The Fourth Keele Meeting on Aluminium, 2001

The Biological Availability of Aluminium: Sources, Sinks and Symptoms.

Sunday 25th February 2001 – Session 1

Platform 1 - The potential of fluorimetry and atomic force microscopy to identify hydroxyaluminosilicate formation in acidic solution

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The unique inorganic reaction of monomeric silicic acid (Si(OH)4) with aluminium (Al) to form hydroxyaluminosilicates (HAS) plays a fundamental role in controlling the biological availability, hence toxicity, of Al in biological systems. For this reason, the development of new methods to successfully identify HAS formation in solution is of great interest to both biologists and geologists. However, up to now the identification of HAS formation in solution has remained fraught with experimental difficulties. At the meeting we will report : (i) the use of the formation of the fluorescent morin-Al complex as an indirect method of successfully identifying HAS formation in solution; (ii) the establishment of Atomic Force Microscopy (AFM) as a reliable technique for the direct observation of HAS of varying shapes and structures in their native, hydrated state; (iii) the confirmation of the applicability of the morin-reactive Al method using the direct observation of HAS by AFM.

Poster 1 - The mechanism of formation of well characterised hydroxyaluminosilicates of biological and geological significance.

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Aluminium, the most abundant metal in the lithosphere and omnipresent in biological systems, is acutely toxic to a number of biota (e.g. fish, plants, man). Our earlier works demonstrated the potential role for silicon , as monomeric $Si(OH)_4$, to influence/eliminate the biological availability, hence the toxicity, of aluminium in biological systems, through the formation of hydroxyaluminosilicates (HAS). The mechanism of HAS formation in soil and surface waters and their role in the biological availability of aluminium are of great interest to biologists, chemists and geologists, and have been subjects of some controversy. Herein we will report the preparation of HAS of biological and geological significance in acid solutions at room temperature and their structural characterisation. These results will be used to demonstrate the hitherto undetermined mechanism of formation of HAS both in the laboratory and in the natural environment.

Poster 2 - Silicic Acid and the Biological Availability of Environmental Aluminium.

Celine Schneider and Christopher Exley.

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Silicic acid has been proposed as a geochemical control of the biological availability of aluminium [1]. Silicic acid reacts with aluminium to form hydroxyaluminosilicates (HAS) [2], [3], [4], thereby ameliorating the toxic effects of aluminium [5], [6], [7]. Very little is known about the kinetics underlying the formation of HAS. The rate at which HAS are formed and achieve stability with respect to their environment will be critical to their role in defining the biological availability of aluminium. We are using a number of different techniques including fluorimetry, pH-metry and GFAAS to try to understand the factors, which influence HAS formation. In establishing these techniques we have made the interesting observation that the presence of silicic acid influences the measurement of aluminium by GFAAS. Standard calibration curves prepared in the presence of different amounts of silicic acid produce different signal absorbances. These differences are significant and suggest that significant errors in the measurement of total aluminium could occur if silicic acid is not included in standards and blanks. The observation that silicic acid enhanced the absorbance signal might suggest that silicic acid could be used as an effective matrix modifier in the measurement of aluminium by GFAAS. [1] Exley, C.; Birchall, J.D. J. Theo. Bio. 1992, 159(1), 83. [2] Exley, C.; Birchall, J.D. Polyhedron 1992, 11(15), 1901. [3] Exley, C.; Birchall, J.D. Polyhedron 1993, 12(9), 1007. [4] Doucet et al., submitted for publication 2000 [5] Birchall, J.D.; Exley, C.; Chappell, J.S.; Phillips, M.J. Nature 1989, 338(6211), 146. [6] Exley, C.; Tollervey, A.; Gray G.; Roberts S.; Birchall, J.D. Proc. Roy. Soc. London. 1993, 253(1336), 93. [7] Exley, C.; Pinnegar, J.K.; Taylor, H. J. Theor. Biol. 1997, 189(2), 133

Platform 2 - Fulvic Acid Complexes with Aluminum, Lanthanum, and Terbium

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Humic substances are the organic material that remains after dead plant tissue has been attacked by micro-organisms and other processes. These affect the pH of natural waters, trace metal aquatic chemistry and bioavailability, and the degradation and transport of hydrophobic organic molecules. Fulvic acids are the lowest molecular weight substances and have the highest oxygen content in the complex humic group. In this study, we have investigated the interaction of three trivalent metals, Al^{3+}, La^{3+}, and Tb^{3+}, with fulvic acids in a standard (Suwannee River) fulvic acid sample (International Humic Substances Society, http://www.ihss.gatech.edu/). Aluminum is a biologically important metal and is potentially bound by the fulvic acids in nature. The trivalent metals form soluble complexes with the fulvic acid at low metal ion concentration while insoluble complexes precipitate out at higher

metal levels. Tb^{3+} and La^{3+} have been used to probe potential Al^{3+} binding using fluorescence, IR and solid state NMR. Formation constants for Ca^{2+} and subsequent Al^{3+} binding to the fulvic acid using a calcium ion selective electrode will also be presented.

Poster 3 - Silicic acid and the precipitation of natural organic materials by aluminium

Helen Taylor & Chris Exley

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The precipitation of natural organic materials (NOM) by metals such as aluminium is a key stage in drinking and wastewater treatment and often determines the effectiveness of the entire treatment process. Despite its ubiquity in natural waters and its known interactions with aluminium, the influence of silicic acid ($Si(OH)_4$) on the precipitation of NOM by aluminium has not previously been investigated. Molecular absorbance, fluorescence and atomic absorption spectroscopy were used to follow the aluminium-induced precipitation of a well-characterised model NOM, humic acid. The pH dependence of the precipitation of humic acid by aluminium was established using three experimental protocols in which the initial hydrolysis conditions were varied. The influence of $Si(OH)_4$ was tested using two of the protocols. This poster reports the results of experiments with the model NOM, and of experiments which compared the influence of $Si(OH)_4$ on HA precipitation by aluminium in the model system, with the precipitation of NOM from natural water samples.

Poster 4 - A New Method of Investigating Coagulation with Aluminium Salts

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Aluminium and iron salts have been employed as coagulants for water treatment for a long time. A new approach to the determination of quantity of coagulant necessarily to promote the aggregation of colloidal particles dispersed in water has been developed and tested for aluminium nitrate. The new method employs the Atomic Force Microscopy for the determination of the surface potential of colloidal particles in water solutions and prediction of aggregation probability.

Platform 3 - Trapid non-equilibrium aluminium-ligand interaction (II):Critical precipitation assay to study aluminium-ligand interaction

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A simple assay is described that can be used to investigate the interaction of aluminium (or any hydrolytic metal) with metal binding ligands at neutral pH. The assay, used here to investigate the interaction of aluminium with weak and strong dietary ligands, was shown to correlate with the literature-derived stability constants, suggesting that non-equilibrium Al-ligand interaction approximate to equilibrium. This assay also gave insight into the nature of the Al-ligand interaction (Al:ligand ratio), which was also investigated here by ^{1}H-nuclear magnetic resonance.

Poster 5 - Some Peculiarities of Aluminium Speciation in Medium and Highly Concentrated Aqueous Solutions

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This study has involved investigation of the speciation of aluminium-ions in medium and highly concentrated solutions (>0.1 M) during neutralisation of inorganic salt solutions with alkalis of different strength (KOH, NH_4OH, $KHCO_3$) and concentration (0.4-4 M). Aluminium nitrate, chloride and sulphate have been chosen as aluminium sources. Investigations have been performed at room temperature, with and without adjustment of ionic strength. Aluminium speciation during neutralisation on a short time scale (2-5 min) has been studied by potentiometry and conductometry. Relaxation curves from potentiometric titrations have also been investigated. The process of $Al(OH)_3$ precipitation on a long time scale (>12 hours) has been studied with ^{27}Al NMR and dynamic light scattering. Particular results include: · When $KHCO_3$ and KOH are used as titrants, the potentiometric curves possess a distinct inflexion at OH/Al ratio 2.4-2.6, which is more pronounced when KHCO3 is used. When NH_4OH is the titrant, the potentiometric curves do not show an inflexion at this hydrolysis ratio. The inflexion at OH/Al=2.4-2.6 is related to the formation of the Al13-mer. Thus, this species is formed faster when $KHCO_3$ and KOH are used as titrants. Relaxation potentiometric curves also support the differences in behaviour observed using conventional potentiometry. · Anion identity and ionic strength both affect the formation of the Al_{13}-mer. Of the anions investigated, sulphate suppresses the formation of the Al_{13}-mer, as does high ionic strength over the short time scale investigated. · ^{27}Al NMR spectra of solutions of aluminium chloride titrated to a range of hydrolysis ratios show no differences after extended equilibration times. For solutions of aluminium sulphate, however, the NMR spectra suggest that dimeric species are stabilised in the presence of sulphate ions. The results obtained will be discussed in reference to the development of materials containing aluminium for industrial application in a biological environment.

Poster 6 - Fast Kinetics of Al Hydrolysis/Condensation in Medium and Highly Concentrated Solutions as Studied by an Advanced Potentiometric Method.

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In this work we have investigated two relatively simple approaches to the elucidation of kinetic effects, in the time range 0-15 min, for aluminium speciation during alkaline

hydrolysis using potentiometry. The first approach is based on the use of relaxation curves produced during the equilibration of the system in a potentiometric titration. We have found that the relaxation curve can exhibit one of three distinct shapes that can be interpreted in terms of the specific kinetic processes occurring during the neutralization of an aluminium salt with base. The other approach makes use of first-derivative titration curves to obtain additional information about speciation and changes in speciation with time. Mathematical deconvolution of the derivative titration curves leads reveals more information than the native curve. Results are presented for the titration of medium- and highly concentrated solutions (> 0.1M) of inorganic aluminium salts (chloride, nitrate, sulphate), with bases of different strength (NH_4OH, $KHCO_3$, KOH). Analysis of the data provides evidence for several peculiarities of aluminium speciation in highly concentrated solutions on a short time scale, and confirms the usefulness of the proposed approaches in potentiometry.

Platform 4 - Studies on aluminium-binding by transferrin in human uremic serum by fast protein liquid chromatography coupled to double focusing icp-ms.

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The binding of Aluminium to High Molecular Mass (HMM) biocompounds in human uremic serum is revisited here by using a hybrid technique consisting of Fast Protein Liquid Chromatography coupled on-line with Double Focusing ICP-MS. It has been well documented that transferrin seems to be the only protein binding aluminium in human serum. However, it is not clear the binding affinity of aluminium for each of the two lobes (the N-lobe and the C-lobe) of transferrin or the type of complexes formed. In the present work the chromatographic separation of transferrin molecular forms by using FPLC (Mono Q HR 5/5 anion exchange) was studied. Proteins were detected spectrophotometrically at 295 nm. Al specific detection was carried out on-line with a DF-ICP-MS (R=3000) in order to identify the preferential transferrin lobes binding the aluminium in unspiked human uremic serum with different Al contents. The binding patterns of co-existing serum Al and Fe in transferrin were also studied.

Poster 5 - Interaction of Aluminium with Commercial Silicas and Magnesium Trisilicate.

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Silicas are widely used in the food and brewing industry, however, the potential of these for binding aluminium in such matrices has not been fully investigated. We now present our experimental data on the interaction of aluminium with silica compounds in water and beer. Known amounts (0-10mg/ml) of two commercial amorphous silica compounds [Gasil 200, average particle pore size 20Å and IJ 45, 80Å] and magnesium trisilicate suspension were added into solutions of $Al(OH)_3$ at pH's 4.0, 5.0, 6.0 and 7.0 (soluble aluminium varying from 0.03-39mg/L) or four different beers (pH range 3.59-4.02, aluminium 0.32-0.38mg/L) to a final volume of 5ml and mixed constantly for 30mins at 25°C. The suspension was then centrifuged for 10mins at 3000rpm and the aluminium measured in the supernatant as an

indicator of free aluminium. Gasil 200, IJ 45 and magnesium trisilicate bound aluminium released by $Al(OH)_3$ in water that was both pH dependent (optimal pH 5.0) and dose responsive, binding all the free aluminium at the concentration of 10mg/ml. The presence of citric acid (100mM/L) in water had no effect on the binding. However, only magnesium trisilicate bound the soluble aluminium in beer with a dose response relationship with 80-99% bound at the highest concentration investigated (4mg/ml). In conclusion, silica has an affinity for aluminium in water, but the nature of the aluminium complex species determines the effectiveness of binding in beer.

Poster 8 - Rapid non-equilibrium aluminium-ligand interactions (i):Studies on the precipitation of aluminium by laser light scattering, ultrafiltration and centrifugation

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The study of aluminium speciation in complex solutions is difficult and, even in simple solutions, often months of ageing are required because equilibrium is so slowly reached. However, the initial seconds or hours of interactions are important; firstly, because they (must) largely dictate the final thermodynamic equilibrium state and, secondly, because most natural aluminium-ligand interactions of relevance occur in dynamic systems that rarely equilibrate. The gastrointestinal tract is a typical example, since, following ingestion of the metal, absorption occurs within a few hours and, furthermore, prior to faecal excretion, aluminium that is not absorbed is within the continually changing chemical environment of the intestinal lumen. The immediate and non-equilibrium precipitation of aluminium hydroxide, in aqueous solution at neutral pH, was therefore studied by laser light scattering (diffraction), ultrafiltration and centrifugation. The interaction of weak ligands, present in the gastrointestinal lumen, on the precipitation of aluminium hydroxide was also investigated.

Monday 26th February 2001 (AM) – Session 2

Platform 5 - Modulation of the TCA Cycle Enzymes by Aluminum Stress and the Production of oxalic acid

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Oxalic acid plays a pivotal role in the adaptation of the soil microbe *Pseudomonas fluorescens* to aluminum stress. Its production via the oxidation of glyoxylate necessitates a major reconfiguration of the enzymatic reactions involved in the TCA cycle. The demand for glyoxylate appears to enhance the activity of isocitrate lyase (ICL), an enzyme that participates in the cleavage of isocitrate to glyoxylate and succinate. A 4-fold increase has been observed in Al-stressed cells. The ICL activity has been shown to increase in a dose-dependent manner in relation to the aluminum concentration in the culture medium. However, the activity of isocitrate dehydrogenase, a competitor for the substrate isocitrate appeared to be markedly diminished in cells exposed to aluminum. This demand for oxalate in Al-stressed cells did also influence the activities of the enzymes α-ketoglutarate dehydrogenase and succinate dehydrogenase. Thus, it appears that the TCA cycle is diverted to generate the necessary precursor for oxalate synthesis as a consequence of aluminum stress.

Platform 6 - Direct Evidence of Silicon Biochemistry

Eric W. Deguns, Ashley-Mae E. Gillson, Robin J. Hamilton, Christopher T.G. Knight, **Stephen D. Kinrade**

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Although silicon cannot be considered "essential" to most plants species (diatoms and Equisetaceae being notable exceptions), there is no doubt that it plays a major role in defending many plants against both biotic and abiotic stress. Evidence is accumulating to show that silicon is no less important in animals, being required for the healthy development of bone and cartilage. Details on the underlying mechanisms have been scarce, however, leading some workers to doubt the very existence of silicon biochemistry in nature.
We recently demonstrated that aqueous Si interacts with certain aliphatic carbohydrate molecules to form five- and six-coordinated organosilicon complexes, even at physiological concentrations and pH. Here, we describe a second mode of carbohydrate-silicon interaction with important biological implications. Also, a preliminary report is given on the first ever characterization of silicon uptake and metabolism *in vivo.*

Platform 7 - Up-regulation of intracellular silicon in response to aluminium in the snail *Lymnaea stagnalis*

CR McCrohan[1], M Desouky[1,3], R Jugdaohsingh[2], JJ Powell[2] and KN White[1]

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Exogenous silica has been shown to ameliorate absorption and/or toxicity of aluminium (Al) in fish and other organisms, due to formation of hydroxy-aluminosilicates. Here we show that, in the freshwater snail, endogenous Si is up-regulated in response to sub-lethal Al exposure. X-ray microanalysis revealed the presence of Si in lysosomal (detoxificatory) granules of digestive gland cells. Exposure of snails to low levels of Al led to Al accumulation, accompanied by up-regulation of Si, in the granules. This response was specific to Al since exposure to cadmium or zinc had no effect on Si levels. Furthermore, intra-lysosomal sulphur, from metallothionein and other sulphur-containing ligands, was significantly increased following exposure to cadmium and zinc but not Al. This finding suggests a specific role for endogenous Si in detoxification of Al.

Poster 9 - Bioavailability and toxicity of aluminium in the fresh water snail *Lymnaea stagnalis* at neutral pH

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The behaviour and bioavailability of aluminium (Al) in fresh water is influenced by ligands, including soluble silica. Here we examine the influence of orthosilicic acid ($Si(OH)_4$) on the behaviour of Al and its bioavailability and toxicity to the snail *Lymnaea stagnalis* over a 30-day period. Orthosilicic acid did not reduce the degree of hydroxypolymerisation of Al and, hence, its rate of removal from the water column. The amount of Al in the digestive gland (the main 'sink' for Al) peaked at day 15, suggesting regulation of elevated Al during continued exposure. Orthosilicic acid did not reduce Al accumulation. However, Al depressed snail behaviour (behavioural state score and latency to bite) but this effect was totally ameliorated by orthosilicic acid. Thus, exogenous silicon ameliorates toxicity (but not accumulation) of Al, whereas regulation of Al by the snail does not prevent toxicity.

Platform 8 - Aqueous Aluminium has a Positive Effect on Atlantic Salmon Infected by the Devastating Ectoparasite *Gyrodactylus salaris*

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Recently it was demonstrated that aqueous aluminium eliminates the devastating ectoparasite *Gyrodactylus salaris* from infected Atlantic salmon. An interesting observation was that aluminium seemed to have no toxic effect on the salmon, even though all parasites were eliminated. The mechanism behind this disinfecting effect, however, might be that aluminium is toxic to the parasite, a direct effect, or influencing the fish resulting in detachment of parasites, an indirect effect. We present our most recent results indicating that aluminium has a direct effect on the parasite rather than an indirect effect through the host. Fish pre-exposed to aluminium showed no altered infection rate compared to control. Parasites seemed vulnerable to aluminium even at pH 6.5, when aluminium is considered not to be toxic to fish, adding support to the suggestion that aluminium is beneficial to salmon infected by G. salaris due to a direct effect on the parasite.

Platform 9 - Growth Pattern in Fish Gills Exposed to Aqueous Aluminium During Exposure and Recovery .

A.G. Hagen, G.E. Nilsson and A.B.S. Poléo.

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Growth and apoptosis patterns in brown trout (Salmo trutta) gills were investigated during and after aqueous aluminium exposure. Gill sections have been prepared and stained by various techniques (BrdU and TUNEL), before examination. The results indicate a growth zone in the primary epithelia, close to the central venous sinus of the gill filament. No cell division was evident in the gill lamellae. Aluminium exposure led to a disruption of this pattern, with cell growth detected randomly within the gill filament. The cell growth rate increases with a factor of 2 to 3. The rate of apoptosis increases during exposure. After a significant aluminium challenge, gill recovery was rapid and the epithelial structure was fully rebuilt.

Poster 10 - Physiological recovery in Atlantic salmon from the river Suldalslågen stock after exposure to acidic Al-rich water

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In this study we have tested the prediction that Atlantic salmon exposed to a heavy aqueous Al-challenge, recover after transfer to optimal water quality conditions. It is a question if this is different under steady-state and non-steady-state chemical conditions. We observed no mortality in Al-exposed fish after transfer to optimal water quality, even though some moribund individuals were observed towards the end of the Al-exposures. All physiological parameters revealed that the Al-exposures caused significant disturbances in the fish. Recovery started immediately after transfer to optimal water quality, and most fish were fully recovered after 7 days. The fish recovered from respiratory disturbances more rapidly than ionoregulatory disturbances, and the recovery rate was highest in fish exposed to aluminium under non-steady-state conditions. In conclusion, our results support the prediction that Al-toxicity in fish is a reversible process. This finding should be of importance for future strategies and mitigating activities in acidified waters.

Poster 11 - Increased seawater tolerance in Atlantic salmon exposed to aluminium and acidic water from a tributary to the river Suldalslågen, western Norway: an evidence of acclimation?

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It is documented that acid water and aqueous aluminium influence parr-smolt transformation and seawater tolerance in Atlantic salmon. Thus, seawater tolerance in salmon has been used as a criteria for water quality and effects of acidification in fish populations. Our preliminary results show that fish exposed to natural water quality fluctuations in an acid stream (pH 5.0-6.0) had significantly higher seawater tolerance than fish exposed to a limed stream (pH 7.0-7.5). These results either indicate that fish acclimates to fluctuating pH and Al-concentration, or that liming does not eliminate all adverse effects of acidification. We also observed that a

pre-exposure to a heavy Al-challenge, caused fish in the limed tributary to increase their seawater tolerance considerably. This adds support to the suggestion that fish can acclimate to sub-optimal water quality condition. At present time, however, we do not dear to conclude that a heavy Al-challenge some time before the parr-smolt transformation period is beneficial for the salmon. On the other hand, we do dear to conclude that seawater tolerance is useless as an indicator of water quality.

Poster 12 - The Toxicity Of Acidic Al-Rich Water Under Non-Steady-State Conditions To Four Different Aquatic Invertebrates.

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Aqueous aluminium is believed to be the principal toxicant killing fish in acidified soft waters. The importance of aluminium for adverse effects of acidification in invertebrates, however, is more debated. In the present study we test the prediction that aluminium is of the same importance for the toxic effect of acid water in invertebrates as in fish. We exposed four different invertebrate species for elevated concentrations of aluminium under non-steady-state chemical conditions. Such conditions have been demonstrated to be acutely toxic to fish, and our results revealed that this was also the case for three of the invertebrates we tested. As in several studies of fish, the mortality in the present study was correlated to the degree of Al-polymerisation. This gives some support to our prediction that aluminium is of similar importance for the negative effects of acidification in most aquatic organisms. Our results also demonstrate for the first time that conditions favouring Al-polymerisation can enhance Al-toxicity in invertebrates.

Monday 26th February 2001 (PM) – Session 3

Platform 10 - Aluminium alters cellular membrane properties without affecting Catacholaminergic Systems

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We previously reported a selective increase in hypothalamic dopamine and metabolites in mice treated with different concentrations of aluminium (Al) in drinking water for 4 weeks. To investigate if Al directly influenced catecholamine turnover, we investigated the effects of in vitro Al exposure in PC-12 (rat pheochromocytoma) cells, a model for neurotransmitter synthesis and release. Incubation of PC-12 cells with 0.001 to 3 mM Al for 6 hr caused no alterations in the production or release of neurotransmitters or their metabolites. Al at >0.1 mM caused an increase in the rate of extracellular acidification, measured by a microphysiometer, indicating stimulation of proton extrusion from cells for up to 6 minutes. Similar concentrations of Al hyperpolarized the cell membrane as determined by decreased $DiBAC_4$ fluorescence. This was associated with a marked reduction of cellular reactive oxygen species, indicated by diminished dihydrorhodamine 123 fluorescence. Results indicate that Al had no direct effect on catecholamine pathways but at a relatively high concentration can alter membrane properties of neuronal cells. The in vivo effects of Al can be explained by its effects on non-neuronal cells (i.e., microglia) that provide important signals for neuronal well-being.

Poster 13 - Aluminium inhibits NF-κB activation leading to decreased proinflammatory cytokine production in murine microglial cells.

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We previously reported a selective increase in tumor necrosis factor a (TNFα) expression in the cerebrum of mice treated with aluminium (Al) in the drinking water for 4 weeks. To further investigate the role of cytokines in Al neurotoxicity, BV-2 murine microglial cells were exposed to Al (0.001 to 1 mM) *in vitro*. Al (> 0.5 mM) exposure resulted in a significant increase in the production of reactive oxygen species (ROS) as evidenced by enhanced dihydrorhodamine 123 fluorescence. Notwithstanding the increase in ROS production, Al caused a dose-dependent decrease in the expression of TNFα and interleukin-1β (IL-1β). Examination of the activation status of nuclear factor-κB (NF-κB) revealed that Al exposure inhibited the nuclear localization of this transcription factor. Results indicate that Al decreases the expression of proinflammatory cytokines by suppressing the activation of NF-κB in isolated microglial cells. Interactions with other cell types in the intact brain may explain the differential effects of Al *in vivo*.

Platform 11 - Inhibition of Parathyroid Hormone mRNA by Aluminium.

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Aluminium reduces parathyroid hormone (PTH) levels in chronic renal failure (CRF). In previous studies we and others have suggested that aluminium inhibited PTH release rather than PTH synthesis. The aim of these complementary studies was to assess the effect of aluminium on PTH synthesis by means of quantifying PTH mRNA by Northern blot analysis . The first study was performed in CRF rats receiving either aluminium or placebo. Biochemical parameters, aluminium content of the gland and mRNA of PTH were measured. The second study was carried out in parathyroid glands from rats cultured "in vitro" with or without aluminium, PTH secreted to the medium and mRNA of PTH were measured. In both studies we found that aluminium reduced PTH release as well as PTH mRNA. So far, previous studies had demonstrated that aluminium suppressed PTH release, our findings suggest that also PTH synthesis is reduced.

Poster 14 - The cytotoxic effects of aluminium ions in solution on a monolayer of renal proximal tubular cells

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The toxicity of aluminium (Al) to various cells is well described. However little is known about its effect on kidney cells. In this study we have investigated the effect of aluminium on a monolayer of kidney proximal tubular cells (PTC). Stock Al solution of 1 mmol/l was prepared in citric acid (3 mmol/l) at pH 7.2 and sterilised by filteration through a filter 0.22 μm. Monolayers of LLC-PK1 cells in 96 well plate were exposed to Al at final concentrations of 0, 25, 50, 75 and 100 μmol/l in cell culture medium (L-glutamine supplemented medium 199). Assessment of cell viability was carried out using; Thiazol blue (MTT) uptake as an indicator of mitochondrial membrane integrity; NAG activity as an indicator of lysosomal damage; LDH as an indicator of plasma membrane integrity and DAPI staining for DNA fragmentation in apoptotic cells. Cell viability studies showed slight reduction of 15 % and 20 % after 24 and 48 hrs incubation respectively at 100 μmol/l aluminium. NAG activity slightly increased after 24 and 48 hrs of exposure, but this increase was not significant. ($p > 0.05$). LDH release in the medium showed significant increase after incubation for 24 (44.67 versus 24 μmol/l) and 48 hrs (50.33 versus 28.33) at 100 μmol/l ($p < 0.001$).> Electron microscopy and DAPI Staining of PTC cells treated with Al at 100 μmol/l, showed no obvious changes compared with control. Conclusion: Al in solution showed only a slight cytotoxic effect on PTC assessed by MTT uptake, NAG activity and LDH release This may be because the cells are not significantly affected by Al (III) ions or there is some protective effect of the culture medium.

Platform 12 - Iron and aluminium homeostasis in the brains of experimentally aluminium loaded rats.

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Iron is an essential trace element which has been associated with many neurodegenerative diseases in man e.g. substania nigra in Parkinson's disease. In early studies, the suggestion that there was an association between Alzheimer's disease and the accumulation of aluminium in the neurofibrillary tangles and/ or the amyloid plaques of brain, precipitated research for an animal model of brain aluminium toxicity. One such model (1) accumulated large amounts of aluminium in specific brain regions after IP injection of aluminium gluconate for a period of 1-2 months, which was accompanied by increases in iron. Such results begged the question as to whether the increased iron was caused by an altered flux of iron across the blood brain barrier via transferrin-transferrin receptor pathway, or that the iron was acquired locally. Such perturbations of iron homeostasis may also be related to changes in the iron regulatory proteins, IRP-1 and IRP-2, which are responsible for controlling the uptake of iron, via transferrin receptors and storage of iron, via ferritin, within cells. Injection of ^{26}Al to rats showed that minimal amount of the tracer accumulated either within ferritin after 24h, or the lysosomal/ mitochondrial fraction. However ^{26}Al was present in the cytoplasmic/microsomal fraction. Perturbation of iron homeostasis may be an important factor in the development of many neurodegenerative diseases including Alzheimer's disease. Investigation of such perturbations may be a key in understanding the initiation and pathology of Alzheimers disease.

1. Florence A., Gauthier A., Ward RJ., Crichton R.R. (1995). *Neurodegeneration* **4** 449-455

Poster 15 - Cellular iron uptake and utilisation are impaired by aluminium

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Aluminium (Al) affects erythropoiesis by inducing anaemia through mechanisms that impair metabolic events in erythroid progenitor cells. In this work we investigated whether Al affects Fe uptake and utilisation in K562 erythroleukaemic cells. Fe uptake diminished when Al was present in the culture medium, recovering control values when Al was taken away. These results provide evidence for a competitive mechanism of binding between Fe-Tf and Al-Tf, as the membrane Tf receptor has similar affinity for both moieties. Fe incorporation increased in cells induced to differentiate with haemin, probably due to a higher expression of Tf receptors. The impairment of Fe utilisation was demonstrated by the decrease of the haemoglobinised cell number. Moreover, Fe incorporation to haem was found depressed when cells were grown in media containing Al. Assays carried out under different experimental conditions led us to suggest that Al might disturb intracellular regulatory mechanisms of Fe homeostasis.

Platform 13 - Impairment of the glutamate-nitric oxide-cGMP pathway in rat brain in vivo by chronic exposure to aluminium

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Activation of glutamate receptors leads to increased intracellular calcium which binds to calmodulin and activates nitric oxide synthase (NOS). The nitric oxide (NO) formed activates soluble guanylate cyclase (GC), increasing cGMP. This pathway modulates important cerebral processes. Chronic exposure to Al of cultured neurons impaired the pathway both at the level of activation of NOS and of formation of cGMP induced by NO. In cultures of neurons from rats prenatally exposed to Al activation of NOS induced by glutamate is inhibited and the content of NOS and of GC cyclase is significantly reduced while the content of calmodulin is increased. Modulation of GC by NO is also altered. Chronic administration of Al also impairs the glutamate-NO-cGMP pathway in rat brain in vivo, as assessed by in vivo brain microdialysis, resulting in reduced activation of NOS by NMDA and increased activation of GC by NO. Aluminium reduced the content of calmodulin and NO, the basal activity of GC and the extracellular content of cGMP. The alteration of this pathway may contribute to the neurotoxic effects of Al. We have also tested whether determination of activation of soluble guanylate cyclase in blood cells may be useful as a peripheral marker of the alteration of the glutamate-nitric oxide-cGMP pathway in brain

Poster 16 - Investigation of Specific and Efficient Preparations of Tetrafluoroaluminate (AlF4-).

Brandon Conley, David A. Atwood.

Department of Chemistry, University of Kentucky

The tetrafluoroaluminate (AlF_4^-) anion has been shown by numerous studies to act as an analog for the phosphate group in biochemical reaction systems, especially with regard to G proteins. The customary method of preparation of AlF_4^- for biochemical studies is a combination of NaF and $AlCl_3$; this procedure, however, has been shown to result not in AlF_4^- specifically, but rather in a complex mixture of aluminum fluorides, hydroxides, and fluoro-hydroxides. The presence of these co-products could have an unforeseen effect upon the studies conducted with AlF_4^-, and presents researchers with an unacceptable number of variables. This has led to the research to be presented, wherein various methods of preparation, both published and novel, have been investigated, along with the analysis and characterization of their respective products.

Platform 14 - Aluminium neurotoxicity: Evidence from in vivo investigations.

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Aluminium (Al) is the third most abundant element on earth, and present in all organs fluids and tissues from birth. While the organism appears to be able to cope with this general Al load, exposure to higher Al concentrations, for instance as a consequence of increased Al contents in the food chain due to acid rain, may lead to serious cognitive and neurological impairments both in humans and animals. In recent investigations, we reported that aluminium (Al) has various neurotoxic actions, which may hamper neuronal communication and signalling. Our studies focussed on the function and susceptibility of the hippocampus in Al exposed rats, since this brain area is known to be crucially involved in learning and memory and also particularly affected in neurodegenerative disease such as Alzheimer's (AD). We found that Al can interfere with hippocampal long-term potentiation (LTP), a cellular correlate of learning and memory mechanisms, in a dose-dependent manner (micromolar range), which could be identified both *in vivo* and *in vitro*. For the *in vivo* situation, impairment of LTP was evident even before any signs of cell death were detected. Further histological analysis indicated that Al particularly targeted one particular transmitter system, namely the cholinergic system. A set of fibres from the basal forebrain to cortex and hippocampus was found to be damaged in rats after Al exposure, leading to a depletion of cholinergic projections, which resembles the situation in AD. Another observation was that Al was able to accumulate in lipid-rich fibre bundles and caused enhanced inflammation, another hallmark of AD. Other transmitters such as the GABAergic system appeared intact. Behavioural analysis commenced to determine putative memory deficits in Al exposed rats. We utilised a delayed-matching-to-place version of the water maze, which requires the animal to remember the position of a hidden platform in a circular pool. Animals have to locate the platform in 4 successive trials within one day, but a new platform position is chosen between days. This test is specifically sensitive to deficits in spatial episodic memory - a type of memory affected in the early stages of AD. Moreover, the animal's performance can be assessed repeatedly and compared with pre-exposure behaviour thus allowing within subject comparison. Under control conditions, animals needed ~ 5 sec to locate the platform after a 30-sec inter-trial delay, and 15 sec after a 4 hr delay. Al exposed animals were highly significantly impaired for the 30 s delay (14.5 sec latency). For the longer (4 hr) delay, Al animals achieved initial latencies similar to controls (14.4 sec) but did not improve their performance in a subsequent 30 sec delay (20 sec latency). The overall performance in all 4 trials was found to be significantly different from controls. Together, our data provide evidence for the susceptibility of the mammalian brain to Al toxicity, which was manifested on the behavioural, cellular and functional level. Our studies also provide evidence for the cholinergic system as a possible connection for the involvement of Al in AD

Platform 15 - The Effect of Aluminum on Early Embryonic Bone Development.

Conrad E. Firling

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Elevated aluminum (Al) in humans and experimental animals is associated with several bone disorders. In this study the chick embryo was used to investigate the effect of Al on early bone formation. Embryos were treated with Al citrate on day 8 and tibias collected on days 10-16 of development. The tibias of Al-treated embryos exhibited (a) a persistent mid-shaft abnormality spatially correlated with Al positive staining, (b) reduced total bone calcium, and (c) a lower rate of ^{45}Ca uptake. Al administration had no effect on levels of serum calcium, phosphorus, osteocalcin, and parathyroid hormone or on the synthesis of tibia collagen. Histomorphometry showed that tibia of Al-treated embryos exhibit normal osteoid volume

coupled with a significant undermineralization of the osteoid (day 10, P below 0.001). These results support the hypothesis that Al perturbs embryonic bone formation by a direct physical inhibition of osteoid calcification sites or by perturbing normal osteoblast function in the mineralization process.

Tuesday 27th February 2001 (AM) – Session 4

Platform 16 - Peptide YY: Structure, Biological Activity and Applications

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Peptide YY (PYY) is one member of the pancreatic polypeptide (PP) family of gastrointestinal and central nervous system peptides that also includes neuropeptide Y (NPY). PYY is produced by the L-cells, which are found in abundance in the terminal ileum. In mammals and avians, PYY has a primary structure of 36 and 37 acids, respectively. Phylogenetically, the primary structure of the PP family is highly conserved with a 75% homology between mammals and chickens. There is also a high degree of homology between members of the family with a 70-80% homology of the primary structure between NPY and PYY. The tertiary structure of PYY is U shaped and includes an N-terminal proline helix and C-terminal a-helix. Members of the PP family bind to six subtypes of the Y receptor series. PYY and NPY bind with similar affinities to the Y1,Y2,Y5,Y6 receptor subtypes with equal affinity. EGF receptor activation may be required for functioning of the Y1 receptor in the intestinal tract. A PYY-preferring receptor has been putatively identified. PYY is believed to be one of the humoral regulatory agents contributing to the functioning of the "ileal brake syndrome" during intestinal malabsorption. The ileal brake is associated with decreased upper gastrointestinal motility, decreased pancreatic and gastric secretion and, possibly, increased intestinal glucose and lipid absorption. Other described biological actions of PYY include gastrointestinal hypertrophy and the ability to deplete the murine brain of Al. In ovo PYY administration has been shown to increase growth and nutrient utilization in poultry. Additionally, reports of alterations in NPY/PYY brain receptor densities and NPY concentrations in brain, cerebrospinal fluid and plasma in Alzheimer's disease suggest a possible role for these and other related peptides. (Supported in part by F.R.I.E.N.D.S. of Trisomy 21 Research Inc. of Los Angeles, CA, USA).

Poster 17 - Rapid non-equilibrium aluminium-ligand interaction (III): Aluminium binding capacity of gastrointestinal fluids

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The regulation of mineral absorption in the gastrointestinal tract is poorly understood. Recent work has identified an intracellular metal-ion transporter but considerable evidence suggests that both soluble and mucosally associated metal-binding ligands regulate initial uptake. Like aluminium many of the biologically important metals exhibit extensive hydrolytic behaviour around the pH of the intestine, however, except for aluminium, their gastrointestinal uptake is high. Previous work has noted that some of the gastrointestinal fluids have their own intrinsic metal binding properties, such as copper binding by bile, gastric juice and saliva, and iron binding by gastric juice. These interactions, which interfere with hydroxypolymerisation have

not, however, been investigated for aluminium and therefore individual components that may be responsible for the intestinal luminal binding of soluble aluminium have not been identified. The endogenous presence of low molecular weight ligands in gastrointestinal fluids and their potential for interaction with aluminium have been studied.

Platform 17 - Deleterious effects of aluminium on mature and immature human erythroid cells.

Daniela Vittori, Graciela Garbossa, Carlos Lafourcade, Gladys Pérez, **Alcira Nesse**.

Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

There has been evidence that anaemia is associated to aluminium (Al) accumulation. We have already reported the sensitivity of erythroid cell populations from animals chronically exposed to Al. The aim of the present work was to investigate the action of Al on human erythroid cells. Erythroid progenitors were concentrated from peripheral blood of healthy donors and stimulated with erythropoietin in cultures with or without Al compounds. Results (Median/Range) expressed as CFU-E number/10^6 cells showed a significant decrease of haemoglobinised colony number produced by Al (C: 12,800/11,200-16,300; Al: 8,700/6,500-9,200; p below 0.05). Scanning electron microscopy of peripheral erythrocytes aged *in vitro* in the presence of Al revealed the presence of abnormal-shaped erythrocytes, particularly acanthocytes and stomatocytes. Membrane proteins of red blood cells were analysed by SDS-PAGE and immunoblotting. An increased membrane protein breakdown due to Al was restricted to band 3, while no changes were observed on actin and spectrin. These in vitro results suggest that Al may disturb human erythropoiesis through combined effects on mature erythrocytes and cellular metabolism in late erythroid progenitors.

Poster 18 - Reference values for trace and ultratrace elements in human serum determined by double focusing ICP-MS.

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Reference values for trace and ultratrace elements concentrations in human serum, measured by double focusing Inductively Coupled Plasma Mass Spectrometry (ICP-MS), are presented. Blood donors from Asturias (Spain) were selected as the reference population (N=59). A total number of 14 elements: Al, Ca, Cr, Mn, Fe, Co, Cu, Zn, Rb, Sr, Mo, Cd, Pb and U were monitored almost simultaneously with detection limits from 0.35 to 0.001 ng g^{-1}. Matrix interferences were corrected using Sc, Ga, Y and Tl as internal standards. The reference values found for Al were always unexpectedly low (<0.35 ng g^{-1}) and explanations for this fact will be discussed. Serum samples from hemodialysis patients (N=14) were also analyzed for comparison. High levels of Al, Cr, Sr, Mo, Mn, Pb, U, Co and Cu and low levels of Fe, Zn and Rb were found in the serum samples of hemodialysis patients when compared to the corresponding reference values observed in this work.

Poster 19 - Computer model to understand Aluminium inter-relationships with other elements in cerebrospinal fluid of normal and Alzheimer's disease: A diagnostic approach

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Normal and Alzheimer's disease (AD) cerebrospinal fluid (CSF) samples were analyzed for Al, S, Na, Mg, Fe, Co, Cu, Mn, Cr, K, Ca, Zn and P using Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES). The results showed that Al, Mg, Mn and Ca levels did not show change between normal and AD CSF. However, K, P, and S were significantly (P below 0.0001) decreased in AD CSF over normal, while Na level was significantly (p below 0.0001) increased in AD CSF. Further, the elements Co and Cr were elevated in AD CSF, while Cu and Fe levels were decreased in AD CSF. Mole percentage ratio of selected elements namely, Na/Fe, Ca/Fe, Al/Fe, Mg/Fe, Na/P, Na/K, Na/S, K/P, Ca/P, K/S, Ca/K, Co/Fe, Ca/S, Al/P, Al/K, Mg/P, Mg/S, Al/Zn, Fe/Cu, Fe/S, Zn/Cu showed a definite increase in AD CSF over normal. The comparative assessment of total percentage of charge distribution between normal and AD CSF indicated that in AD CSF, the percentage charge distribution of divalent and trivalent ions were moderately decreased, while monovalent charge distribution was moderately increased compared to normal. The comparison these CSF results with AD and normal brain showed definite relations (direct or inverse) for selected elements, and these findings are new and novel. We developed Algorithmic computer model to understand the inter-relationships between Al and other metals so as to arrive at metal homeostatic pool. To arrive at this we used symbolic data analysis model. To our knowledge this is the first such computer model available in neuroscience. This information provides a clue in understanding the role of trace elemental homeostasis in neurodegeneration

Platform 18 - Aluminium-induced inflammation in cells of glial origin, and it's role in Alzheimer's disease

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The pathogenesis of Alzheimer's disease (AD) is multifactorial. Aluminium (Al) has been proposed to play a role in the etiology of AD although the issue remains unresolved. In the present study, the rate of generation of reactive oxygen species (ROS) in human glioblastoma (T98G) cells increased markedly after treatment with aluminum salts. However, a parallel exposure of human neuroblastoma (SK-N-SH) cells to Al did not significantly change oxidative status. After a 4 h exposure to aluminium salts, the expression of NF-kB was also increased in the T98G cells. In addition, levels of interleukin-6 were elevated by treatment. This implied activation of an innate immune cascade involving cytokines. Eliciting such a stress response by Al complexes may underlie the increased production of ROS of the T98G

cell line. There are thus parallels between amyloid plaques and colloidal aluminium in that both may cause deleterious events within the nervous system by constituting an irresolvable inflammatory focus.

Platform 19 - 26Al investigations at the AMS-laboratory in Lund

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At the Accelerator Mass Spectrometry (AMS) laboratory in Lund, an ultra sensitive facility for ^{26}Al analysis is under development. The sensitivity is expected to be several orders of magnitude higher than with standard mass spectrometry. The planned biomedical program includes studies of the kinetics of various aluminium compounds in man. The initial work has been concentrated on the construction and testing of a new dedicated injector for the accelerator and on the preparation of blood and tissue samples for aluminium analysis. The quality of the facility will be presented and the first results reported at the meeting.

Platform 20 - Long term physiological behaviour of aluminium in humans.

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Aluminium toxicity is still an important consideration for human health. The purpose of the study now reported is to quantify long term accumulation of aluminium in humans from the normal levels of aluminium encountered in everyday diet. The essential parameters needed have been obtained from an uptake-elimination study using the tracer isotope, Al-26, coupled with the extreme sensitivity of accelerator mass spectrometry, over (up to now) a four year period following a single dose. Following the ingestion of the isotopically labelled aluminium citrate solution in 1996, blood plasma, red blood cells and urine were sampled, initially at regular short intervals and then progressively at increasing intervals; this programme is continuing. Total Al and the Al-26:Al-27 ratio in these substrates, and in chromatographically separated plasma fractions, have been determined. A multi-compartment physiological model has been used to represent the observed behaviour over nearly four years, and parameters of long term retention and elimination have been derived. There are sufficient data to support a 4-compartment retention model, which allows the prediction of long term aluminium accumulation in healthy people from a continuous exposure. These projections compare well with current knowledge of Al body burdens in humans as a function of age. The implications of these conclusions in relation to normal and abnormal (e.g. from the use of Al-containing medications) aluminium intakes in healthy humans will be discussed. Eventually (it is intended), the body burden and Al-26 organ distribution in this subject will be determined *post mortem*, the results to be reported (by others) at a later (hopefully, much later) Keele Meeting.

Poster 20 - Prevention of aluminium toxicity in renal patients: the results of a 20 year study on the effect of controlling aluminium exposure in a major renal unit.

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Aluminium accumulation in renal failure patients causes three serious conditions: a fatal encephalopathy, a crippling osteodystrophy, and a microcytic anaemia. Initially epidemiology, and later more direct chemical studies, showed by 1980 that a major Al source is the water supply used to prepare dialysis solutions. Prior to about 1978, untreated drinking water was used for this purpose, but since that time water purification has been progressively introduced, and patient exposure monitored. This paper reports the results of the monitoring programme at a major renal unit in Manchester, UK. The Al content of dialysis water supplies to over 750 patients has been determined at monthly intervals over the course of 20 years, together with plasma-Al measurements at 3-monthly intervals. The effectiveness of the water aluminium control programme in terms of reduction in patients' plasma-Al levels is clearly demonstrated, and the incidence of Al-related dialysis conditions has been largely eliminated.

Poster 21 - Long-Term Retention and Elimination of Aluminum from Vaccine Adjuvants

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Aluminum-containing adjuvants are added to many vaccines to increase the immune response to the vaccine. These adjuvants are given internally to the body. But since they take several months to dissolve, they represent a very small daily exposure to aluminum. Recently there has been a growing concern about long-term effects which aluminum may have on the body following the injection of an aluminum-containing vaccine. Some muscle related adverse effects have been attributed to aluminum-containing adjuvants. The onset of these effects have even been observed several years after the vaccination leading to the belief of a long-term retention of aluminum from vaccine adjuvants. In this study, aluminum hydroxide is labeled with aluminum-26 and injected into the deltoid muscle of a human subject. Decay counting techniques are then used to analyze how much aluminum hydroxide adjuvant remains localized at the injection site over a long period of time while accelerator mass spectrometry is used to measure the amount of aluminum-26 which is cleared from the body in urine. The injection site and urine will be monitored for a total of five years after the injection.

Tuesday 27th February 2001 (PM) – Final Session

The JD Birchall Memorial Lecture

Aluminium and cell suicide: Oh what a tangled web we weave.

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Aluminium neurotoxicity *in vivo* provides unique opportunities for investigations of how brain cells die via programmed cell death or apotosis. By injecting an appropriate Al compound, such as Al maltolate, directly into the brain of the rabbit, it is possible to induce neurofibrillary pathological features that possess many biochemical and immunohistological similarities to similar lesions observed in Alzheimer's disease. However, these neuropathological changes do not necessarily induce neuronal death. Oxidative stress and mitochondrial injury play important roles in such cell death via apoptosis. Also, crosstalk between mitochondria and endoplasmic reticulum appears to be a key factor in determining whether cells survive or are programmed to die. Proapoptotic proteins such as Bax and its antiapoptotic counterpart, Bcl-2, control Al-induced release of cytochrome c from mitochondria via the mitochondrial permeability transition pore, and such release results in caspase activation and apoptosis. By using this animal model system, therapeutic strategies to provide neuroprotection can be devised. Although the role of Al in human neurodegenerative disease remains controversial, the use of this highly neurotoxic metal in experimental encephalomyelopathy is providing important new information on the complex mechanisms by which brain cells die.

Poster 22 - Neurotransmitter dopamine applied in electrochemical determination of aluminum in drinking waters and biological samples

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An indirect electroanalytical method for the determination of aluminum (Al) in drinking waters and biological samples has been established in this paper. It is based on the linear decrease of the differential pulse voltammetric anodic peak current of the ligand, 3,4-dihydroxyphenylethylamine (dopamine, DA), with the increase of Al concentration. Under optimum experimental conditions, pH 4.6, 1.2x10-3 M dopamine, and 0.04 M NaAc-Hac buffer solution, the detection limit of Al in the linear range $4x10^{-7}$ - $8x10^{-5}$ M is $1.4x10^{-7}$ M, and the relative standard deviation for $4x10^{-5}$ M Al is 3.5%(n=8). Many low molecule weights foreign species such as common anions and cations and organic substances have been chosen for interference. Satisfactory recoveries and accuracy have been obtained by applying this method to the determination of Al in drinking waters and biological samples, including synthetic renal dialysate, sodium chloride injection, sucrafate, human blood, urine and hair. The proposed method is anticipated being used in monitoring Al in vivo. The

principle of the method for indirect measurement of Al has been investigated by examining the mechanisms of the electrooxidation of dopamine in the presence and absence of Al with aid of UV-vis, ^{13}C-NMR and Raman spectrometries.

Poster 23 - Modeling the distribution of aluminum speciation in natural waters equilibria with the mineral phase imogolite

Shuping Bi, Rui Xue, Lixiong Wen and Wei Tang

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Speciation of aluminum (Al) is a critical issue when evaluating the environmental and biological significance of elevated Al concentrations in natural waters caused by the acidic precipitation, because not all chemical forms of Al are equally toxic. Free Al^{3+}, $Al(OH)^{2+}$ and $Al(OH)^{2+}$ are the most toxic species while Al-fluoride and Al-organic acids complexes are less harmful. During the last two decades, most researches are only dealing with the natural water equilibria with mineral phase gibbsite because of its widespread presence in many soils. The total dissolved Al concentrations are generally controlled by the solubility of gibbsite. However, there is a combination of aluminisilicate and oxides in soils that undergo weathering, such as, Kaolinite $Al_2Si_2O_5(OH)_4$, and imogolite $Al_2O_3SiOH(OH)_3$. With the increased concentrations of SiO_4^{2-}, the activity of Al and the relative stability of minerals in the soils are greatly modified by the SiO_4^{2-} concentrations, but so far there has been little through investigation about it. This paper reports the computer simulation of the distribution of Al speciation in acidic natural water equilibria with the mineral phase imogolite based on the chemical equilibrium calculation. Some unique characteristics are discovered, such as the dissolved silica has a remarkable influence on the speciation of Al, and the concentrations of polymeric alumino-hydroxo species are insignificant.

Poster 24 - The toxicity of acidic Al-rich water in crayfish

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It has been proposed that the noble crayfish (Astacus astacus) is among the most acid sensitive freshwater species in Scandinavia. Despite this, the knowledge about the influence of aqueous aluminium on crayfish and its life stages is still scarce. Different stages of crayfish were therefore exposed to various combinations of pH, temperature and concentrations of aluminium. Our results revealed that all stages of crayfish were resistant to heavy Al-challenges to which they were exposed. No mortality was observed until water temperature was raised to 19°C in order to induce moulting. Extensive mortality was evident during moulting, suggesting that moulting is the Achilles heal of crayfish inhabiting acidified waters. However, since moulting only takes place when water temperature is above 15°C, and episodes with lowered pH and elevated concentrations of aluminium are most likely to occur during spring and autumn floods, we dispute the fact that crayfish belongs to the group of most acid sensitive freshwater species.

Poster 25 - Episodic acidification during spring floods in high-toc streams in Northern Sweden: physiological responses in brown trout.

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Episodic acidification in high-TOC streams during spring floods is largely driven by an increase in TOC, in combination with ANC dilution. Along with this, the concentration of aqueous aluminium increases substantially. Thus, organic acids can cause both increased mobilisation of aluminium from the catchment and icreased Al-detoxicfication due to complexation. Based on this, it is unclear whether acidic episodes in high-TOC streams is critical to fish or not. We have exposed brown trout to streams varying in pH and TOC-content. Our results indicate that acidic episodes in high-TOC streams are toxic to fish, but to a lesser extent than in low-TOC streams. The results from this study is also discussed in terms of anthropogenic impacts on episodic acidification in Northern Sweden.