves. . . . Reports of toxicity in animals describe two different conditions — an acute illness characterized by severe gastro enteritis, and [a] chronic condition characterized by a peculiar paralysis of the hindquarters which, from all accounts, appears to be progressive and irreversible [reviewed by Spencer and Dastur].

The only medical use I ever found on Guam for the cycad, is for what is termed “tropical ulcers”, which frequently occur on the leg or foot. The fresh fruit is grated and spread on as a fresh poultice for two or three successive days. Reputedly this remedy is effective when all other treatment fails.”

Thus, in this short exchange, Whiting linked the differential consumption of cycad flour in World War II to the inter-island and inter-village distribution of ALS/P-D and showed that people, notably children, fell sick from eating (inadequately prepared) cycad-based foodstuffs. Elsewhere she states that many families reported daily use of cycad starch during World War II. “Today [1964] with more wage employment on the island, use of native plants of all kinds is diminishing because fewer individuals have time for the long procedures required for processing.” Kurland and Molgaard amplifies in 1981 in response to Whiting’s question: “It is my impression that [cycad flour] usage, essentially 100% during World War II, is down; even by 1965, it was probably down to about 25% of what it had been and, currently, would be 10% or less. There seems to be a parallel decrease of cycad usage and ALS, but other changes have also occurred.” Today, flour derived from washed cycad seed (now considered a delicacy) is still used by some people on Guam and Rota.

The second cycad conference, held at N.I.H. on August 17, 1962, continued to address (among other issues) the suggested relationship between ALS/P-D and lathyrism. At that time, the etiology of both human (neuro)lathyrism and experimental (osteo- and angio-)lathyrism were attributed to aminonitrile components in culpable Lathyrus seed. However, it is now known that the experimental disorders of skin, bone, and blood vessels, result from feeding seed of Lathyrus odoratus (a species unassociated with human neurolathyrism) which contains a γ-glutamyl derivative of β-aminopropionitrile (BAPN), a non-neurotoxic entity that inhibits lysyl oxidase required for collagen biosynthesis. By contrast, seed of those species of Lathyrus (sativus, clymenum, ciceria) that induce human (neuro)lathyrism contain little or no BAPN; thus, bone changes are rarely described in association with the spastic paraparesis that characterizes the human disease. The neurotoxic species of Lathyrus contain the potent convulsant amino acid BOAA, isolated in the early 1960s by three groups in India22,53 (Nagarajan, Roy et al., unpubl.) and by Bell in Britain.54 Bell noted the difference between human (neuro)lathyrism and experimental (osteo)lathyrism at the last two cycad conferences in 1967 and 1972.55 The role of BOAA in the human disease was challenged in 198356 but later shown to induce clinical and electrophysiologic signs of corticospinal dysfunction in Old-World primates.4

In 1963, Whiting6 published a scholarly review of the literature on cycad toxicity demonstrating the possible link between acute and chronic cycadism in humans and animals, both domestic and experimental. However, the same year, Laqueur and colleagues57 reported their experimental feeding studies with rodents: they failed to demonstrate any neurological disease but, instead, induced anemia, pancreatic edema, bile-duct proliferation, cirrhosis, hepatocellular carcinomas and reticuloendothelial neoplasms in liver, and adenomas in kidney, in animals fed the endosperm of cycad seed that is processed on Guam for flour. Similar results with cycad seed or flour were reported by other research teams at the third cycad conference held in Chicago, April 17, 1964.58 This theme occupied much of the fourth conference (N.I.H.: April 15, 1965) in which the chemical properties and biological effects of the cyad glucoside Lathyrus odoratus were addressed. The unpublished proceedings also report the experimental induction of hindlimb dysfunction in grazing animals, a disease (cycadism) considered by Mason and Fredrickson (p. 156) to correspond to an upper-motor-neuron disorder (reminiscent of (neuro)lathyrism). The possibility of basal ganglia compromise in these animals was also raised but never studied.

The fifth conference on Cycad Toxicity,9 Miami, April 24-25, 1967, brought together a number of significant contributions on the hepatotoxic, pancreatic, carcinogenic and teratogenic properties of cycasin and cycad components. Sanger and colleagues (pp. XVI-1-21) discussed the results of feeding five animal species either cycad seed, husks, or unwashed endo-
Lathyrus sativus, L.

for BMAA), a convulsant amino acid freely present in cycad seed. They had purposefully looked for agents comparable to BOAA because, they noted, cycad-induced cattle paralysis was reminiscent of lathyrism, "a disease produced in man and domestic animals by eating the seeds of Lathyrus sativus, L. clymenum and L. cicera, all of which contain α-amino-β-oxalamino-propionic acid" (synonym for BOAA).

The presence of a non-cycasin neurotoxic entity had been predicted three years earlier by Dastur:59 At the third cycad conference, Dastur demonstrated Betz cell and anterior horn cell degeneration, with skin atrophy and collagen degeneration, in a single young monkey with severe weakness and wasting of one arm that appeared between months 4-9 after commencing a diet of cycad flour containing no detectable cycasin. Unfort­unately, the enormous significance of these findings in relation to the neurological and dermal changes of Guam ALS/P-D, and their suggested link to lathyrism and odoratism (osteolathyrism), was never cited, nor were Dastur's preliminary studies con­firmed or refuted. Bell returned for the Sixth International Cycad Conference (N.I.H.: April 17, 1972) with the disappoint­ment news that prolonged (78 days) feeding of rats with subcon­vulsive doses of BMAA failed to induce observable neurolog­i­cal changes.60 BMAA, his research team concluded, was unlikely to be important in Guam ALS. Additional papers disclosed the neurotoxic effects of cycad components in postnatal animals, but these were confined to retinal and cerebellar abnormalities that bore no apparent relationship to Guam ALS/P-D. Thereafter, there was little interest in the possible link between oral and percutaneous exposure to cycad and the development of human neurological disease, and a 1975 lead61 of the induction of delayed-onset paralysis in guinea pigs fed an extract of cycad (Encephalartos altensteineii) was not pursued. However, the cycad hypothesis was kept alive by Kurland who, with Mulder, became increasingly persuaded62 that cycad was the principal etiologic candidate for Guam ALS/P-D.

LATHYRISM AND ALS/P-D: EXPLORING THE LINK

In June 1981, at the Scottsdale, Arizona, conference on motor neuron disease, I restated1 the possibility relationship between lathyrism and Guam ALS and lathyrism and called for an evaluation of the effects of prolonged administration of BMAA (Cycas spp.) and BOAA (Lathyrus spp.) in a suitable laboratory species. The first step, however, was to define the neurology of human lathyrism and to produce a satisfactory primate model in which the action of BOAA could be examined. Thus, over succeeding years, the clinical and neurophysiological features of lathyrism were studied (with Schaumburg, Dwivedi and other clinical colleagues noted below) in Bangladesh, India, and Israel.2-5 These investigations revealed that heavy con­s­umption of Lathyrus sativus resulted in diffuse and transitory CNS excitation of somatic motor and autonomic function, including muscle cramping, myoclonus, urgency and frequency of micturition, and nocturnal erection and ejaculation. One or more of these clinical manifestations may precede develop­ment of leg weakness and then wane or disappear once intake of the toxic diet is reduced or abandoned. The individual either recovers or is left with varying degrees of spastic paraparesis (to paraplegia in flexion) indicative of permanent dysfunction of selected regions of the corticomotoneuronal system. A small percentage shows additional lower-motor-neuron changes later in life.62 Neuropathological studies conducted decades after onset of spastic paraparesis have revealed degeneration of long ascending and descending tracts, and, to a lesser extent, spinocerebellar tracts and dorsal columns. Severe loss of Betz cells may occur, most noticeably in the upper part of the precentral sulcus and in the paracentral lobule. Anterior horn cells display small, filamentous aggregates and crystalloid inclusions (reviewed in 2-5,50).

The next challenge was to develop a model of lathyrism in well-nourished primates and to compare the effects of repeated, subconvulsive doses of BOAA. Conducted in collaboration with Dr. Dwijendra Roy, Dr. Albert Ludolph and Dr. Jacques Hugon, these studies demonstrated that signs of myoclonus and corticospinal dysfunction appeared in cynomolgus mon­keys fed for 3-10 months with an exhaustively analyzed diet of lathyrus sativus seed (LS) that had been supplemented beyond the minimum nutritional requirements for that species. Morpho­logical examination of motor cortex revealed mild chromato­lytic changes of motor neurons. Clinical and neurophysiological signs of corticomotoneuronal dysfunction were also found in animals fed LS plus BOAA, or BOAA alone. Cessation of dosing led to disappearance of characteristic hindlimb extensor posturing, indicating the successful modeling of the early, reversible stage of human (neuro)lathyrism. Moreover, elevation of the blood calcium-phosphate ratio and increased alkaline phosphatase activity in animals fed fortified LS (but not BOAA) suggested the presence of additional changes in bone function. Further details of this study will be reported elsewhere.

Once the primate studies had confirmed BOAA as the likely culpable agent of (neuro)lathyrism, my attention turned to the relationship between the acute neurotoxic properties of BOAA and BMAA, and then to the chronic primate toxicity of the latter. Initial studies were performed with mouse spinal cord explants, with or without attached dorsal root ganglia and striated muscle. Application of micromolar concentrations of BOAA resulted in the appearance of discrete, post-synaptic CNS edematous vacuolation60 of the type described by Olney64 in the circumventricular organs of post-natal rodents administer­ed convulsive doses of BOAA. Armed with these data, in September 1983, I approached Dr. Peter Nunn, a member of Bell's team that had discontinued work on the Cycas amino acid (BMAA) more than a decade earlier. Nunn kindly provided samples of BMAA which, at higher concentrations than BOAA, proved to induce comparable, stereospecific neurotoxic pathology in mouse CNS explants.10 Subsequent studies with Dr. Stephen Ross demonstrated that BMAA- and BOAA-induced neuronotoxic pathology in mouse cortex explants was attenuated dose-dependently and differentially by glutamate-receptor antagonists.11 BOAA, a direct-acting glutamate agonist that induces seizures promptly after injection, appears to act largely via the A2 and/or A3 receptor, while the convulsant and neuronotoxic properties of BMAA are attenuated dose-depen­dently by AP7, a specific antagonist at the A1 receptor.11,12,65 Since BMAA lacks the dicarboxylate structure of most excitotoxic amino acids, induces seizures in rodents after a delay period (hours), and requires higher doses than BOAA to cause comparable dendritic vacuolation, the Cycas amino acid probably exerts its neurotoxic effects indirectly, either through a metabolite,
by interrupting a metabolic pathway causing the accumulation of an endogenous excitant amino acid, or by some other mechanism.

Since pathological changes induced in mouse cord and cortex treated with BMAA were similar to those seen with BOAA an agent that precipitated spastic paraparesis in macaques (vide supra) it was predictable, by 1984, that repeated subconvulsive doses of BMAA would induce some type of motor-system dysfunction in primates. These studies began with Nunn and my other colleagues (vide supra) in summer 1985. Within a few weeks of daily oral dosing with BMAA, the pilot animal developed profound motor dysfunction attended by Betz and anterior horn cell degeneration and/or central chromatolysis. Subsequent animals received lower daily doses of BMAA, and their motor function was compromised more slowly. In the majority of animals, the forelimbs were affected first, with wrist-drop, clumsiness and difficulty 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 primate sign associated with impairment of corticospinal function (as in primate lathyrism), a stooped posture, unkempt coat, and tremor and weakness of the lower extremities. Both resting and action hand tremor were noted in the same animal, a feature also reported in the human disease on Guam. More prolonged intoxication led to periods of immobility with an expressionless face and blank stare, a crouched posture and a bradykinetic, shuffling, bipedal gait performed with legs flexed and rump close to the ground. Two such animals treated with an oral antiparkinson drug showed, within 30 minutes, selective recovery of marked facial movement and spontaneous activity. Additional clinical 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 decrements in the entire motor pathway (Unlike primate lathyrism, vide supra), 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 cortex, underwent central chromatolysis, neurofilament accumulation and chronic cell degeneration similar to that seen in ALS. Similar though perhaps less marked abnormalities of motor neurons were found in 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 in the pars compacta of the substantia nigra (Figure 3). Otherwise, the basal ganglia, hippocampus and cerebellum of BMAA-treated animals were similar to controls.

These ongoing studies provide neuropathological, neurophysiological and/or clinical evidence that BMAA induces a primate motor-system disorder with involvement of upper and lower motor neurons, the extrapyramidal system, and possibly other CNS regions regulating patterns of behaviors. Present experience is limited to the early development of this primate disorder. However, under the dosing conditions employed, it is evident that pyramidal compromise precedes extrapyramidal dysfunction, and that clinical signs attributable to these systems surface before the onset of presently detectable pathological changes. A more prolonged period of BMAA administration will be required to determine if neuronal damage is the sequel to functional compromise of the extrapyramidal system, as shown for the pyramidal neurons of motor cortex.

These data demonstrate an intriguing parallelism between chronic BMAA intoxication in primates and human ALS/P-D. While the experimental disorder presently lacks certain features of the human disease, notably nigral degeneration and plentiful paired helical filaments, it is noteworthy that animals received relatively huge amounts of BMAA, the motor-system
disease evolved with great rapidity, and the neuropathology was studied weeks to months — rather than years to decades — after initial exposure to the neurotoxin and commencement of the pathological process. Furthermore, primates were not exposed to whole cycad seed, to other environmental factors proposed as causally related to Guam ALS/P-D, or to the combined effects of toxic damage and decades of age-related attrition of susceptible neurons. 68

ALS/P-D AND CYCADISM: TESTING THE HYPOTHESIS

In addressing the hypothesis that oral (in food) and/or percutaneous (as a topical medicine) exposure to cycad components is etiologically associated with ALS/P-D, it will be important to test for other adverse health effects that can be anticipated from existing knowledge of (controlled and uncontrolled) cycadism in animals. While the present limited space obviates detailed-treatment of this subject, the following questions might be usefully incorporated in the design of future epidemiological and other studies of ALS/P-D. These questions imply that the causation of Guam ALS/P-D should be sought in acute or subclinical cycad intoxication occurring during early life or in adulthood.

1. Do patients with P-D, or more likely (vide infra), ALS, have a history of febrile (previously common in Merizo, Figure 1;69,70 see also 41,72) or afebrile seizures during the first 10 years of life or thereafter? (Cycad poisoning in humans and animals is associated with seizures and/or increased CNS excitation: in animals, the latter is followed weeks later by progressive locomotor difficulties and even frank paralysis.650 Note that BMAA and BOAA are experimental convulsants,54,55,54,65 and myoclonic-like events may herald the onset of human and primate lathyrism).4,5

2. Are Kurland and Mulder’s impressions30,73 of an unusual prevalence of “congenital” anomalies on Guam supportable, and is there a familial relationship between the incidence of ALS/P-D and birth defects? (Cycads contain cycasin, a potent teratogen in many experimental species). Early developmental abnormalities of the cerebellum are suggested by the observation of multinucleated and dislocated cells in the human disease,42 also noted in mice treated with the aglycone of cycasin.74

3. Do Guam ALS/P-D patients have an unusual frequency of multiple exostosis, and has the incidence of diaphyseal aclasis decreased and the sex ratio narrowed in parallel with that reported for ALS/P-D? Are legs bowed or spines scoliotic? Are aneurysms or unexplained hemorrhages more common? (These include some of the expected consequences of a yet-to-be-demonstrated BAPN-like compound present in or derived from cycads that could explain the connective tissue abnormalities and hemorrhages found in cycadism).72 Do bone fractures occur more frequently prior to onset of ALS (as in sporadic ALS) or P-D on Guam? Is there a link with reported abnormalities of calcium and vitamin D metabolism,40 with reduction in cortical bone mass,76 and with osteoporosis20 in P-D patients?

4. Do Guam ALS/P-D patients have an early history of cycad-related jaundice, and are they more prone to liver cirrhosis, hepatomas, gastrointestinal tumours or other types of malignancy or dysplasia? (These include the known effects of cycadism in human and/or animals, both in the natural and experimental setting).8,50,77,78

5. Since 50% of P-D patients and half of the ALS patients on Guam exhibit frank diabetes mellitus, and 70-80% of both groups show subclinical glucose intolerance,79,80 is there a relationship with neurodegenerative disease? (Cycad intoxication causes interstitial pancreatic edema in experimental species).8,54

6. Are there abnormalities of retinal function in ALS/P-D? (Retinal changes and necrosis are reported in experimental cycadism4,81 and, a priori, exposure to BMAA should result in damage to retinal neurons containing glutamate receptors).82

7. Is there a link between the male predominance of lathyrism, Guam ALS, P-D, and diaphyseal aclasis, and does the sex ratio narrow toward unity as these diseases become less frequent?

TYPE AND TIMING OF EXPOSURE TO PLANT TOXINS

Type of Exposure While many pieces of data can be assembled to incriminate cycads in the etiology of Guam ALS/P-D, there are lines of argument that demand consideration of the role of other potentially toxic food plants, notably the cyanogenetic tuber cassava. Like the cycads, cassava was a source of food for Guamanians during World War II,83 and instances of poisoning by ingestion of bitter cassava have been noted by Whiting (vide supra). Heavy reliance on unprocessed cassava has been indicted in the acute onset of spastic myeloneuropathy among children and others in Mozambique,84 and a uniform diet of detoxified cassava is held responsible by Osuntokun for the insidious development of ataxic myeloneuropathy in older Nigerian subjects.85 Notably, some 4% of Osuntokun’s cohort were reported to exhibit various combinations of a “motor neuron-parkinsonism-cerebellar-dementia” complex. Moreover, a former period of heavy reliance on cassava has been noted in relation to the high and declining incidence of ALS in Surabaya, Indonesia.86

In the absence of a primate model of cassava-induced neurodegenerative disease, none of these intriguing pieces of data is sufficient to demonstrate a cause-and-effect relationship. On the other hand, there are strong epidemiological associations to suggest that subsistence on cassava can induce seemingly disparate neurological disorders in early and later life. In conclusion, studies designed to explore the role of cassava intoxication in human neurodegenerative disease in the Western Pacific foci of ALS/P-D will also have to consider cassava exposure, and vice versa.

Timing of Exposure Calne, Eisen, Meagher and Spencer68 have argued that the causation(s) of ALS, Parkinson and Alzheimer diseases, are likely to be found in unusual exposures to culpable environmental agents (toxins, trauma, viruses) at any early stage of life. If these exposures are sufficiently large, disease will promptly appear (as in MPTP intoxication). More commonly, exposures are insufficiently large to precipitate signs shortly after exposure and must be combined with age-related attrition of compromised neurons to find their clinical expression. Viewed from this perspective, therefore, the “exposure dose” determines the temporal expression of the disease.

How does this hypothesis apply to Guam ALS/P-D? During the wartime Japanese occupation of Guam, food was scarce and Chamorros relied heavily on native foods, notably cycad. Over the following 10 years, rates (5-year average incidence) for ALS increased and then declined from approximately 1955. Similarly, rates for P-D among males reportedly began to drop approximately from 1965. These peak-incidence data are con-

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sistent with a minimum "incubation" period from the end of World War II of approximately 10 years for ALS and 20 years for P-D. Other data suggest that 18 years of exposure to the Chamarro environment are needed to establish the conditions for ALS or P-D to appear up to three decades later. The youngest patients with Guam ALS have been in their 20s and those with P-D in the 30s, the mean age for onset having increased for both diseases over the preceding 30 years as reliance on indigenous food crops has diminished (vide supra). Viewed from this perspective, therefore, early-onset ALS might be an expression of severe toxicity leading to a degree of compromise of motor neurons (and sub-clinical parkinsonism) that is incompatible with life, while less severe intoxication damages the principal motor pathway without fatal consequences and therefore allows the clinical expression of nigral (coupled with sub-lethal motor-neuron compromise) damage later in life. This is compatible with the observation that motor neurons undergo pathophysiological changes prior to the appearance of extrapyramidal signs in primates repeatedly dosed with BMAA.

If Guam ALS, P, D, and sub-clinical neurofibrillary degeneration, are no more than four points on a dose-response curve for long-latency plant toxicity, then it follows that with larger doses, the disease will be shifted to an earlier stage of onset. The short delay period (weeks) between acute cychadism in animals and onset of progressive locomotor abnormalities encourages this view, as does the differential clinical expression and age of onset of cassava-related illness in African populations. If this idea has substance, it should be possible to demonstrate motor disorders in younger age groups of other populations more heavily exposed to the toxic plant products in question. The precise clinical expression of these diseases may vary with age at onset. In this regard, it is noteworthy that among cychad- and cassava-consuming aborigines of Groote Eylandt, Northern Territory, Australia, motor neuron disease is found in individuals with a history of abnormalities from birth, while others display mixed cerebellar and pyramidal-tract involvement, supranuclear and internuclear palsy, mild dementia or parkinsonism.

To assist with the challenging task of exploring the possible relationship between cychad/(cassava) toxicity and neurodegenerative disease on Guam, nature may have provided other biological markers of plant toxicity, including diaphyseal aclasis (a disorder that arises between ages 4-8), changes in skin collagen, various terata that indict prenatal exposure, and other age-related adverse health effects. While the pointers are there, much additional investigation is required to determine whether neurodegenerative disease of old age may result from "silent" exposure in the first part of life to chemical agents ("slow toxins") intrinsic to certain plants that are innocently used by mankind for food.

Note Added in Proof

Reed and colleagues recently reported a cohort study of ALS/P-D on Guam and Rota. Preference for traditional Chamarro food was the only one of 23 selected variables found to be significantly associated with an increased risk of P-D.

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