UC Irvine
UC Irvine Previously Published Works

Title
Aluminum as a toxicant

Permalink
https://escholarship.org/uc/item/0sq384df

Authors
Becaria, A
Campbell, A
Bondy, SC

Publication Date
2002-08-01

DOI
10.1191/0748233702th157oa

Peer reviewed

Aluminum as a toxicant

A Becaria, A Campbell and SC Bondy

Department of Community and Environmental Medicine, Center for Occupational and Environmental Health Sciences, Irvine, CA 92697-1820, USA

Although aluminum is the most abundant metal in nature, it has no known biological function. However, it is known that there is a causal role for aluminum in dialysis encephalopathy, microcytic anemia, and osteomalacia. Aluminum has also been proposed to play a role in the pathogenesis of Alzheimer's disease (AD) even though this issue is controversial. The exact mechanism of aluminum toxicity is not known but accumulating evidence suggests that the metal can potentiate oxidative and inflammatory events, eventually leading to tissue damage. This review encompasses the general toxicology of aluminum with emphasis on the potential mechanisms by which it may accelerate the progression of chronic age-related neurodegenerative disorders. *Toxicology and Industrial Health* 2002; **18**: 309–320.

Key words: *inflammation; metal; neurodegenerative disease; neurotoxicology; oxidative stress*

Introduction

Aluminum is the most abundant metal found in the earth's crust. However, it was not until 1825 that the metallic form of the element was first isolated. The metal has a long history of being used for water purification and in medications. For example, in ancient Rome, aluminum salts were used for the purification of water, and in the Middle Ages it was combined with honey for the treatment of ulcers (Crapper and De Boni, 1980). Aluminum sulfate is still used today as a flocculent in water purification and aluminum salts are used in medications such as antacids and buffered aspirin.

The advent of modern industry and the introduction of a variety of chemicals into the atmo-sphere led to the formation of acid rains. These have mobilized aluminum from the soil. Strong mineral acids, such as sulfur and nitrogen oxyacids, found in acid rain, can solubilize aluminum (Smith, 1996). Until recently it was generally believed that this released aluminum is harmless to the environment. This is because in solution, Al^{3+} salts form monomeric hydroxy compounds, which form polymeric and colloidal particles as the solution ages. Because of the formation of these insoluble aluminum species, it was assumed that absorption would be limited and thus the metal would be relatively innocuous. However, aluminum has been shown to be toxic to both plants (Kochian and Jones, 1997) and animals (Sparling and Campbell, 1997).

In humans, aluminum plays a causal role in dialysis encephalopathy, osteomalacia and micro-cytic anemia. Furthermore, several studies have suggested a possible link between aluminum neu-rotoxicity and Alzheimer's disease (AD). However, there is much controversy regarding this relation-ship (Campbell and Bondy, 2000).

Address all correspondence to: Angelica Becaria, Department of Community and Environmental Medicine, Faculty Research Facility, Irvine, CA 92697-1825, USA
E-mail: abecaria@uci.edu

Sources of aluminum exposure

The main source of aluminum intake is food, where the major contributors are products containing aluminum as food additives, such as processed cheese, baked goods and grain products. Aluminum-containing compounds are also used as preservatives, coloring agents, and leavening agents (Soni *et al.*, 2001).

The presence of aluminum in drinking water is due both to natural sources and to water purification procedures. Water treatment generally increases the content of soluble, low molecular weight, chemically reactive, and possibly more readily absorbed aluminum species.

Occupational exposure to aluminum occurs mainly due to processing of the metal (Sjögren *et al.* 1997). Antiperspirants containing aluminum chlorohydrate are another source of exposure. Vaccines, antacids, phosphate binders, dialysis, and total parenteral nutrition solutions are common and can result in a significant increase in aluminum exposure (Yokel and McNamara, 2001).

Aluminum chemistry

By weight, 8.3% of the earth's crust is composed of aluminum. It is the thirteenth element in the periodic table and its electronic configuration is $Ne3s^23p^1$. Al^{27} is the naturally occurring isotope although there is an artificial radioactive Al^{26} with a half-life of 7.2×10^5 years. Because of its long half-life and the fact that it is a β and γ emitter, the use of this isotope as a scientific probe is limited.

In most natural systems, a very small fraction of aluminum is found as the simple Al^{3+} ion. Thus, the toxicokinetics of aluminum will depend on the physical, chemical and biological properties of the various Al^{3+} complexes (Harris *et al.*, 1997). The solubility of Al^{3+} is lowest at pH 6.2 but increases with acidic or alkaline solutions and by some complexing ligands (Soni *et al.*, 2001).

In biological systems, the trivalent cation forms extensive complexes with many available ligands. Since aluminum has a high charge to radius ratio, thermodynamically it is predicted that the metal prefers electrostatic rather than covalent binding (Berthon, 1996). The aqueous chemistry of aluminum is complicated because free Al^{3+} hydrolyzes to form a wide range of complexes with water. There are three main categories of aluminum species relevant to biological availability. These are monomeric, polymeric (formed through activation of coordinated OH groups becoming deprotonated and bridging between the metal centers), and metastable polynuclear aluminum complexes, which grow in size and ultimately form microcrystalline gibbsite (Smith, 1996). At neutral pH, the salt undergoes extensive hydrolysis and $Al(OH)_3$ is produced. As the solution ages, $Al(OH)_4^-$ is also present and this leads to precipitation of aluminum in solutions. The pH of a solution determines the aluminum species and ionic forms present (Corain *et al.*, 1996). Thus, in basic media, aluminum exists as the anionic form while in acidic solutions it is found in the cationic form. Since aluminum is a very strong oxygen acceptor, it also tends to bind to other oxygen donors such as citrate, phosphate, lactic acid, oxalic acid, citric acid and catecholamines (Harris *et al.*, 1996).

Aluminum toxicokinetics

Absorption

Aluminum is absorbed by several routes. These include oral, intranasal, transdermal, and parenteral pathways. The bioavailability of aluminum from drinking water is approximately 0.3% (Yokel *et al.*, 2001). In humans, exposure from food and beverages containing aluminum comprise the major daily intake. It is not known exactly what percentage of the metal gets absorbed from food sources because composition and acidity of the diet modulates availability of the metal.

Absorption of aluminum is increased by low pH, which enhances the solubility of aluminum species. The presence of small organic acids, such as citrate and lactate, elevates the bioavailability of aluminum, whereas phosphorus and silicon appear to reduce absorption (Yokel and O'Callaghan, 1997). The mechanism by which organic acids enhance aluminum absorption is likely to involve the formation of an aluminum–organic acid complex. Citrate is known to increase the gastrointestinal absorbance of aluminum. In three human volunteers drinking a solution containing sodium citrate, the peak increase in blood aluminum was approxi-

mately 45–60 minutes after the peak increase in blood citrate. Thus, citrate and other organic acids may not form a complex with the metal but rather may increase aluminum absorption through a paracellular mechanism by opening tight junctions that are present between mucosal cells (Taylor *et al.*, 1998).

It has been hypothesized that aluminum is absorbed either transcellularly (internalization by cell-mediated endocytosis, simple diffusion, or facilitative diffusion through ion channels) or paracellularly (between adjacent cells) from the extracellular milieu (Exley *et al.*, 1996). Soluble gelatinous mucus in the gastrointestinal tract may be important in the regulation of aluminum absorption (Powell *et al.*, 1999).

Aluminum may be capable of entering the brain through the olfactory neurons located in the roof of the nasal cavity, which project to the olfactory bulb. The axons of these receptors form nerve bundles that pass through the cribriform plate of the ethmoid bone and synapse at the olfactory bulb. Through a series of complex neuronal networks, these cells then project to the olfactory cortex, cortex and the hippocampus. These are the areas that are most effected in AD. Absorption of aluminum from the olfactory pathway has been studied in rats exposed to aluminum acetylacetonate. Aluminum deposits were found in the pons-medulla, olfactory bulb and hippocampus of the aluminum acetylacetonate treated rats (Zatta *et al.*, 1993). In another study, using nose-only exposure to aluminum chlorohydrate, it was found that aluminum distributed to the brain stem of rats (Divine *et al.*, 1999). These studies suggest that olfactory nerve uptake and transneuronal distribution of aluminum is an important exposure route for the brain.

Transdermal absorption of aluminum has been reported after a single underarm application of [26]Al chlorohydrate. The absorption was found to be 0.012% of [26]Al applied (Flarend *et al.*, 2001). Anane *et al.* (1995) demonstrated that shaved mice, exposed dermally to low concentrations of aluminum chloride for a period of 130 days, had high aluminum content in both the brain and the serum. In the brain, the metal principally accumulated in the hippocampus. The fact that the mice were shaved prior to application of the aqueous aluminum chloride solution may have enhanced the systemic absorption of aluminum. Nonetheless, aluminum-containing antiperspirants, especially if applied to the skin after shaving, may be another route by which aluminum can enter into the systemic circulation.

Distribution

The chemistry of aluminum is similar to that of iron and the metal is transported to tissues principally by the iron-binding protein transferrin. Approximately 90% of the total aluminum is bound to this transport protein and since it is only partly saturated, all of the aluminum present in the serum is bound (Harris and Sheldon, 1990; Cabezuelo *et al.*, 1997). The main mechanism by which aluminum enters cells is via transferrin receptor-mediated endocytosis (Shi and Haug, 1990). The 10% of the aluminum that is not bound to transferrin is believed to be associated with low molecular mass aluminum biocompounds. It is believed that aluminum-citrate is the predominant low molecular mass species in the serum (Cabezuelo *et al.*, 1997).

Intravenous aluminum exposure from dialysates, total parenteral nutrition, vaccines and immunotherapy constitute a significant source of absorbed aluminum. In rabbits injected intramuscularly with [26]Al hydroxide or [26]Al phosphate adjuvants, [26]Al was distributed to multiple organs, including the brain (Flarend *et al.*, 1997). The tissue distribution of aluminum in rats killed three weeks after a single intravenous injection of trace amounts of [26]Al, was found to be 0.9% in bone, 0.2% in kidney, 0.06% in liver, 0.03% in heart, and 0.02% in brain and muscle (Walker *et al.*, 1994).

The brain is an important target organ for aluminum toxicity. This metal is capable of crossing the blood–brain barrier (BBB). Aluminum clearance from brain is much slower than that of other organs possibly due to the lack of neuron turnover. The elimination half-life of aluminum from human brain is calculated to be seven years (Yokel and McNamara, 2001). Aluminum–protein complexes are unlikely to permeate the BBB directly because of their large size, although a possible mechanism is by transferrin-receptor mediated endocytosis (Yokel, 2002). Transferrin receptors are present on brain capillary endothelial cells, choroid plexus

epithelial cells, neurons, and also glial cells (Moos and Morgan, 2000). Another mechanism of aluminum entry into the brain may be by nonspecific processes such as diffusion or pinocytosis of small molecules bound to the metal. The permeation of aluminum into the brain could be a combination of both specific and nonspecific processes (DeVoto and Yokel, 1994). The concentration, particular biochemical form, and localization of the metal may be the ultimate determinant of toxicity.

Excretion

Since aluminum forms highly insoluble hydroxy compounds in neutral pH, a substantial amount of the dietary aluminum is excreted in the feces without ever being absorbed. A recent study demonstrates that much orally ingested aluminum is recovered in the feces after seven days (Priest *et al.*, 1998). Once the metal is absorbed, it is either excreted in the urine or in the bile. The liver is an important early sink for absorbed aluminum and a single dose of aluminum citrate by gavage is rapidly cleared by the biliary system (Exley *et al.*, 1996). The favored route of aluminum excretion is dependent on the route of administration and the dose of the metal (Sutherland and Greger, 1998). For normal dietary levels of aluminum exposure, the biliary system appears to be the major route of excretion while at about 1 mmol aluminum, this system is saturated and renal excretion begins to take an important role. Thus, a large aluminum load, such as that often used in experimental settings, requires excretion of the major proportion of the aluminum by the urine. Approximately 90% of the excretion of aluminum by the kidney takes place 48 hours after exposure (Jouhanneau *et al.*, 1993).

Aluminum exposure and human health effects

Osteomalacia

The skeletal system is a target for aluminum toxicity. Rats fed diet drinks packaged in aluminum cans had a higher concentration of aluminum in the blood, liver and bone, as well as a weight reduction in the femur, compared to rats fed diet soft drinks

packaged in glass bottles and distilled water (Kandiah and Kies, 1994). Robertson *et al.* (1983) reported an aluminum-induced osteomalacia in rats chronically injected with aqueous aluminum chloride. This condition was worsened by both chronic renal failure and parathyroid hormone deficiency. Bone biopsy of a 39-year old woman with peptic ulcer and gastritis without renal insufficiency, who took large doses of an antacid containing a high concentration of aluminum, revealed that 27.6% of the total bone surface was composed of aluminum deposits (Woodson, 1998).

Aluminum incorporates into the bone and causes physiochemical mineral dissolution as well as cell-mediated bone resorption (Bushinsky *et al.*, 1995). Another study substantiates this finding by demonstrating that aluminum-induced osteomalacia is due to disruption of bone mineralization and resorption (Jablonski *et al.*, 1996). Interestingly, there is an increased risk of bone fragility fractures in people suffering from senile dementia. Since there is a higher concentration of aluminum in bone biopsies from patients with AD, it has been hypothesized that chronic aluminum intoxication may play a role in the etiology of this neurodegenerative disease (Mjoberg *et al.*, 1997).

Microcytic anemia without iron deficiency

Aluminum plays a role in the blood toxicity seen in patients with chronic renal failure. Uremic patients on chronic dialysis together with a phosphate binding buffer regimen, can suffer from severe aluminum intoxication and develop microcytic anemia without iron deficiency (Short *et al.*, 1980; Touam *et al.*, 1983). To prove that the increase in aluminum is the principal causative factor, Touam *et al.* injected uremic rats with aluminum salts and showed that there was a significant reduction in both the hematocrit and the average hemoglobin content of erythrocytes compared to uremic rats injected only with saline. Aluminum may also cause hemolysis by effecting peroxidative damage of the erythrocyte cell membrane (Zaman *et al.*, 1993).

The adverse effect of aluminum in erythropoiesis is not merely a secondary effect of renal failure. Normal rats treated with different concentrations of aluminum citrate develop a dose-dependent increase in bone aluminum as well as a decrease

in colony-forming units – erythroid development. The latter parameter is used as a measure of the ability of hematopoietic progenitor cells to develop. The rats also had decreased haematocrit and a shorter red blood cell life span compared with control rats (Garbossa *et al.*, 1998). Aluminum may be directly affecting the bone marrow progenitor cells and rendering them defective, or the metal may be affecting the function of the mature cells by damaging the cell membrane.

Neurologic deficits

In 1976, Alfrey *et al.* proposed aluminum salts as the possible cause of encephalopathy in uremic patients on chronic hemodialysis, who routinely received aluminum-containing phosphate binders (Alfrey *et al*., 1976). The study measured the aluminum content of various tissues of control subjects and uremic patients. In uremic patients, muscle and bone had elevated levels of aluminum (14.8 and 98.5 ppm, respectively) relative to control subjects (1.2 and 2.4 ppm, respectively). Values of aluminum in brain gray matter in a group of uremic patients on dialysis who died of neurologic syndrome of unknown cause were 25 ppm, compared to 6.5 ppm in a group of uremic patients on dialysis who died of other causes, and 2.2 ppm in control subjects. Symptoms of severe encephalopathy and high brain aluminum concentration were also reported in a uremic boy who was not undergoing dialysis, but was treated with oral aluminum hydroxide (Nathan and Pedersen, 1980). Another case study further implicated aluminum as the cause of the dialysis encephalopathy syndrome by demonstrating that the symptoms completely disappeared after oral aluminum intake was stopped (Russo *et al.*, 1991). In a more recent study, a patient with advanced obstructive nephropathy displayed symptoms of encephalopathy after continuous bladder irrigation with 1% alum for two days, and these symptoms were associated with elevated serum aluminum levels (Phelps *et al.*, 1999).

Studies in other populations also suggest aluminum induction of neurological damage. Infants subjected to prolonged feeding with aluminum-containing intravenous solutions exhibited impaired neurological development and increasing aluminum exposure was associated with a reduction in the Mental Development Index, with an adjusted loss of one point per day of intravenous feeding for infants receiving the standard solutions (Bishop *et al.*, 1997).

'McIntyre Powder' (finely ground aluminum and aluminum oxide) was used as a prophylactic agent against silicotic lung disease between 1944 and 1979 in miners in northern Ontario. A morbidity prevalence study conducted between 1988 and 1989 demonstrated that miners exposed to aluminum performed less well on cognitive examinations compared to unexposed workers. Furthermore, the likelihood of scores in the impaired range increased with duration of exposure (Rifat *et al.*, 1990). These studies have prompted considerable investigation into the mechanisms by which aluminum may be linked to neurodegenerative events.

The aluminum and Alzheimer's disease controversy

One of the first reports linking aluminum to AD found that there was an elevated level of aluminum in necropsy and biopsy samples of the brains of patients with histopathologically confirmed AD when compared to necropsy samples of normal brains (Crapper *et al.*, 1973). In contrast, a study by McDermott *et al.* (1979) found no significant changes in the brain aluminum concentration of patients with AD compared to normal age-matched controls. However, the region with the highest aluminum content was the hippocampus, which is the most critical site for brain lesions in AD. These authors also found an increase in aluminum concentration with increasing age. Another study reported that the level of aluminum is not elevated in the frontal cortex, temporal cortex, liver or the head of femur of patients with AD (Bjertness *et al.*, 1996).

It has been proposed that the root of this apparent discrepancy is in the methodology used to measure the metal. If there is an elevated level of aluminum in the brain of patients with senile dementia, it could be confined to small areas which are most affected. This increase may be masked when bulk brain tissue is analysed (Savory *et al.*, 1997). Indeed, direct analysis of the aluminum content in neurofibrillary tangles shows an increase

in the concentration of the metal (Perl and Brody, 1998).

Epidemiologic studies indicate a positive relationship between AD frequency and aluminum concentration in drinking water (Neri and Hewitt, 1991; Altmann *et al.*, 1999). However, other studies have been unable to confirm this association (Wettstein *et al.*, 1991; Martyn *et al.*, 1997). Subsequently, a Canadian study investigated the effects of aluminum exposure through municipal drinking water systems, using a 10-year residential history. The study correlated the relative risk of developing AD with residence in areas where the concentration of aluminum in drinking water was $\geq 100\,\mu g/L$ (McLachlan *et al.*, 1996). Similar results were obtained in a French study that included over 3700 subjects aged 65 and over, where it was found that the adjusted relative risk for dementia was 1.99 and that for AD was 2.14 in subjects exposed to aluminum concentrations $> 0.1\,mg/L$ (Rondeau *et al.*, 2000).

Mechanisms of aluminum-induced neurodegeneration

Oxidative stress

Oxidative stress is an event resulting from the formation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and the superoxide radical (O_2^-). These species are generated by electron leakage from enzymes involved in the mitochondrial electron transport chain, which contain transition metal ions at their active sites (Halliwell 1992). Most of the cell damage caused by ROS is due to their conversion into a relatively short-lived but highly reactive intermediate such as the hydroxyl radical ($^\bullet OH$), which can damage DNA, as well as cytosolic and membrane-bound macromolecules.

Oxidative events have frequently been linked to neurodegenerative disorders such as AD (Zhou *et al.*, 1995; Smith, 1996; Marcus *et al.*, 1998; Nunomura *et al.*, 1999); whether their presence is the cause or the end result of the disease is unresolved (Hensley *et al.*, 1998). Smith *et al.* (1998) further established the association between oxidative stress and components of the aging brain by demonstrating that 4-hydroxynonenol, the anti-

oxidant enzyme heme oxygenase-I, and τ-reactive dystrophic neurites are all located at the periphery of amyloid plaques. Events that chronically increase the normal production of these reactive intermediates may lead to a reduction in antioxidant defense and thus compromise cell integrity. Total plasma antioxidant capacity is decreased in AD patients while the activity of copper-zinc superoxide dismutase in erythrocytes and the lymphocyte mRNA level of manganese superoxide dismutase are significantly increased perhaps due to compensatory mechanisms (De Leo *et al.*, 1998). The activity of catalase and superoxide dismutase is decreased in the brain of AD patients, together with an increase in lipid peroxidation (Marcus *et al.*, 1998). It has been suggested that metals without redox capacity, such as aluminum can make fatty acids more available to attack by free radicals, thus facilitating the propagation of lipid peroxidation (Oteiza *et al.*, 1993; Ohyashiki *et al.*, 1998).

In isolated systems aluminum can potentiate the ROS production by iron (Bondy and Kirstein, 1996) and copper (Bondy *et al.*, 1998b). Fe and Cu, which are present in most cell compartments, are known to be pro-oxidant metals and aluminum potentiates the capability of these transition metals to produce oxidative stress. It is hypothesized that colloidal aluminium may bind these metals and thus modulate their ability to promote metal-based oxidative events (Campbell *et al.*, 2001). This may give further impetus to the concept that colloidal compounds (including aggregated β-amyloid peptide) may complex pro-oxidant metals, and thus allow them to participate in Fenton reactions for a longer period (Yang *et al.*, 1999).

Aluminum treatment can exacerbate ROS formation *in vivo* and *in vitro*. Intraperitoneal injection of aluminum gluconate increased the rate of ROS formation in cortical tissue (Bondy *et al.*, 1998a), and the brain of rats treated with aluminum lactate for four weeks showed an increase in lipid peroxidation and a significant decrease in antioxidants (Julka and Gill, 1996). Exposure of cells to aluminum sulfate increased ROS formation, accompanied with elevated mitochondrial activity and glutathione depletion, in glial but not neuronal cell lines (Campbell *et al.*, 1999). Because aluminum salts, complexed to strong acids such as sulfate and chloride, have a tendency to form colloidal

particles as they age (Corain *et al.*, 1996), it is possible that the glial-specific aluminum-induced increase in oxidative parameters is due to activation of these cells by extracellular aluminum complexes.

Inflammatory response

The number of activated astrocytes is increased in AD. These astrocytes are associated with both senile plaques and cerebral microvessels (Cullen 1997). Cytokines such as interleukin (IL)-1, IL-6 and IL-8 are primarily synthesized by activated microglia and macrophages in response to pathogens and trauma. Chronic production of these chemotactic factors can result in cytotoxicity because they recruit and activate macrophages that produce high concentrations of ROS (Dunn, 1991). In the hippocampus of AD patients, there is an upregulation of proinflammatory genes (Colangelo *et al.*, 2002) and the levels of the proinflammatory cytokine IL-6 is elevated in the brain (Cadman *et al.*, 1994) as well as the cerebrospinal fluid (CSF) and plasma (Sun *et al.*, 2003) of AD patients. An animal model of chronic inflammation induced by infusion of lipopolysaccharide produces astrogliosis as well as an increase in the levels of β-amyloid precursor protein (APP), IL-1, and TNFα mRNA levels. This is followed by hippocampal cell loss and impairment of spatial memory. These changes parallel those seen in the AD brain (Wegrzyniak *et al.*, 1998). Thus it may be that extended cerebral inflammatory events can lead to neuronal cell loss and subsequent neurodegeneration.

The ability of aluminum to promote peripheral inflammation is unquestionable. This metal has a long history of use as an adjuvant in vaccines. The mechanism includes formation of a depot, efficient uptake of aluminum-antigen particles by antigen presenting cells, and stimulation of immune competent cells of the body through activation of complement, induction of eosinophilia and activation of macrophages (Gupta, 1998). Sensitization can develop in individuals injected with vaccines containing aluminum hydroxide as an adjuvant and aluminum-induced inflammatory nodules are formed in adults revaccinated for hepatitis B (Cosnes *et al.*, 1990). Alum-precipitate allergenic extracts used for immunotherapy have also been associated with subcutaneous nodule formation at injection sites (Orfan *et al.*, 1995). Administration

of aluminum phosphate adjuvant induces transcription of inflammatory markers, such as IL-2 receptor gamma subunit, c-jun and c-fos in lymphoid organs that are distant from the injection site (Regnstrom *et al.*, 2002). Authier *et al.* (2001) described a persistent local inflammatory reaction to intramuscular injection of aluminum containing vaccines in patients and accumulation of this metal in macrophages at these sites. Furthermore, aluminum present in parenteral nutrition formula accumulated in the liver of rats and produced hepatobiliary dysfunction characterized by portal inflammation (Demircan *et al.*, 1998).

The ability of aluminum to cause an inflammatory response in the brain has been reported both *in vitro* and *in vivo*. Gene expression analysis of neural cells exposed for 72 hours to aluminum maltolate, led to a concentration-dependent increase in TNF-α and macrophage inflammatory protein-1 alpha (MIP-1α) (Johnson and Sharma, 2003). In glial cells, a three-day aluminum treatment resulted in an increase in TNF-α secretion together with activation of the immunologically relevant transcription factor NF-κB (Becaria *et al.*, 2003). Intraventricular injections of aluminum in the brain of adult mice produced gliosis in the striatum of exposed animals, a region where aluminum accumulated (Platt *et al.*, 2001). Chronic aluminum lactate treatment increased glial fibrillary acidic protein (GFAP) concentrations in the cerebral cortex of rabbits (Yokel and O'Callaghan, 1997) and intracisternal injection of aluminum maltolate in aged rabbits induced NF-κB activation (Ghribi *et al.*, 2001). We have found that extended administration of very low levels of aluminum in the drinking water of mice (10 μM), paralleling those found in some residential waters, can lead to microglial and astroglial activation (Campbell *et al.*, in press). Thus the capability of aluminum to promote various inflammatory events in the brain occurs over a range of aluminum species and in a wide range of experimental systems. A feature common to all these studies was a relatively extended exposure to aluminum. The neurotoxicity of aluminum may rely on its potential to chronically upregulate inflammatory cascades and oxidative events and this may eventually contribute to progression of pathological processes in neurodegenerative diseases (Figure 1).
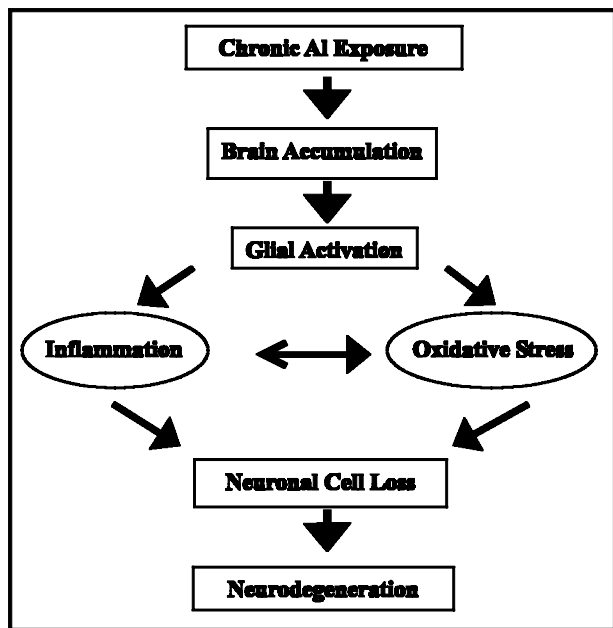
Figure 1. Chronic exposure to Al leads to accumulation of the metal in the brain. This then triggers activation of glial cells, which then produce proinflammatory cytokines and ROS to resolve a stimulus that the cells perceive as pathogenic. Prolonged secretion of these factors can diminish neuroprotective mechanisms such as antioxidant defense mechanisms, which lead to neuronal cell loss, eventually culminating with neurodegeneration.

Conclusions

Many neurological disorders, as well as brain aging in the absence of specific disease, are characterized by enhancement of two processes. One of these is the upregulation of inflammatory events in the absence of a well-defined exogenous stimulus. Unless triggered by an infection, the healthy younger animal has very low basal levels of inflammatory cytokines, inducible nitric oxide synthetase and other indices of immune reactivity. These indices are upregulated with age and are even more prominently associated with neurodegenerative disease. Aluminum salts may exacerbate such intrinsic inflammatory activity by providing an irresolvable chronic stimulus for microglial and phagocytic activation within the brain.

The second process which is reported as elevated in both the aging brain and especially in those disorders associated with aging is increasing oxidative damage to macromolecules due to enhanced formation of active oxygen species. This trend can be largely attributable to progressive deterioration of the efficiency of mitochondrial oxidative phosphorylation. As described in this review, aluminum

has the potential to catalyse the formation of iron-based oxidant events. This is also likely to be due to the colloidal nature of aluminum salts at neutral pH. Oxidative and inflammatory events associated with reduced cerebral functioning are intimately associated but the complex temporal and causal relationships between them remain unclear.

Exposure to low levels of aluminum has been regarded as harmless and reports to the contrary have often been met with skepticism. The thrust of the evidence described in this article is that, while the acceleration of neurological deficits by low levels of aluminum may be very difficult to unequivocally demonstrate, there is a good experimental basis to believe that such a hazard exists. Significantly, our studies using a rodent model, have not yet determined the 'no observable effect' levels of aluminum in drinking water (Campbell *et al.*, in press). Although deleterious effects of aluminum are likely to be subtle and unspectacular, there is a clear potential for the involvement of large populations in these adverse health changes. This constitutes an important reason for a detailed re-examination of this issue.

It is well accepted that aluminum, when present in the circulation in relatively high doses, can act as a toxicant, especially with respect to bone, blood, and the nervous system. Thus, although there is no consensus as to whether dietary aluminum can lead to elevations in plasma levels of this element that are sufficient to lead to acute toxicity, both epidemiological and experimental findings described here strengthen the possibility that a prolonged exposure to relatively low levels of aluminum may be neurotoxic.

References

Alfrey, A.C., LeGendre, G.R. and Kaehny, W.D. 1976: The dialysis encephalopathy syndrome. Possible aluminum intoxication. *The New England Journal of Medicine* 294, 184–88.

Altmann, P., Cunningham, J., Dhanesha, U., Ballard, M., Thompson, J. and Marsh, F. 1999: Disturbance of cerebral function in people exposed to drinking water contaminated with aluminium sulphate: retrospective study of the Camelford water incident. *British Medical Journal* 319, 807–11.

Anane, R., Bonini, M., Grafeille, J.M. and Creppy, E.E. 1995: Bioaccumulation of water soluble aluminium chloride in the hippocampus after transdermal uptake in mice. *Archives of Toxicology* 69, 568–71.

Authier, F.J., Cherin, P., Creange, A., Bonnotte, B., Ferrer, X., Abdelmoumni, A., Ranoux, D., Pelletier, J., Figarella-Branger, D., Granel, B., Maisonobe, T., Coquet, M., Degos, J.D. and Gherardi, R.K. 2001: Central nervous system disease in patients with macrophagic myofasciitis. *Brain* 124, 974–83.

Becaria, A., Bondy, S.C. and Campbell, A. 2003: Aluminum and copper interact in the promotion of oxidative but not inflammatory events: implications for Alzheimer's disease. *Journal of Alzheimer's Disease* 5, 31–38.

Berthon, G. 1996: Chemical speciation studies in relation to aluminum metabolism and toxicity. *Coordination Chemistry Review* 149, 241–80.

Bishop, N.J., Morley, R., Day, J.P. and Lucas, A. 1997: Aluminum neurotoxicity in preterm infants receiving intravenous-feeding solutions. *The New England Journal of Medicine* 336, 1557–61.

Bjertness, E., Candy, J.M., Torvik, A., Ince, P., McArthur, F., Taylor, G.A., Johansen, S.W., Alexander, J., Gronnesby, J.K., Bakketeig, L.S. and Edwardson, J.A. 1996: Content of brain aluminum is not elevated in Alzheimer disease. *Alzheimer Disease and Associated Disorders* 10, 171–74.

Bondy, S.C. and Kirstein, S. 1996: The promotion of iron-induced generation of reactive oxygen species in nerve tissue by aluminum. *Molecular and Chemical Neuropathology* 27, 185–94.

Bondy, S.C., Ali, S.F. and Guo-Ross, S. 1998a: Aluminum but not iron treatment induces pro-oxidant events in the rat brain. *Molecular and Chemical Neuropathology* 34, 219–32.

Bondy, S.C., Guo-Ross, S.X. and Pien, J. 1998b: Mechanisms underlying aluminum-induced potentiation of oxidant properties of transition metals. *Neurotoxicology* 19, 65–72.

Bushinsky, D.A., Sprague, S.M., Hallegot, P., Girod, C., Chabala, J.M. and Levi-Setti, R. 1995: Effects of aluminum on bone surface ion composition. *Journal of Bone and Mineral Research* 10, 1988–97.

Cabezuelo, A.B.S., González, E.B. and Medel, A.S. 1997: Quantitative studies of aluminum binding species in human uremic serum by fast protein liquid chromatography coupled with electrothermal atomic absorption spectrometry. *Analyst* 122, 573–77.

Cadman, E.D., Witte, D.G. and Lee, C.M. 1994: Regulation of the release of interleukin-6 from human astrocytoma cells. *Journal of Neurochemistry* 63, 980–87.

Campbell, A. and Bondy, S.C. 2000: Aluminum induced oxidative events and its relation to inflammation: a role for the metal in Alzheimer's disease. *Cellular and Molecular Biology* 46, 721–30.

Campbell, A., Prasad, K.N. and Bondy, S.C. 1999: Aluminum induced oxidative events in cell lines: Glioma are more responsive than neuroblastoma. *Free Radical Biology and Medicine* 26, 1166–71.

Campbell, A., Hamai, D. and Bondy, S.C. 2001: Differential toxicity of aluminum salts in human cell lines of neural origin: implications for neurodegeneration. *Neurotoxicology* 22, 63–71.

Campbell, A., Becaria, A., Sharman, K. and Bondy, S.C. (in press): Chronic exposure to aluminum in drinking water increases inflammatory parameters selectively in the brain. *Journal of Neuroscience Research*.

Colangelo, V., Schurr, J., Ball, M.J., Pelaez, R.P., Bazan, N.G. and Lukiw, W.J. 2002: Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. *Journal of Neuroscience Research* 70, 462–73.

Corain, B., Bombi, G.G., Tapparo, A., Perazzolo, M. and Zatta, P. 1996: Aluminum toxicity and metal speciation: established data and open questions. *Coordination Chemistry Review* 149, 11–22.

Cosnes, A., Flechet, M.L. and Revuz, J. 1990: Inflammatory nodular reactions after hepatitis B vaccination due to aluminum sensitization. *Contact Dermatitis* 23, 65–67.

Crapper, D.R., Krishnan, S.S., Dalton, A.J. 1973: Brain aluminum distribution in Alzheimer's disease and experimental neurofibrillary degeneration. *Science* 180, 511–13.

Crapper, D.R. and De Boni, U. 1980: Aluminum. In Spencer, S.S. and Schaumburg H.H., editors, *Experimental and clinical neurotoxicology* Baltimore, MD: Williams & Wilkins, 326–35.

Cullen, K.M. 1997: Perivascular astrocytes within Alzheimer's disease plaques. *Neuroreport* 8, 1961–66.

De Leo, M.E., Borrello, S., Passantino, M., Palazzotti, B., Mordente, A., Daniele, A., Flippini, V., Galeotti, T. and Masullo, C. 1998: Oxidative stress and overexpression of manganese superoxide dismutase in patients with Alzheimer's disease. *Neuroscience Letters* 250, 173–76.

Demircan, M., Ergun, O., Coker, C., Yilmaz, F., Avanoglu, S. and Ozok, G. 1998: Aluminum in total parenteral nutrition solutions produces portal inflammation in rats. *Journal of Pediatric Gastroenterology and Nutrition* 26, 274–78.

DeVoto, E. and Yokel, R.A. 1994: The biological speciation and toxicokinetics of aluminum. *Environmental Health Perspectives* 102, 940–51.

Divine, K.K., Lewis, J.L., Grant, P.G. and Bench, G. 1999: Quantitative particle-induced X-ray emission imaging of rat olfactory epithelium applied to the permeability of rat epithelium to inhaled aluminum. *Chemical Research in Toxicology* 12, 575–81.

Dunn, C.J. 1991: Cytokines as mediators of chronic inflammatory disease. In Kimball, E.S., editor, *Cytokines and inflammation.* Boca Raton, FL: CRC Press, 1–35.

318

Exley, C., Burgess, E., Day, J.P., Jeffery, E.H., Melethil, S. and Yokel, R.A. 1996: Aluminum toxicokinetics. *Journal of Toxicology and Environmental Health* 48, 569–84.

Flarend, R., Hem, S.L., White, J.L., Elmore, D., Suckow, M., Rudy, A.C. and Dandashli, E. 1997: In vivo absorption of aluminium-containing vaccine adjuvants using Al^{26}. *Vaccine* 15, 1314–18.

Flarend, R., Bin, T., Elmore, D. and Hem, S.L. 2001: A preliminary study of the dermal absorption of aluminium from antiperspirants using aluminium-26. *Food and Chemical Toxicology* 39, 163–68.

Garbossa, G., Gálvez, G., Castro, M.E. and Nesse, A. 1998: Oral aluminum administration to rats with normal renal function. 1. Impairment of erythropoiesis. *Human and Experimental Toxicology* 17, 312–17.

Ghribi, O., Herman, M.M., DeWitt, D.A., Forbes, M.S. and Savory, J. 2001: A beta(1-42) and aluminum induce stress in the endoplasmic reticulum in rabbit hippocampus, involving nuclear translocation of gadd 153 and NF-kappaB. *Brain Research. Molecular Brain Research* 96, 30–38.

Gupta, R.K. 1998: Aluminum compounds as vaccine adjuvants. *Advanced Drug Delivery Reviews* 32, 155–72.

Halliwell, B. 1992: Reactive oxygen species and the central nervous system. *Journal of Neurochemistry* 59, 1609–23.

Harris, W.R., Sheldon J. 1990: Equilibrium constants for the binding of aluminum to human serum transferrin. *Inorganic Chemistry* 29, 119–24.

Harris, W.R., Berthon, G., Day, J.P., Exley, C., Flaten, T.P., Forbes, W.F., Kiss, T., Orvig, C. and Zatta, P.F. 1996: Speciation of aluminum in biological systems. *Journal of Toxicology and Environmental Health* 48, 543–68.

Harris, W.R., Berthon, G., Day, J.P., Exley, C., Flaten, T.P., Forbes, W.F., Kiss, T., Orvig, C. and Zatta, P.F. 1997: Speciation of aluminum in biological systems. In Yokel, R.A. and Golub, M.S., editors, *Research issues in aluminum toxicity*. Washington, DC: Taylor and Francis, 91–116.

Hensley, K., Pye, Q.N., Tabatabaie, T., Stewart, C.A. and Floyd, R.A. 1998: Reactive oxygen involvement in neurodegenerative pathways. In Wood, P.L., editor, *Neuroinflammation.* New Jersey, NJ: Humana Press Inc., 265–80.

Jablonski, G., Klem, K.H., Danielsen, C.C., Mosekilde, L. and Gordeladze, J.O. 1996: Aluminum-induced bone disease in uremic rats: effect of deferoxamine. *Bioscience Reports* 16, 49–63.

Johnson, V.J. and Sharma, R.P. 2003: Aluminum disrupts the pro-inflammatory cytokine/neurotrophin balance in primary brain rotation-mediated aggregate cultures: possible role in neurodegeneration. *Neurotoxicology* 24, 261–68.

Jouhanneau, P., Lacour, B., Raisbeck, G., Yiou, F., Banide, H., Brown, E. and Drueke, T. 1993: Gastrointestinal absorption of aluminum in rats using 26Al and accelerator mass spectrometry. *Clinical Nephrology* 40, 244–48.

Julka, D. and Gill, K.D. 1996: Effect of aluminum on regional brain antioxidant defense status in Wistar rats. *Research in Experimental Medicine* 196, 187–94.

Kandiah, J. and Kies, C. 1994: Aluminum concentrations in tissues of rats: effect of soft drink packaging. *Biometals* 7, 57–60.

Kochian, L.V. and Jones, D.L. 1997: Aluminum toxicity and resistance in plants. In Yokel, R.A. and Golub, M.S., editors, *Research issues in aluminum toxicity*. Washington, DC: Taylor & Francis, 69–89.

Marcus, D.L., Thomas, C., Rodriguez, C., Simberkoff, K., Tsai, J.S., Strafaci, J.A. and Freedman, M.L. 1998: Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease. *Experimental Neurology* 150, 40–44.

Martyn, C.N., Coggon, D.N., Lacey, R.F. and Young, W.F. 1997: Aluminum concentrations in drinking water and risk of Alzheimer's disease. *Epidemiology* 8, 281–86.

McDermott, J.R., Smith, A.I., Iqbal, K. and Wisniewski, H.M. 1979: Brain aluminum in aging and Alzheimer's disease. *Neurology* 29, 809–14.

McLachlan, D.R.C., Bergeron, M.D., Smith, J.E., Boomer, D. and Rifat, S.L. 1996: Risk for neuropathologically confirmed Alzheimer's disease and residual aluminium in municipal drinking water employing weighted residential histories. *Neurology* 46, 401–405.

Mjoberg, B., Hellquist, E., Mallmin, H. and Lindh, U. 1997: Aluminum, Alzheimer's disease and bone fragility. *Acta Orthopaedica Scandinavica* 68, 511–14.

Moos, T. and Morgan, E.H. 2000: Transferrin and transferrin receptor function in brain barrier systems. *Cellular and Molecular Neurobiology* 20, 77–95.

Nathan, E. and Pedersen, S.E. 1980: Dialysis encephalopathy in a non-dialysed uraemic boy treated with aluminium hydroxide orally. *Acta Paediatrica Scandinavica* 69, 793–96.

Neri, L.C. and Hewitt, D. 1991: Aluminium, Alzheimer's disease and drinking water. *Lancet* 338, 390.

Nunomura, A., Perry, G., Pappolla, M.A., Wade, R., Hirai, K., Chiba, S., Smith, M.A. 1999: RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. *The Journal of Neuroscience* 19, 1959–64.

Ohyashiki, T., Suzuki, S., Satoh, E. and Uemori, Y. 1998: A marked stimulation of Fe^{2+}-initiated lipid peroxidation in phospholipid liposomes by a lipophilic aluminum complex, aluminum acetylacetonate. *Biochimica et Biophysica Acta* 1389, 141–49.

Orfan, N.A., Dykewicz, M.S. and Barnowsky, L. 1995: Extensive subcutaneous fibrosis in a patient treated with alum precipitated allergenic extract. *Annals of Allergy, Asthma and Immunology* 75, 453–56.

Oteiza, P.I., Fraga, C.G. and Keen, C.L. 1993: Aluminum has both oxidative and antioxidant effects in mouse brain membranes. *Archives of Biochemistry and Biophysics* 300, 517–21.

Perl, D.P. and Brody, A.R. 1998: Alzheimer's disease: X-ray spectrometric evidence of aluminum accumulation in neurofibrillary tangle-bearing neurons. *Science* 208, 297–99.

Phelps, K.R., Naylor, K., Brien, T.P., Wilbur, H. and Haqqie, S.S. 1999: Encephalopathy after bladder irrigation with

alum: case report and literature review. *The American Journal of the Medical Sciences* 318, 181–85.

Platt, B., Fiddler, G., Riedel, G. and Henderson, Z. 2001: Aluminium toxicity in the rat brain: histochemical and immunocytochemical evidence. *Brain Research Bulletin* 55, 257–67.

Powell, J.J., Whitehead, M.W., Ainley, C.C., Kendall, M.D., Nicholson, J.K. and Thompson, R.P. 1999: Dietary minerals in the gastrointestinal tract: hydroxypolymerisation of aluminium is regulated by luminal mucins. *Journal of Inorganic Biochemistry* 75, 167–80.

Priest, N.D., Talbot, R.J., Newton, D., Day, J.P., King, S.J. and Fifield, L.K. 1998: Uptake by man of aluminium in a public water supply. *Human and Experimental Toxicology* 7, 296–301.

Regnstrom, K., Ragnarsson, E.G., Rydell, N., Sjoholm, I., Artursson, P. 2002: Tetanus antigen modulates the gene expression profile of aluminum phosphate adjuvant in spleen lymphocytes in vivo. *Pharmacogenomics Journal* 2, 57–64.

Rifat, S.L., Eastwood, M.R., McLachlan, D.R. and Corey, P.N. 1990: Effect of exposure of miners to aluminium powder. *Lancet* 336, 1162–65.

Robertson, J.A., Felsenfeld, A.J., Haygood, C.C., Wilson, P., Clarke, C. and Llach, F. 1983: Animal model of aluminum-induced osteomalacia: role of chronic renal failure. *Kidney International* 23, 327–35.

Rondeau, V., Commenges, D., Jacqmin-Gadda, H. and Dartigues, J.F. 2000: Relationship between aluminum concentrations in drinking water and Alzheimer's disease: an 8-year follow-up study. *American Journal of Epidemiology* 152, 1.

Russo, L.S., Beale, G., Sandroni, S. and Ballinger, W.E. 1991: Aluminum toxicity in undialyzed patients with chronic renal failure. *Journal of Neurology, Neurosurgery and Psychiatry* 55, 697–700.

Savory, J., Exley, C., Forbes, W.F., Huang, Y., Joshi, J.G., Kruck, T., McLachlan, D.R.C. and Wakayama, I. 1997: Can the controversy of the role of aluminum in Alzheimer's disease be resolved? What are the suggested approaches to this controversy and methodological issues to be considered? In Yokel, R.A. and Golub, M.S., editors, *Research issues in aluminum toxicity*. Washington, DC: Taylor & Francis, 185–205.

Shi, B. and Haug, A. 1990: Aluminum uptake by neuroblastoma cells. *Journal of Neurochemistry* 55, 551–58.

Short, A.I., Winney, R.J. and Robson, J.S. 1980: Reversible microcytic hypochromic anaemia in dialysis patients due to aluminum intoxication. *Proceedings of the European Dialysis and Transplant Association* 17, 226–33.

Sjögren, B., Elinder, C.G., Iregren, A., McLachlan, D.R.C. and Riihimaki, V. 1997: Occupational aluminum exposure and its health effects. In Yokel, R.A. and Golub, M.S., editors, *Research issues in aluminum toxicity*. Washington, DC: Taylor & Francis, 165–83.

Smith, M.A., Hirai, K., Hsiao, K., Pappolla, M.A., Harris, P.L.R., Siedlak, S.L., Tabaton, M. and Perry, G. 1998: Amyloid-β deposition in Alzheimer transgenic mice is associated with oxidative stress. *Journal of Neurochemistry* 70, 2212–15.

Smith, R.W. 1996: Kinetic aspects of aqueous aluminum chemistry: environmental implications. *Coordination Chemistry Review* 149, 81–93.

Soni, M.G., White, S.M., Flamm, W.G. and Burdock, G.A. 2001: Safety evaluation of dietary aluminum. *Regulatory Toxicology and Pharmacology* 33, 66–79.

Sparling, D.W. and Campbell, P.G.C. 1997: Ecotoxicology of aluminum to fish and wildlife. In Yokel, R.A. and Golub, M.S., editors, *Research issues in aluminum toxicity*. Washington, DC: Taylor & Francis, 47–68.

Sun, Y.X., Minthon, L., Wallmark, A., Warkentin, S., Blennow, K. and Janciauskiene, S. 2003: Inflammatory markers in matched plasma and cerebrospinal fluid from patients with Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders* 16, 136–44.

Sutherland, J.E. and Greger, J.L. 1998: Effect of the size of an oral dose of aluminum on the relative importance of biliary v. urinary aluminum excretion in conscious rats. *Food and Chemical Toxicology* 36, 505–12.

Taylor, G.A., Moore, P.B., Ferrier, I.N., Tyrer, S.P. and Edwardson, J.A. 1998: Gastrointestinal absorption of aluminium and citrate in man. *Journal of Inorganic Biochemistry* 69, 165–69.

Touam, M., Martinez, F., Lacour, B., Bourdon, R., Zingraff, J., Di Giulio, S. and Drueke, T. 1983: Aluminium-induced, reversible microcytic anemia in chronic renal failure: clinical and experimental studies. *Clinical Nephrology* 19, 295–98.

Walker, V.R., Sutton, R.A., Meirav, O., Sossi, V., Johnson, R., Klein, J., Fink, D. and Middleton, R. 1994: Tissue disposition of 26aluminum in rats measured by accelerator mass spectrometry. *Clinical and Investigative Medicine* 17, 420–25.

Wegrzyniak, B.H., Dobrzanski, P., Stoehr, J.D. and Wenk, G.L. 1998: Chronic neuroinflammation in rats reproduces components of the neurobiology of Alzheimer's disease. *Brain Research* 780, 294–303.

Wettstein, A., Aeppli, J., Gautschi, K., Peters, M. 1991: Failure to find a relationship between amnestic skills of octogenarians and aluminum in drinking water. *International Archives of Occupational and Environmental Health* 63, 97–103.

Woodson, G.C. 1998: An interesting case of osteomalacia due to antacid use associated with stainable bone aluminum in a patient with normal renal function. *Bone* 22, 695–98.

Yang, E.Y., Guo-Ross, S.X. and Bondy, S.C. 1999: The stabilization of ferrous iron by a toxic beta-amyloid fragment and by an aluminum salt. *Brain Research* 839, 221–26.

Yokel, R.A. 2002: Brain uptake, retention, and efflux of aluminum and manganese. *Environmental Health Perspectives* 110 Suppl 5, 699–704.

Yokel, R.A. and O'Callaghan, J.P. 1997: An aluminum-induced increase in GFAP is attenuated by some chelators. *Neurotoxicology and Teratology* 20, 55–60.

Yokel, R.A. and McNamara, P.J. 2001: Aluminum toxicokinetics: An updated minireview. *Pharmacology and Toxicology* 88, 159–67.

Yokel, R.A., Rhineheimer, S.S., Brauer, R.D., Sharma, P., Elmore, D. and McNamara, P.J. 2001: Aluminum bioavailability from drinking water is very low and is not appreciably influenced by stomach contents or water hardness. *Toxicology* 161, 93–101.

Zaman, K., Zaman, A. and Batcabe, J. 1993: Hematological effects of aluminum on living organisms. *Comparative Biochemistry and Physiology* 106, 285–93.

Zatta, P., Favarato, M. and Nicolini, M. 1993: Deposition of aluminum in brain tissues of rats exposed to inhalation of aluminum acetylacetonate. *Neuroreport* 4, 1119–22.

Zhou, Y., Richardson, J.S., Mombourquette, M.J. and Weil, J.A. 1995: Free radical formation in autopsy samples of Alzheimer and control cortex. *Neuroscience Letters* 195, 89–92.