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Nutrient and toxin interactions in neurodegenerative disease

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The brain is the most complex organ of the body. Its effective function is dependent on its unique anatomical structure and neuronal cell morphology, together with the efficient coordination of its metabolic and physiological processes. The degenerative diseases of the brain, for example Huntington's, Parkinson's and Alzheimer's disease (AD), are generally characterized by an associated loss of functional neurones, with accompanying motor, memory and cognitive deficits.

In the case of familial amyotrophic lateral sclerosis (ALS; Deng *et al.* 1993), Huntington's disease, early-onset dementia of familial AD (Martin, 1993), and the prion diseases (the spongiform encephalopathies, i.e. Creutzfeldt-Jacob disease and the Gerstmann-Sträussler-Scheinker syndrome; Prusiner, 1991), a genetic aetiology has been demonstrated. The larger number of so-called sporadic cases of AD which occur in the 8th and 9th decades of life, suggests that environmental factors are also operative. Even so, recent findings concerning apolipoprotein E $\epsilon 4$ allele indicate genetic polymorphisms are significant risk factors in the development of late-onset AD as well (Saunders *et al.* 1993). Hence, senile dementia of the Alzheimer type, the most common of the neurodegenerative diseases, appears to be of multifactorial origin, presenting a complex interplay of genetic, environmental, and age-related factors (Calne *et al.* 1986).

BRAIN RESEARCH

Undertaking studies into the toxicological and nutritional aspects of neurodegeneration, poses a range of ethical, organizational, technical and financial, challenges. Since there is no laboratory test for AD and the definitive diagnosis is dependent on postmortem identification and quantification of the pathognomic intracellular neurofibrillary tangles and extracellular senile plaques within the brain, epidemiological and clinical investigations into AD are extremely problematic. However, tests for cognitive function, and development of new *in vivo* imaging techniques, namely magnetic resonance imaging and positron emission topography scans, do permit a degree of clinical assessment.

The inherent difficulty of research into the brain is compounded not only by the diverse range of neuronal and glial cell types and the complexity of the integrated neural network, but also by the properties of redundancy and plasticity exhibited by the brain. Brain damage related to degenerative change may not become apparent until the loss of neurones reaches a particular threshold level. Hence, cognitive deficits appearing in later life may not be directly caused by senility or the ageing process itself, but may be the result of developmental deficits or toxic damage which has occurred several decades earlier. Studies indicating the major significance of pre- or early postnatal nutrition to infant brain development (Lucas, 1993) and the subsequent development of disease in adult life (Barker *et al.* 1989), are of particular importance in relation to the non-replicative nature of neuronal cells. While the significance of proper nutrition in early brain development is well appreciated, evidence of nutritional influences on intelligence

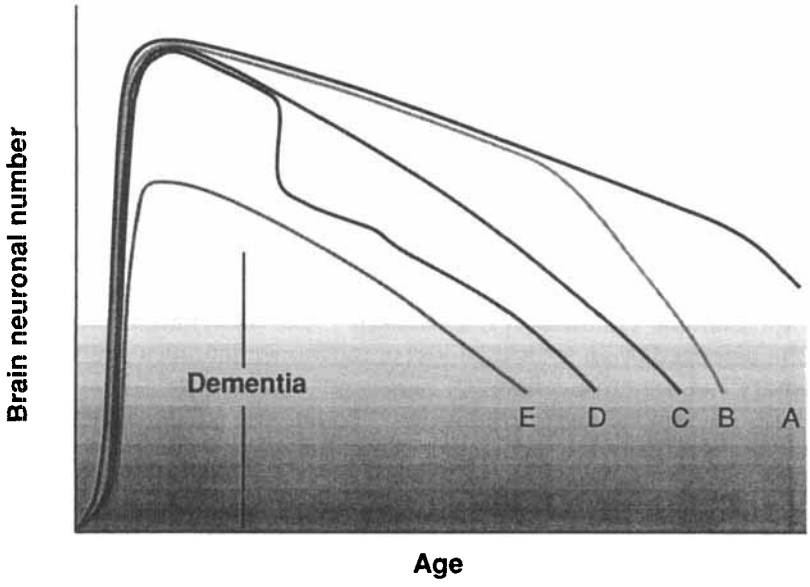

Fig. 1. Loss of neurones with age. (A), Normal ageing; (B), senile Alzheimer's disease; (C), familial Alzheimer's disease; (D), early-life neurotoxic insult; (E), developmental neuronal deficit.

which has been presented with respect to micronutrient supplementation in school-children, continues to arouse controversy (Benton, 1992). As illustrated in the generalized scheme (Fig. 1), the loss of neurones in later life, may be the consequence of neuronal degeneration occurring at various life stages, caused by a variety of adverse developmental, toxic, genetic and senile processes.

The plasticity of the brain means that such neuronal loss may be offset by various semi-adaptive modifications which allow a degree of compensatory retention of brain function. Such processes as proliferation of dendritic outgrowths, enchanced neurotransmitter synthesis, increased number of synaptosomes, and increased receptor density, may all contribute to an amelioration of adverse effects following a decline in neuronal number. Factors which inhibit neuronal loss may be expected to delay the onset of early dementia and reduce its rate of progression, thus contributing to a prolongation of active cognitive function. It may be envisaged that nutrients are of benefit both from their intrinsic biological effect on brain metabolism, and also by counteracting the injurious effects of environmental neurotoxins.

ENVIRONMENTAL NEUROTOXINS

The potential aetiological role of neurological toxins in degenerative brain diseases, has received considerable scientific and media prominence. Of the suggested causative agents, heavy-metal pollutants have attracted particular attention. In particular, Al, a known neurotoxic agent (Wisnieweski *et al.* 1982), has aroused controversy with regard to its putative aetiopathogenic role in AD (Rifat, 1994). Several epidemiological studies have revealed an association of AD with drinking-water Al content (Martyn, 1992). Analysis of brain tissue utilizing a variety of sensitive multi-elemental and microprobe

techniques including: neutron activation (Ward & Mason, 1987), electron microscope X-ray energy dispersion (Singhrao *et al.* 1990), and laser (Lovell *et al.* 1993) and proton (Landsberg *et al.* 1992) microprobe, have produced inconsistent findings. Although bulk brain concentrations of Al appear not to be generally raised, solid-state NMR analysis has revealed increased accretion of co-localized Al and Si to form plaque core deposits of aluminosilicate (Candy *et al.* 1986). Elevated levels of Si in cerebrospinal fluid have been found to occur in senile forms of AD (Hershey *et al.* 1984).

Al accumulates slowly within the body, small amounts being absorbed from the diet in the small intestine and rapidly excreted (Powell & Thompson, 1993). Ingress may also occur via inhalation of aluminosilicate clay dust, or even as Al–Al_2O_3 (McIntyre powder), once used in the prophylactic treatment of silicosis (Rifat *et al.* 1990). Access across the blood–brain barrier is mediated by binding to transferrin (Roskams & Connor, 1990). More speculatively, pathological studies indicating early involvement of the olfactory tract in AD (Mann *et al.* 1988), together with experimental investigations into the olfactory uptake of Al (Perl & Good, 1987), suggests that the nose may provide an alternative direct route of entry of toxins into the brain.

Neutron activation has also revealed increased concentrations of Hg in AD brain regions (Thompson *et al.* 1988), a finding pertinent to the controversy concerning the stability of dental amalgam. Likewise, Sn has also been implicated in dementia (Corrigan *et al.* 1991). Early exposure to Pb has been associated with dysfunction of the hippocampus, an area of the brain important in memory function (Petit *et al.* 1983), and also with the subsequent adult appearance of neurofibrillary tangles (Nicklowitz & Mandybur, 1975). Exposure of Australian aborigines to Mn-rich soils is associated with the Angurugu Syndrome, a Parkinson's-like disease similar to that found in Chilean Mn miners (Florence & Stauber, 1989). In addition, increased Al and Fe occurs in the substantia nigra of Parkinson's disease brains (Hirsch *et al.* 1991).

TOXICO-DIETETICS

Research concerning the modulation of toxicity by diet has grown considerably with the appreciation of the specific nutritional factors and mechanisms involved (Netter, 1986). Interactions of toxic and nutrient elements are many, varied and complex. Absorption of Pb is enhanced by dietary deficiencies of Ca, Fe, Zn and Cu, and dietary Se is protective against the toxic effect of Hg and Cd (Couzy *et al.* 1993). Experimental dietary deficiency of Ca and Mg enhances Al uptake in monkeys, a finding of relevance to the pathogenesis of ALS, a parkinsonian-dementia prevalent in the Pacific islands of Guam (Yasui *et al.* 1991). Animal studies have revealed enhanced memory in aged rats fed on a diet high in Mg, mediated possibly by cellular Ca interactions (Landfield & Morgan, 1984), and suboptimal dietary Zn increases Al accumulation in the brains of rats (Wenk & Stemmer, 1983). Zn has an important role in neuronal function, being found in high concentrations in the hippocampus (Dreosti, 1989). Zn concentrations in blood plasma decrease with age (Lindeman *et al.* 1971) and are further decreased in AD brains (Ward & Mason, 1987). Confirmation of the hypothesis that gastrointestinal absorption of Al is inhibited by dietary Si levels (Birchall, 1993), has been provided both in humans (Edwardson *et al.* 1993) and in brains of aged rats (Carlisle & Curran, 1987). Interestingly, Al deposition was actually increased in rat spleen, possibly due to uptake of colloidal microprecipitates of aluminosilicates (Quartley *et al.* 1993). However, the

question of whether the association of Al and Si in serum exerts a protective effect, is unanswered (Fahal *et al.* 1994). The role of citrate and ascorbate in promoting absorption and excretion of Al, awaits clarification (Domingo *et al.* 1991). Absorption of aluminium citrate in humans increases with age, and in younger AD subjects compared with age-matched controls (Taylor *et al.* 1992).

BRAIN OXIDATIVE STRESS

The prevalence of dementia of the AD type is strongly correlated with old age, while dementia in familial AD and Down's syndrome, generally appears several decades earlier. A proposed mechanism of the ageing process involves the injurious activity of free radicals and associated reactive O metabolites (ROM), namely superoxide and hydroxyl radicals, and H_2O_2 (Harman, 1984). As a result of reactivity with DNA, proteins and lipids, and the consequent tissue injury to vital cellular functions, ROM have been implicated in various pathological and age-related disease processes. An age-dependent increase in superoxide generation (Sawada *et al.* 1992), lipid peroxides (Mizuno & Ohta, 1986), and cerebral glutathione susceptibility to oxidant-induced stress (Benzi *et al.* 1989), has been demonstrated in rats.

The brain is particularly susceptible to oxidant-mediated damage, exhibiting high metabolic activity, and contains high levels of readily-oxidizable polyunsaturated fatty acids. Antioxidant protection in the brain is largely provided by vitamin E, glutathione, ascorbate and carnosine (Kohen *et al.* 1988; Sokol, 1989; Grünewald, 1993). In addition, participation of 'catalytic' Fe in free-radical reactions within the brain (Gutteridge, 1992), has stimulated interest in its neuropathogenic role (Sachdev, 1993). Fe-mediated peroxidation of brain membrane lipids may be augmented in the presence of Al ions (Oteiza *et al.* 1993). ROM have been implicated in a variety of neuropathological disorders, including Parkinson's disease, AD, trauma, and ischaemia (Halliwell, 1992; Evans, 1993).

Pathogenic reactions of ROM in brain tissues include peroxidation of synaptosomes (Binkova *et al.* 1990), and inhibition of mitochondria (Hillered & Ernster, 1983). ROM also compromise blood–brain barrier functions (Greenwood, 1991), and act as mediators of neurotoxicity (LeBel & Bondy, 1991). However, in addition to their role in inducing pathological changes, age-related studies of superoxide dismutase (*EC* 1.15.1.1; SOD) show ROM involvement in foetal brain development, indicating a physiological function (Takashima *et al.* 1990).

ALZHEIMER'S DISEASE AND FREE RADICALS

A clue to the pathogenesis of dementia was the finding that Down's syndrome subjects, who exhibit chromosome-21 trisomy, possess an extra copy of the Cu–Zn SOD gene, suggesting perturbation of the redox balance (Kedziora & Bartosz, 1988). Evidence of redox changes in AD brains is indicated by the increased O-stimulated peroxidation (Götz *et al.* 1992), accumulation of lipofuscin (Dowson, 1989), and oxidatively-modified dysfunctional glutamine synthetase (*EC* 6.3.1.2; Smith *et al.* 1991). Such changes are possibly related to a disruption of Fe homeostasis (Connor *et al.* 1992), and to monoamine oxidase B-related generation of H_2O_2 (Zetzsche & Chan-Palay, 1992). Brain antioxidant homocarnosine is decreased, although cortical levels of glutathione

and bulk brain concentrations of vitamin E are reportedly normal in AD (Perry *et al.* 1987; Metcalfe *et al.* 1989). However, the absence of altered brain vitamin E levels in experimental ischaemia–reperfusion oxidative injury in rats, indicates that vitamin E may not be a sensitive index of ROM-mediated brain damage (Yue *et al.* 1993). In peripheral tissues, blood plasma concentrations of vitamins A and E, and carotenoids, are decreased (Zaman *et al.* 1992).

AMYLOID, MICROGLIA AND FREE RADICALS

Pathological studies of AD indicate that deposition of the β/A4 protein and accompanying formation of insoluble amyloid fibrils, is associated with the consequent accumulation of brain macrophages–microglia at the plaque site (Mann *et al.* 1992). Indeed, β/A4 protein is chemotactic for microglia *in vitro* (Davis *et al.* 1992). Activation of microglia and related release of inflammatory mediators has been implicated in the pathogenesis of degenerative neurological diseases (McGeer *et al.* 1993). Of especial significance is the finding that activated microglia are potent producers of ROM in response to a number of immunological and chemical stimuli (Sonderer *et al.* 1987). The question as to whether the accumulation of Al and Si within the plaque cores represents merely an inconsequential epiphenomenon, or is indeed of aetiopathogenic importance, is a key one to understanding the potential toxic role of Al in AD. Pertinent to this issue, *in vitro* chemiluminescent studies have shown microglia to generate ROM when exposed to various model synthetic and mineral aluminosilicate particulates of differing composition, size, and fibrillar morphology (Evans *et al.* 1992*a*). This finding, akin to pneumoconiosis, is consistent with the so-called cephaloconiosis hypothesis of AD (Evans *et al.* 1991), namely that analogous *in vivo* aluminosilicate–amyloid fibril plaque deposits act as a persistent reactive nidus, and induce the chronic generation of injurious ROM by endogenous brain microglial cells. Inhibition of macrophage-derived ROM production by vitamin E (Sakamoto *et al.* 1990), confirms the therapeutic potential of antioxidant micronutrients to modify and prevent the proposed cephaloconiotic oxidative injury (Evans *et al.* 1992*b*).

In vitro aggregation of β/A4 protein to form insoluble amyloid fibrils is enhanced by oxidative reactions (Dyrks *et al.* 1992). It is interesting to speculate whether the enhanced binding of β/A4 protein to oxidant-modified apolipoprotein allele E ε4 (Strittmatter *et al.* 1993), may be exacerbated by environmental factors, namely Al and aluminosilicates, by the production of microglial ROM. Oxidized lipoproteins, together with impaired endothelial cell vasodilator function (Keaney *et al.* 1993), may thus contribute to the pathogenesis of AD as well as to cerebrovascular dementia. Of allied interest, *in vitro* neurotoxicity of synthetic β amyloid (Behl *et al.* 1992), and systemic casein-induced amyloidosis in mice (Harman *et al.* 1976), are inhibited by vitamin E. Cytotoxicity of microglial cells to neurones in culture is inhibited by catalase (*EC* 1.11.1.6), indicating the adverse effects of H_2O_2 (Théry *et al.* 1991). Immunostimulation of microglia with the resultant activation of nitric oxide synthase and consequent production of reactive NO, has also been shown to be cytotoxic to co-cultured neuronal cells (Boje & Arora, 1992). The ambivalent neurotoxic and neuroprotective effects of nitric monoxide have been related to the activity of the alternative redox states of nitric oxide ($NO\bullet$) and the nitrosonium ion (NO^+) respectively (Lipton *et al.* 1993).

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NEUROTOXIC XENOBIOTICS

The brain, despite the general effectiveness of the blood–brain barrier, which may be compromised in disease and in the aged, is exposed to a wide variety of environmental foreign agents. Metabolism of xenobiotic organic chemicals by brain NADPH-cytochrome P450 generates superoxide radicals and, hence, contributes to oxidative stress (Ghersi-Egea *et al.* 1991). Dietary excitotoxins, i.e. the amino acids glutamate and aspartate, are also associated with oxidative stress, and have been implicated in the pathophysiology of AD (Maragos *et al.* 1987), stroke, trauma and seizures (Coyle & Puttfarken, 1993). Nutritional toxicology, and the study of food-borne neurotoxins, for example cycasin, a component of the cycad palm and a possible cause of endemic ALS found in Guam, has provided significant clues to understanding specific neuropathogenic mechanisms (Meldrum, 1993). Similarly, the identification of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine as the contaminant in synthetic heroin responsible for the parkinsonian syndrome which occurred in Californian drug addicts, illustrates the important potential causal role of environmental redox chemicals in idiopathic Parkinson's disease (Adams & Odunze, 1991).

DIET IN THE ELDERLY

The challenge to determine and meet the nutritional requirements of the elderly in order to optimize health and minimize disease, is a substantial one. Dietary studies of the elderly undertaken in the UK (Campbell *et al.* 1989), and Europe (SENECA, 1991), have revealed wide variations in micronutrient intake, with significant proportions of the elderly possibly marginally deficient in antioxidant vitamins and minerals.

Nutrition is important for cognitive function in the elderly (Rosenberg & Miller, 1992), and epidemiological investigations have linked malnutrition with an increased risk of late-onset AD (Henderson *et al.* 1992). In addition to dietary deficiencies, maladsorption of vitamin B_{12} has been demonstrated in psychogeriatric patients (Burns *et al.* 1986). The degree of cognitive impairment in AD correlates with the decrease in serum vitamin B_{12}, and would appear to be disease-related and not only diet-related (Levitt & Karlinsky, 1992). The finding that nitrous oxide and associated hydroxyl radical inactivates cobalamin (Haurani, 1989), suggests that the depletion of vitamin B_{12} in AD may be caused by microglial ROM. Allied studies of vitamin status in the elderly also have shown a correlation of cognitive dysfunction with decreased erythrocyte folic acid (Sommer & Wolkowitz, 1988). At the other end of the age spectrum, maternal deficiency of vitamin B_6 impairs foetal brain development (Guilarte, 1993), abnormalities that may predispose to adult neurodegeneration.

PHARMACOLOGY OF NEURODEGENERATION

The partial success of various pharmacological agents, namely metal chelators, and anti-inflammatory and antioxidant drugs used in treating dementia, illustrate the significance of the aetiopathogenic mechanisms involved. The reported beneficial effects of treating AD with the chelator desferrioxamine, instigated for the removal of the putative aetiological toxin Al, may have been due also, to chelation of redox-active Fe (McLachlan *et al.* 1991). The 21-aminosteroidal lazeroid, which inhibits experimental brain ischaemia injury by its antioxidant sparing action on brain vitamins C and E (Sato

& Hall, 1992), may be expected to be of therapeutic worth in AD. The finding that the anti-inflammatory drug indomethacin is of value, supports the view that inflammatory microglial cells are of pathogenic pertinence in AD (Rogers *et al.* 1993). Treatment of Parkinson's disease with the monamine oxidase inhibitor drug deprenyl has been shown to slow the deterioration; however, co-administration of vitamin E appeared not to produce additional benefits (Parkinson Study Group, 1993).

MICRONUTRIENTS, COGNITION AND NEURODEGENERATION

The clinical use of antioxidant micronutrients, i.e. vitamins C and E, β-carotene, Se and Zn, in the treatment of a variety of diseases involving immune deficiencies, inflammation, ischaemia and vascular thrombosis, has long been advocated (Crary *et al.* 1984). Trials using various combinations of antioxidants, have been shown to be of some clinical value in the treatment of geriatric patients (Clausen *et al.* 1989) and in subjects with early Parkinson's disease (Fahn, 1992). Therapeutic investigations in AD subjects with vitamins B_1, B_2, B_6 and C cocktails including thiamin, riboflavin, pyridoxine and ascorbic acid (Burns *et al.* 1989), and others containing Zn, Se and fatty acids (Van Rhijn *et al.* 1990; Constantinidis, 1992), have reported varying degrees of improvement in psychological and cognitive function. The need for additional carefully controlled clinical trials in this field of nutritional medicine and prevention, is of special importance.

CONCLUSION

Predicted demographic change in the next few decades indicate a large increase in the number and proportion of the elderly, and particularly of the very old. With the anticipated rapid rise in the prevalence of dementia and other age-related neurodegenerative diseases, severe medical and social problems will be encountered. Recognition of the importance of research into function and diseases of the brain, has resulted in pronouncements in the USA and the EEU of the planned 'Decade of the Brain', and initiation by the USA National Academy of Sciences' Institute of Medicine of the 'Human Brain Project'. The National Institute of Health of the USA has identified the prevention of neurodegenerative diseases as a medical research priority. In the UK, similar moves to address these pressing matters are beginning to enter the medical, governmental and public consciousness. The important role which nutritional factors play in modulating cognitive function and neurodegeneration provides a significant challenge and opportunity for worthwhile research endeavour in the future.

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