

## Research Report

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# The John Walton Muscular Dystrophy Research Centre in the University of Newcastle and the BMAA Theory of Motor Neuron Disease

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### INTRODUCTION: MY EARLY YEARS IN NEWCASTLE

I first met John Walton in 1966 when he picked me up from Newcastle railway station to interview me for a neurology registrar position in the Newcastle General Hospital. The senior clinicians for whom I had worked in Oxford and London would never have thought to do such a thing. John took me all round Newcastle and the General Hospital Regional Neurological Center and offered me the job. I gladly accepted it and so was launched my career in neuromuscular diseases.

I had been drawn to Newcastle because I had done some research in Oxford on what we then called disseminated sclerosis and had heard Henry Miller give a talk on the research that he and E.J. Field were doing at the MRC Demyelinating Disease Center. Henry was a charismatic figure and I immediately asked him for a job. He offered me a research registrar position with EJ, but it was the wrong time in my early career to get into full-time research; I needed more clinical experience. Hence, I applied to John Walton, having previously seen only one case of muscular dystrophy in my life.

It was a very fast learning curve. The joint Friday morning meetings of the RVI and NGH neurology staff

were particularly harrowing for a newcomer. The format where the newest, most junior person started the discussion was the reverse of what I had been used to. Until I understood the rules of the game, I was thrown when the home team would leave out a crucial piece of information on the case. This was just part of the competitiveness of the two departments that started from the top. Henry Miller complained at one of the Friday meetings that he had woken that morning and looked at his shoes to see if John Walton was standing in them.

At the end of the 1-year clinical registrar position, John offered me a research registrar position in the Muscular Dystrophy Group Research Labs. I was now thrown into the world of muscle pathology, EMG, muscular dystrophy clinics and genetic counseling. I was soon doing muscle biopsies and research that included collecting at-risk fetuses of Duchenne carriers to discover the earliest muscle changes in muscular dystrophy.

Albert Aguayo was visiting Newcastle that year from Montreal and he encouraged me to work up a family with what we now know was CMT type 1. I read all the papers of Peter Dyck and PK Thomas, and created all the morphological and electrophysiological techniques that were needed to study peripheral nerve biopsies. From this work came a number of interesting papers and my first monograph, "Disorders of Peripheral Nerves." Jack Foster suggested that I work up his family with what turned

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out to be hyperkalemic periodic paralysis. That generated an interesting first-in-the-field intraoperative studies of sarcolemmal membrane physiology with Alan McComas.

Towards the end of the year, John Walton sprung on me that he was sending me to do a year's neuropathology fellowship in the Massachusetts General Hospital with Raymond Adams. He also suggested that I start a program of research into motor neuron disease (MND), since we had no research in that disease in the Muscular Dystrophy Research Labs. I discovered that Falconer in Edinburgh had just reported a mouse model of MND, the wobbler mouse. Falconer kindly sent me 3 breeding pairs, and with Brian White, our animal house technician, I set up a thriving colony of wobbler mice that was waiting for me when I returned from the fellowship year in the Mass General Hospital.

Those were halcyon days. Newcastle Neurology was in the ascendancy and Queen Square was in the doldrums. We had so many bright people like Peter Hudgson and Margaret Johnson working on muscle diseases, Alan McComas and a series of fellows working on the electrophysiology of motor units, Ron Pennington and Dorothy Park adding the biochemical dimension to muscle research, David Barwick doing EEGs and EMGs, and David Gardner-Medwin, who was one of the first bona fide pediatric neurologists in the UK. We had superb technical assistance from Meg Jenkison and John Fulthorpe, who taught us the techniques and were active participants in all the research. A series of senior fellows from all around the world joined us for six or 12 months at a time, which broadened our perspectives of the world and helped increase our productivity.

With John Walton as President, we held the Third International Congress in Muscle Diseases in Newcastle in 1974. By that time, John Walton and the rest of us in Newcastle were the world leaders in neuromuscular diseases. We were being invited to conferences all over the world. Also, we had a never-ending parade of eminent neurologists coming to visit the Muscular Dystrophy Labs and Newcastle General Hospital, like Fred Plum and Ray Adams. It was all very exciting, though we did tire of the old saw of bringing coals to Newcastle.

John was asked by Lord Brain at his death to take over editing Brain's *Diseases of the Nervous System*. I remember John asking me what he should do with the 40 or so files of library cards that Lord Brain bequeathed to him. In 1975, John and a number of us decided that we would plan a Newcastle Textbook of Neurology. This never saw the light of day, but the ideas

that we developed eventually morphed into Bradley's *Neurology in Clinical Practice* that is now approaching its 7th edition.

When I returned from Boston in 1969, John quickly found a senior lectureship, NHS consultant position and a Wellcome Foundation Fellowship for me. In 1974, he succeeded in getting the National Fund for Crippling Diseases to endow the Research Chair of Experimental Neurology, of which I was the first incumbent. By that time the Neurology Departments of the NGH and RVI were now conjoined. John Walton was now both Professor and Head of the Department, and also Dean. The University of Newcastle upon Tyne went from strength to strength. In 1979 then Sir John Walton was knighted. In 1983, to Newcastle's loss but Oxford's gain he became the second warden of Green College, which is centered in Osler House, where I did my clinical medical school training. It was an interesting turn of the wheel.

Lord Walton of Detchant has played a pivotal role in medical matters in the House of Lords for more than 30 years and continues to be very active both in the House of Lords and in his physical life. John was my mentor and is my friend, and I will forever be grateful for his leadership, support and advice.

In 1977, I decided to join Ted Munsat in Tufts-New England Medical Center and to help him set up a neuromuscular center focusing on MND and peripheral nerve diseases. My career in the United States has included being chairman for 25 years of the Departments of Neurology in the Universities of Vermont and of Miami. During that time I developed an increasing concentration on research into the miserable disease called MND, which across the pond is known as amyotrophic lateral sclerosis (ALS). It is this search for the causes and potential treatment of MND that I now want to review. I will be brief and quote only a few key references.

### **THE BMAA THEORY OF MOTOR NEURON DISEASE**

Despite a great deal of productive research in the last two decades elucidating the genetic basis of familial MND, only 10–15% of cases have a family history of the disease. Much less attention has been given to the cause of the disease in the 85+% of the patients who have no family history and are said to have sporadic MND (sMND). The search for environmental factors responsible for sMND has incriminated a number of risk factors: lead, mercury, agricultural

and industrial chemicals, certain occupations, military service and head injuries.  $\beta$ -methylamino-L-alanine (BMAA) derived from cyanobacteria has recently been added to the list, though its history as the probable cause of Guamanian ALS/parkinsonism/dementia complex (ALS/PDC) extends back nearly 70 years. Though some of these risk factors may have a linked common basis, it is likely that an individual patient develops MND because of an increased genetic susceptibility to just one or two of these risk factors. The goal of my research is to find the environmental and genetic factors that produce MND in my patients.

Soon after the Americans recaptured Guam, one of the Mariana Islands in the central Pacific, from the Japanese in 1944 they found a very high incidence of MND on the island. Epidemiological studies suggested that an environmental toxin with a very long incubation period was responsible. From the outset, the indigenous cycad, *Cycas micronesica*, the seeds of which are the source of flour for the natives, was considered a likely source of that yet-to-be identified toxin. However, despite two decades of NIH-funded research on Guam and six NIH-sponsored conferences the toxin was not definitively identified.

Following the discovery that a non-protein amino acid,  $\alpha$ -amino- $\beta$ -oxalylaminopropionic acid (BOAA) in the seeds of *Lathyrus sativus* was the likely cause of the human upper motor neuron disease of lathyrism, attention turned to cycad seeds to see if they contained BOAA. Instead they were found to contain a hitherto unknown non-protein amino acid, BMAA. Spencer and Nunn demonstrated that BMAA is neurotoxic *in vivo* both in mice and non-human primates and suggested that it was the cause of Guamanian ALS/PDC [14, 18]). Duncan and colleagues rejected this theory because they found only low concentrations of free BMAA in washed cycad flour [7]. However, Cox and colleagues later found that BMAA in cycads originates from symbiotic cyanobacteria resident in specialized coralloid roots of *Cycas micronesica*, that it is biomagnified up the food chain (cyanobacteria – cycad seeds – flying foxes, pigs and deer - man), that it occurs at much higher levels in the protein fraction of cycad flour than in the free form (explaining Duncan's problem with the BMAA theory), and that BMAA is concentrated in the brains of patients with ALS/PDC but not control brains [12, 13].

BMAA is just one of more than a thousand substituted amino acids that are not normally present in proteins, but can be misincorporated producing various diseases (Allende and Allende 1964, Rodgers and Shiozawa 2008). Methodological disputes arose about

BMAA assay techniques and whether BMAA was present in human brain samples and continue to plague the field (Montine et al. 2005, Snyder et al. 2009). However, using the same assay techniques as Murch et al. [12, 13], Mash and colleagues confirmed Cox's results in Alzheimer's disease brains, and also found BMAA in the brains of patients dying from ALS, but not in control brains [16]. Later studies of our Miami group have linked the BMAA in human brains to cyanobacterial BMAA in the Florida ecosystem and consumption of fish and crustaceans [1].

Cyanobacteria are ubiquitous and are found in almost all conceivable habitats, from blooms on the surface of lakes affected by eutrophication, to the depths of the ocean, to thermal hot springs, bare rocks and desert. BMAA is biosynthesized both by symbiotic and free-living cyanobacteria. The potential therefore exists for humans throughout the world to be exposed to BMAA derived from cyanobacteria. BMAA produces neurotoxicity via several different mechanisms: glutamatergic toxicity; depletion of glutathione; synergism with other neurotoxins including methylmercury; and misincorporation into proteins via L-seryl tRNA synthetase, producing protein misfolding, intracellular protein aggregates and neuronal cell death [8]. Models of BMAA neurotoxicity are now available in several animal species including *Drosophila* and rats [6, 9]). BMAA misincorporation into proteins and neurotoxicity can be prevented *in vitro* and in *Drosophila* by L-serine [8].

## ENVIRONMENTAL EPIDEMIOLOGY AND MOTOR NEURON DISEASE

The demonstration of BMAA in the food chain and of BMAA in patients' brains but not controls is strong evidence linking cyanobacterial BMAA to MND. However, the demonstration of a linkage of cyanobacteria in the environment to an increased incidence of MND would greatly strengthen the BMAA theory.

Humans may potentially be exposed to cyanotoxins like BMAA through several routes, including the food chain, drinking water, recreational use of waterbodies, aerosolization and desert dust storms. Studies have pointed to cyanobacteria and BMAA in water and deserts as risk factors for MND in northern New England, Florida, Chesapeake Bay, France, Scandinavia and the Persian Gulf ([5, 10, 11]). Stommel has hypothesized that aerosolization of cyanobacteria and BMAA is responsible for the higher incidence of MND in

people living around lakes with cyanobacterial blooms [19].

The geographic distribution of MND cases offers clues to potential environmental risk factors. Spatial “clustering” of MND cases has been argued about for decades. Geospatial clusters have not been found in every epidemiological study of MND and authors of those negative studies have suggested that “clusters” result from analysis of numbers of cases and geographic areas that are too small to exclude random variation. However, it is important to realize that studies that look at case distribution using large areas, such as counties, may fail to identify micro-environmental exposures that only extend over half-a-mile or so.

We have recently linked clusters of MND cases to exposure to cyanobacteria. We initially identified an area of high ALS incidence around Lake Mascoma in New Hampshire and later correlated 11 locations of high incidence of MND in northern New England with proximity to waterbodies that are subject to frequent cyanobacterial blooms [5, 20]. We are now extending our studies of the distribution of MND patients in regions of the United States that are affected by frequent cyanobacterial blooms and also by other types of pollution that have been incriminated for MND. The target areas are Florida, northern New England and Ohio, where we already have large databases of patients with MND and where increasingly massive algal blooms have occurred in waterbodies as the result of eutrophication.

We are using a specially designed environmental exposure questionnaire recording lifetime histories of residences, occupations, fish consumption and other incriminated risk factors for MND. We recently analyzed completed questionnaires from 157 of MND patients in our northern New England ALS database and compared them with 373 control subjects (Stommel E and Andrew A, 2015, personal communication). The latter were a combined group of neurology clinic patients and a previously collected population-based control group of North Central NH and VT residents without neurodegenerative illness. Analysis of these data adjusted for age and gender found that two items were statistically significantly higher in the MND patients than in control subjects at  $P < 0.05$  level: residing near a waterbody at the time of diagnosis (odds ratio 1.97), and estimated methylmercury consumption in fish in upper quartile vs. lower quartiles (odds ratio 6.08. MND patients 770  $\mu\text{g}/\text{yr}$ , neurology clinic controls 390  $\mu\text{g}/\text{yr}$ , general-population-controls 510  $\mu\text{g}/\text{yr}$ ). The following showed non-significant

trends of increased odds ratios from 1.20 to 1.58: ever having lived near waterbodies with algal blooms; watersport participation; and self-reported exposures to lead and to mercury from hobbies and occupations.

We are also investigating the proximity of the homes of subjects to sources of environmental toxins, using geomapping techniques. We are comparing residential exposure of MND patients and control subjects to cyanobacteria, to sources of industrial toxicants from hazardous waste sites, and to sources of agricultural pesticides and herbicides. We are geomapping the residential addresses of patients with MND and population-based random controls, and doing a case control study of residential exposures to these environmental toxins/toxicants. We are applying novel techniques to investigate cyanobacterial content of waterbodies, using a combination of direct sampling of water from these waterbodies and satellite remote sensing, and applying GIS analytical tools [20].

It is widely believed that sMND results from the interaction of environmental factors with genetic predisposing factors. Investigation of gene-environment interactions will require genome-wide sequencing of DNA from a large series of patients and controls who have provided detailed information on environmental risk factor exposures. This is likely to require collaborative efforts across many institutions and countries. However, this effort is important. Identification of genetic predisposing factors would allow screening of populations for individuals at increased risk from exposure to an individual environmental toxin/toxicant. Such susceptible individuals could then be protected from such exposures. Identification of environmental toxins/toxicants would give added impetus to mitigation strategies to reduce exposures of the general population to environmental toxins like cyanobacterial BMAA.

#### **THERAPEUTIC TRIAL OF L-SERINE IN MOTOR NEURON DISEASE**

As I indicated earlier, *in vitro* and preclinical *in vivo* evidence indicates that L-serine can block the neurotoxic action of BMAA to produce protein misfolding, neuronal cell death and resultant motor disorders. We are currently completing an FDA-approved phase 1 double-blind safety and tolerability clinical trial of L-serine in varying doses up to 30 g/day in 20 sMND patients, and a phase 2B trial in 60 MND patients is in development.

## ENVIRONMENTAL EPIDEMIOLOGY AND OTHER NEURODEGENERATIVE DISEASES

If you survey the literature, you will find that many of the risk factors for MND are also incriminated in other non-familial neurodegenerative diseases, like Alzheimer's and Parkinson's diseases. Moreover, in single gene inherited neurodegenerative diseases, there are examples of multiple disease phenotypes, both clinical and pathological, arising in the same family. For instance, Alzheimer-like dementia, frontotemporal dementia, MND, primary progressive aphasia and autosomal recessive neuronal ceroid lipofuscinosis-11 may all result from mutations of GRN, the gene for progranulin [15]. There are many other examples of multiple phenotypes arising from single mutations in familial diseases. If a single gene can cause multiple phenotypes, then it is conceivable that a single environmental toxin might cause several neurological diseases. We plan to extend our studies of environmental epidemiology to the investigation of other neurodegenerative diseases, in addition to MND.

### SUMMARY

Cyanobacteria are ubiquitous and produce many neurotoxins, including BMAA. This non-protein substituted amino acid becomes misincorporated into neuronal proteins, producing neuronal cell death. BMAA misincorporation via L-seryl tRNA synthetase can be prevented in preclinical models by high-dose L-serine. BMAA is bioconcentrated up the food chain and concentrated in the brain of patients with MND, as well as that of patients with Alzheimer's and Parkinson's diseases. Our environmental epidemiological work is directed to establishing the link between exposure to cyanobacteria and the risk of developing MND, and to investigating the relative risk of BMAA exposure compared to exposure to other environmental risk factors like lead, mercury, and chemicals like pesticides. The BMAA theory of MND has already led to the launching of therapeutic trials of L-serine in MND.

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