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Brain aluminum, magnesium and phosphorus contents of control and Alzheimer-diseased patients

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Abstract. A study was undertaken to determine Al, Mg and P concentrations in 5 different brain regions of 3 control and 3 Alzheimer-diseased patients. One of the aims of this work was to evaluate the performance of applied analytical techniques. The digested samples were analyzed by inductively coupled plasma atomic emission spectrometry for Al, Mg and P. The dried samples were measured by instrumental neutron activation analysis for Al and Mg. The determination of human brain Al levels is complicated by the interfering reaction of P. We have previously worked out an analytical method which can eliminate this interference. The accuracy of the measured data was investigated by the analysis of biological standard reference materials. Our second goal was to study the possible elemental concentration changes in Alzheimer-diseased patients. Significantly higher Al and lower Mg and P values were found in some AD brain regions compared to the controls.

Keywords: Alzheimer disease, aluminium, magnesium, phosphorus, brain regions

1. Introduction

Alzheimer's disease (AD), a progressive degenerative disease of the central nervous system (CNS), is the most common cause of dementia comprising over 50–70 per cent of cases. AD is characterized clinically by a diffuse deterioration of cognitive function and pathologically by the presence of neurofibrillary tangles (NFTs), neuritic (senile) plaques (NPs) and granulovascular degeneration [3,32,45,50,51,66–69,78,85]. It has been suggested in the past few years that elements may be involved in the etiology and/or pathogenesis of several age-related diseases of the CNS [1,20–25, 27,30,31,35,42,55,61]. Our previous works have been shown that some trace elements are imbalanced in AD compared to the controls [6–9,11,19]. However, a role for Al in AD is an unproven and controversial hypothesis. It was postulated that Mg dietary deficience might increase the neurotoxicity of metals (e.g. Al) [61]. It has been previously shown that Al and P are antagonistic to each other [64]. Therefore the present study was initiated to determine Al, Mg and P concentrations in control and Alzheimer-diseased brain parts.

There is a worldwide recognition today that the accumulation of Al in the body plays a fundamental role in the pathogenesis of some clinical disorders identified in patients undergoing regular dialysis. Encephalopathy, anaemia and osteomalacic osteodistrophy are some clinical abnormalities associated to Al loading in human body [1,20,41,48,58,82]. Al administration to animals causes a neurotoxicity that is accompanied by neurofibrillary tangle formation [16,47,63,79]. AD is associated with the formation of tangles, and, while

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tangles do not appear in patients suffering from dialysis dementia, AD is associated with some of the same behavioral changes as dialysis dementia [27]. These similarities have suggested a possible common etiology for the two diseases. While both experimental Al and AD cause formation of neurofibrillary tangles, the morphology differs [63]. Furthermore, while it is agreed that Al does not accumulate in AD to the extent seen in dialysis dementia, whether or not Al accumulates at all in the brains of AD patients is still a question [15].

Mg is essential to all organisms [25,43]. It has electrochemical, catalytic and structural functions. It activates numerous enzymes (e.g aminopeptidase, glucokinase). It is especially important in activating the large class of phosphate transferases, and a number of decarboxylases, but it also activates acetyltransferases and one reductase. It plays a role in muscle function and in nerve stimulus. Probably it holds ribosomes together. It is moderately toxic when injected intravenously into mammals, otherwise relatively harmless [25].

P is vital constituent of nucleic acids and organic phosphates. It probably activates some enzymes. It is important for energy – rich molecules as ATP. P is structural atom of apatite in vertebrate bones and teeth. White phosphorus and phosphor-hydrogen are highly toxic to mammals, phosphates are relatively harmless [64].

Evidence of the clinical consequences related to an increased Al body burden and the parallel lack of knowledge on the metabolism of this element are facts justifying the present demand for analytical methods to determine very low levels of this metal in biological materials (e.g. in human brain) [16,17,28–31,53, 56,60,83]. In their present state, neither X-ray fluorescence nor flame atomic emission methods are sensitive enough to measure trace levels of Al in biological samples. Atomic absorption spectrometry (GF-AAS) is the technique of choice for clinical chemistry laboratories [14,37,46,52,71–74,88]. Instrumental conditions which should be stressed to obtain reliable results at very low levels of Al are: the use of the L'Vov platform-pyrolitic graphite combination, peak area measurement of the signal and the use of fast circuiting and read-out system able to follow the transient absorption signal without distortion [72,73]. At the same time any contamination risk along the different steps of analysis should be avoided. It has been demonstrated that cleanroom conditions allow a substantial improvement in the detection limits obtained for measuring low Al levels [74]. Although the detection limits are mostly adequate, throughput of the technique is rather slow. Therefore, the use of inductively coupled plasma atomic emission spectrometry (ICP-AES) has been investigated as an alternative method for routine control in renal patients [57,70,71,74]. Using the atomic line at 396 nm and a conventional cross flow nebulizer the detection limit was clearly poorer (by an order of magnitude) than those reached with the GF-AAS. This sensitivity limitation of conventional ICP-AES prompted the researchers to investigate more efficient alternatives of sample presentation to the plasma. Electrothermal vaporization (ETV) systems and direct sampling insertion devices (DSID) have been tried [74]. The observed comparative analytical performance of the three ICP-AES sample introduction techniques studied has been shown that the detection limits observed by ETV-ICP-AES or DSID-ICP-AES were not significantly better than those observed by ICP-AES conventional nebulization. Phosphorus has been shown to interfere seriously with the determination of Al by GF-AAS [88]. Human brain tissue is very rich in P and alkali and alkaline earth metals [4,5]. In an investigation of the effect of P species on the determination of Al in human brain, a substantial amount of molecular absorption was found. Potassium dichromate as chemical modifier helps to minimize the interference caused by comprehensive effect of P species and alkali and alkaline earth metals and can provide an optimal ashing temperature at which the P species are eliminated [88].

Instrumental neutron activation analysis (INAA) has been used for the non-destructive determinations of trace elements and main components of biological samples. A number of elements (e.g. Al, Na, K) could be measured after short-time irradiation via short-lived radionuclides. This has the advantage that radiation damage and warming of the biopsies are restricted to an acceptable degree so that the latter can subsequently be used for further clinical tests. When biological samples are irradiated with reactor neutrons the short-lived radionuclide ^{28}Al is produced from Al via ^{27}Al (n, α) ^{28}Al, whereas the same radionuclides are produced from P and Si. Some authors took into account the P interference, while others ignored it [28,29,31,36,39]. Thermal neutrons are eliminated to a large extent by irradiating the samples under a Cd or a BN-shield [7,36, 40,86]. The elimination of thermal neutrons reduces the presence of other induced activities by about two orders of magnitude and thus facilitates the determination of the ^{28}Al activity. Investigations have shown that INAA is a suitable method to determine Al in human brain [28,53,54,75,83]. In one study for multi-element analysis by thermal INAA, brain samples for

Table 1
Function and dry weight content of studied brain parts ($n = 3$)

Brain part	Function	Dry weight content (%)	
		Control group	AD group
Ammon's horn	autonom system	18.50	14.50
Cortex entorhinalis	autonom system	16.37	16.18
Cortex frontalis parasagittalis	cognitive	16.90	16.76
Cortex frontalis basalis	cognitive	16.71	15.63
Globus pallidus	movement (facilitation)	21.06	17.01

The relative standard deviation is $\pm$ 2%.

short radiations were digested with concentrated nitric acid in a teflon bomb and subsequently subjected to pre-irradiation chemical separation for the removal of sodium by hydrated antimony pentoxide [83]. In another work P levels were determined in the sample of brain using 14 MeV neutrons to correct for interference by P on Al determination. Since the P content of each individual brain sample was already known from the 14 MeV INAA procedure, the contribution to gross ^{28}Al from the (n_e, a) reaction on P was calculated and substracted to obtain the net ^{28}Al activity from aluminium [53].

One of our aims of this work was to investigate the suitability of ICP-AES to the determination of Al in human brain using ultrasonic nebulization. In our previous study we have worked out an INAA method for Al determination in human brain which can eliminate the interference reaction of P [7]. The silicon content of brain being very low it causes insignificant interference in analysis of brain tissue [7,53,83]. Mg and P occurs at μg/g level in human brain. Therefore there is no major analytical problem in the determination of these elements by ICP-AES (Mg and P) and by INAA (Mg). Our second goal was to examine possible Al, Mg and P alterations in the brain of AD patients.

2. Materials and methods

2.1. Sample preparation

Brain samples were obtained from Institute of Neuropathology, University of Munich, where samples were pathologically classified, and stored at $-70\,^{\circ}$C until required. Tissue samples were dissected from the brains about 24 hours after death. As the samples were received, they were lyophilized in a freeze – dryer for about 5–6 days until constant weight. Weights before and after the drying procedure were recorded and dried to wet weight ratios were determined for each sample (Table 1). Instruments used for collecting tissue, storing and transporting were of the same material in all cases and care was taken to avoid the contamination of samples during collection and treatment (ceramic-, Ti-tools, high-purity laboratory). All containers used at ICP-AES analysis were soaked in 1:1 nitric acid for at least 24 hours before thorough rinsing with large amounts of deionized water. All containers were tested for background Al, Mg and P levels and showed no detectable levels. Exceptions were polyethylene vials used for INAA: even a virgin polyethylene vial contains Al impurity. This problem could be eliminated by blank measurement and its correction.

The samples were collected from three control patients between 55–71 years of age (mean age: 61 years). All subjects taken into consideration were diseased for reasons not involving the nervous system. Three AD subjects (age range: 72–80 years, mean age: 77 years) were also followed. Longitudinal clinical studies of patients with dementia may give a definite diagnosis. The histological alterations can be grouped in two main categories: neuronal alterations (which involve NFTs and granulovascular degeneration) and extraneuronal changes (which are characterized by NPs). These findings proved pathognostic in AD, but they can also be found in to a lesser degree within the hippocampus in normal old age. It must be emphasized that among the pathologic markers not only qualitative, but also a quantitative factor (the number of alterations) must be taken into account [3,12,51,81].The pathological signs of AD were demonstrated both by biochemical and electronmicroscopic methods [12,45,51]. Five brain parts were investigated from both groups (Table 1).

The dried samples were analyzed directly by INAA. Two digestion methods were applied for the removal of organic substances for ICP-AES analysis. The first one was carried out in a high-pressure Parr-bomb (sample mass: 20–50 mg; digestion conditions: 2.5 ml 65% nitric acid (Suprapur, Merck), 150 °C; 2 h). In the second case microwave heating was used for sample dissolution (Milestone MLS 1200 Mega Microwave Instrument; maximum power: 1000 W; internal pres-

Table 2
Experimental conditions of ICP-AES method

RF generator	40 MHz (fee-running)
Power output	1300 W
Plasma view	axial
CETAC U 5000 AT + ultrasonic nebulizer	
AS 90 autosampler	sample flow rate: 2.0 ml/min
Plasma gas flow rate	15 l/min
Auxiliary gas flow rate	0.5 l/min
Aerosol carrier gas flow rate	0.8 l/min
UV detector (+ Li, K, Sr wavelengths in the visible range)	167–395 nm

sure up to 110 bar). Dried samples (20–50 mg) were transferred into TFM vessels and 2.5 cm^3 65% HNO_3 (Suprapur, Merck) plus 0.5 cm^3 30% H_2O_2 (Suprapur, Merck) were added as digesting solvents. The brain samples were digested at 250 W for 1 min, then after a cooling period of 1 min the digestion was continued at 250 W for 8 min, at 400 W for 5 min and at 650 W for 5 min. A digestion run included 6 vessels: 1 blank, 5 samples (including 1 biological standard reference material (SRM)). All digested samples were clear and colourless. After the bombs cooled to room temperature, the contents were diluted to 5 ml with high purity water (resistance: 18 MΩ/cm).

2.2. Applied techniques

The Al, Mg and P concentrations were determined by ICP-AES. The experimental conditions can be found in Table 2. A Perkin-Elmer Optima 3200DV instrument equipped with AS-90 autosampler and CETAC U5000 AT+nebulizer was applied for ICP-AES measurements. The digested samples were measured in 10-fold dilution. Ultrasonic nebulization was used to increase the sensitivity. The six most sensitive emission lines for the determination of Al are the 396, 394, 309, 308, 237 and 226 nm lines [13]. The 394 nm line was selected for the evaluation of Al. 279 nm, 280 nm and 285 nm lines were detected and the 285 nm line was selected for Mg analysis. For P analysis the 214 nm and 215 nm lines can be used. The 214 nm P line was choosen for P evaluation. Blanks and standards were prepared and run the same way as the samples. Three parallel measurements were carried out.

The Al and Mg contents were measured by INAA. The irradiations were performed in the Institute of Nuclear Techniques, University of Technology and Economics, Budapest. The swimming-pool type thermal reactor ($\Phi_{\mathrm{th,max}} = 2 \cdot 10^{12}$ n $cm^{-2}s^{-1}$, $P_{\mathrm{th,max}} =$ 100 kW) is operated with aluminium-clad UO_2 fuel element at 10% enrichment. The moderator and the coolant are light water. The reflector is a combination of graphite and water. Dried samples (20–50 mg) were placed in high-purity polyethylene (PE) vials and irradiated for 300 sec (Al) and 600 sec (Mg). The cooling time was between 90 and 100 s for Al analysis and 190–240 sec for Mg measurement, while the measuring time were 300 sec (Al) and 600 sec (Mg). The measuring system consists of a HPGe well-type detector (FWHM = 1.95 keV at 1333 keV) connected to a Canberra S100 multichannel analyzer. The γ-spectra were evaluated by the Sampo 90 program. Single comparator method was applied. Al-foils containing 0.1% Au (comparator) and Zn foils (flux monitor) were irradiated together with the samples.

Two major problems are associated with the determination of Al in biological tissues by INAA: spectral and primary interference reactions. The first is interference from the Compton scattered γ-rays and spectral lines from pair production events in the detector produced by internal high energy γ-rays emitted by ^{24}Na ($t_{1/2} = 15.02$ h), together with intense low energy bremsstrahlung radiation from the decay of ^{32}P ($t_{1/2}$ = 14.28 d). In order to address the first problem, short irradiation time has been selected. The β-rays from ^{32}P decay can be absorbed by a 0.5 mm copper plate placed between the sample and the detector. The second problem is the primary epithermal neutron (n_e) interference reactions: ^{31}P (n_e,α) ^{28}Al and ^{28}Si (n_e,p)^{28} Al, which produce the same analytical radionuclide used for determination of Al.

Determination of Al levels in human brain parts by INAA using reactor thermal neutrons (n_{th}) was performed utilizing the nuclear reaction ^{27}Al (n_{th},γ)^{28} Al. Radioactive ^{28}Al has a life-time of 2.3 min and β decay with accompanying emission of a single gamma ray at 1778 keV. Fortunately, the epithermal neutron reaction on Si was found to cause insignificant interference in analysis of brain tissue due to the very low Si content of the brain [53,83]. However, according to our experiments, the INAA determination of human

Table 3
Comparison of Parr-bomb (P) and microwave (M) digestion for the analysis of three elements in control human brain parts (ICP-AES; n = 3; μg/g dry weight)

Element	Cortex frontalis basalis		Ammon's horn	
	P	M	P	M
Al	2.2	1.8	1.4	1.5
Mg	651	639	693	718
P	13000	13300	13920	13740

The relative standard deviations is $\pm$ 2%.

brain Al levels is complicated by the interfering reaction of P. The epithermal neutrons which accompany the desired thermal neutron flux are responsible for this reaction. In our reactor the epithermal/thermal neutron flux ratio is 0.3. Therefore, it was necessary to work out a correction method to solve this problem (using Cd-shield) [7].

The 843.8 keV γ line of the ^{27}Mg ($t_{1/2}$ = 586 s) was used for the analysis of Mg (^{26}Mg(n_{th},γ) ^{27}Mg) which can interfere with the 846.9 keV γ-line of the ^{56}Mn($t_{1/2}$ = 2.58 h) but the software system can resolve the two peaks. The effect of primary interference reactions: ^{27}Al(n_e,p) ^{27}Mg and ^{30}Si(n_e,α) ^{27}Mg can be neglected (cannot be measured experimentally) because concentrations of Al and Si are very low in human brain samples [53,83].

3. Results

The total lipid content of the dried human brain samples accounts for ca 30% of white matter and 50% of gray matter [19]. This matrix requires the application of agressive digestion techniques. In our previous work we applied two closed – vessel digestion methods for the removal of organic substances: high pressure Parr-bomb and microwave-assisted digestion [5]. The decomposition efficiency of the two methods was compared (Table 3). Good agreement of the parallel digestions (the relative standard deviation (SD): 2%) showed adequate homogeneity of samples.

In the case of ICP-AES ultrasonic nebulizer was applied to get the appropriate sensitivity. This method increases the sample requirement (10 ml/sec). All the measurements were carried out after tenfold dilution of digested samples in order to decrease acidity. The calibration standard solutions were acidified according to the diluted samples. The selected wavelength for the Al evaluation according to standard measurements was the most intensive spectrum line at 394 nm. The interference of Ca emission lines (393 and 396 nm) is not a problem, provided that background correction is done on both sides of Al peak. Mg (285 nm) and P (214 nm) measurements did not pose any difficulties by ICP-AES method. Corresponding detection limits are 2 μg/l (Al and Mg) and 10 μg/l for P. After the analysis sequence samples and standards were remeasured. Our precision was approximately 10% for Al and 5% for Mg, while about 7% in case of P.

Al concentrations determined by INAA using our correction method compared with ICP-AES results for a few control human brain parts are shown in Table 4 [7]. In our earlier experiments the samples were analyzed only in PE vials by INAA, but now knowing the Al amount formed from 1 μg P, we could perform the correction of Al values. Since the P content of each indiviual brain sample is already known from ICP-AES measurements, the contribution of ^{28}Al from the (n_e,α) reaction on P can be calculated and substracted to obtain the true Al concentration from the total (measured) Al concentration [7]. The P content of the control brain samples varied only narrowly among the parts. Therefore we can use an average P content, 11500 μg/g, and in consequence of this an average Al concentration formed from P, 30.8 μg/g. The measured and corrected Al concentrations can be seen in Table 5. Comparing the corrected Al values determined by INAA with the analytical results of ICP-AES method, good agreement was found. The limit of quantitative determination is 1 μg/g dry weight for Al and 39 μg/g dry weight for Mg. The SD was 10% for Al and 5% for Mg.

In our earlier works and the present investigation it was found that regions corresponding to each other in both hemispheres show almost identical concentrations of essential trace elements [4,5]. Mg concentrations of left and right hemispheres for three control human brain parts can be seen in Table 6.

Results of ICP-AES and INAA measurements for control and AD subjects are summarized in Table 7. As the results of independent techniques did not show significant differences, the averages of Al and Mg concentration data were used for further calculation. Values given for brain parts are the mean $\pm$ SD of results for individuals. The t-test was applied for the three determined elements to check for significant difference between the control and the AD patients. The significancy must be handled carefully because of the low n value but the tendency (increasing or decreasing elemental concentrations in AD cases) can be established.

Table 7 clearly illustrates that the concentration of Al is consistently higher in AD specimens than in control human brain parts. The mean Al concentration in brain

Table 4
Al concentrations measured in PE vials and in Cd capsule by INAA and comparison with ICP-AES results in different control human brain parts (μg/g dry weight)

Brain part	$c_{P(ICP-AES)}$	$c_{totalAl(INAA)}$	$c_{Al(from\ P)}$	$c_{Al(true)}$	$c_{Al(ICP-AES)}$
Cortex entorinalis					
left	13170	35.4	35.3	< 0.1	< 0.1
right	11800	33.9	31.6	2.3	2.4
right	12850	35.9	34.4	1.5	1.1
Globus pallidus					
left	13270	36.9	35.6	1.3	1.5
right	11630	34.4	31.2	3.2	3.6

The relative standard deviation is $\pm$ 7% for P and 10% for Al.

Table 5
Applying our correction method for Al concentration data (AD) determined in PE vials by INAA and comparison with ICP-AES results (μg/g dry weight)

Brain part	c_{Al} without correction (INAA)	c_{Al} with correction (INAA)	c_{Al} (ICP-AES)
Ammon's horn	35.8	5.0	5.3
Cortex entorhinalis	39.9	9.1	8.9
Globus pallidus	34.4	3.6	4.0

The relative standard deviation is $\pm$ 10%.

parts ranged from 1.4–2.5 μg/g dry weight for control and from 3.5 to 10.2 μg/g dry weight for AD samples. Statistical analysis revealed significant difference between AD samples and controls at 95% confidence level.

The data in Table 7 show that the Mg content is significantly lower (95% confidence level) in three brain parts (cortex entorhinalis, Ammon's horn, globus pallidus) of AD patients than in control human brain parts. The mean Mg concentration in the investigated brain areas ranged from 628–680 μg/g dry weight for control and from 540–625 μg/g dry weight for AD subjects.

In the case of P the values of the AD patients were found significantly lower for the 5 studied brain parts (t (95), $p < 0.05$). The control mean concentration is in the range 12040–13190 μg/g dry weight, while for AD samples is between 10650 and 11000 μg/g dry weight.

Unfortunately, no biological certified or SRM exists for Al at the concentration levels typical of brain tissue. Accuracy can be evaluated using National Institute of Standards and Technology (NIST) Bovine Liver 1577 and 1577/a SRMs. The elemental composition of these reference materials and the human brain is similar. The data of these measurements can be seen in Table 8.

4. Discussion

Using dry/wet weight ratios from Table 1, our results can be converted and compared to literature values expressed on wet weight basis. The differences in dry weight content between AD and controls are significant indicating a slightly greater water content in some AD brain parts.

Both sample preparation methods have the same advantages: small risk of contamination and loss of trace elements, and they indicated successful decomposition of the organic matrix. Microwave heating yielded elemental values which are in good agreement with those obtained by a Parr-bomb digestion (Table 3). Microwave heating enabled us to decrease the digestion time by a factor of six. Another important advantage of the microwave digestion is the possibility of digesting blanks and standards parallelly with the samples.

Bovine Liver 1577 and 1577/a (NIST) SRMs were the best available to evaluate our methods because of their similar composition and trace element content to human brain. Our results are in good agreement with the certified Mg and P and information/literature values of Al (Table 8) [2,49,53].

As it may be seen from our data the ICP-AES method is currently one of the most powerful analytical methods for determination of Al, Mg and P in dissolved human brain samples. The method is almost insensitive to disturbing effects, resulting in a high detection power and a wide linear range. Mg and P measurements did not pose any difficulties by ICP-AES method, and by means of an ultrasonic disintegrator, the sensitivity could be increased sufficiently to quantify Al. The advantages of the method are the high speed of analysis and the good precision. The disadvantages of ICP-AES technique are: the dilution and the neces-

Table 6
Control Mg concentrations of right and left hemisphere (INAA; n = 3; μg/g dry weight)

Patient	Brain part	Hemisphere[a]		Mean value ± SD	
		left	Right		
1.		568	595	left:	678 ± 96
2.	Ammon's horn	718	693	right:	703 ± 114
3.		748	822		
1.		578	619	left:	588 ± 68
2.	Cortex frontalis parasagittalis	660	769	right:	642 ± 117
3.		525	538		
1.		651	639	left:	694 ± 65
2.	Cortex frontalis basalis	663	626	right:	652 ± 35
3.		769	692		

[a]The relative standard deviation is ± 10% for individual values.

Table 7
Al, Mg and P contents in five control and AD brain parts measured by ICP-AES and INAA methods (mean ± SD; μg/g dry weight)

Brain part	Control value			AD value		
	Al	Mg	P	Al	Mg	P
Ammon's horn	1.4 ± 0.6	680 ± 100	13190 ± 960	4.9 ± 3.0	557 ± 82	11000 ± 480
Cortex entorhinalis	1.5 ± 0.9	666 ± 106	12560 ± 900	10.2 ± 9	540 ± 55	10700 ± 500
Cortex frontalis parasagittalis	1.8 ± 0.6	606 ± 89	12040 ± 850	6.8 ± 4.3	625 ± 35	10860 ± 550
Cortex frontalis basalis	2.5 ± 0.7	673 ± 48	12500 ± 940	6.4 ± 2.9	623 ± 53	10650 ± 730
Globus pallidus	1.8 ± 0.7	628 ± 80	13000 ± 1000	3.5 ± 0.4	552 ± 48	10850 ± 860

Table 8
Al, Mg and P analysis in NIST SRMs and in one control human brain part measured by INAA and ICP-AES methods (n = 5; μg/g dry weight)

NIST SRM	Al		Mg		P	
	Literature values	Our value INAA[a] (with P correction)	Certified value	Our value	Certified value	Our value
Bovine Liver 1577	2.21 ± 0.15 (53)	2.3	604 ± 9	595 ± 10	–	–
Bovine Liver 1577/a	2.5 (2,53)	1.8	600 ± 15	600 ± 10	11100 ± 400	10520 ± 500
Cortex entorhinalis (control)	2.33 ± 0.16 (53)	2.0		656 ± 88		10800 ± 360

[a]The relative standard deviation is ± 10%.

sity of the ultrasonic nebulisation increases the sample requirement.

This work confirmed that INAA can be used as an accurate and precise method for human brain analysis when all the relevant parameters are accurately measured and controlled (single comparator method). Mg can be quantified interference free in human brain samples. Measurement of Al introduces methodological problems as a result of increased γ and β-background induced by major matrix elements. These interference can be eliminated by choosing suitable cooling and measuring time and using a copper plate placed between the sample and the detector. The contribution of P to the ^{28}Al activity could be determined in each case with two irradiations of the samples, once in a Cd capsule. So we could set up a correction formula for further Al determination and correction of our earlier Al values [7]. The sensitivity, as expressed by the detection and quantification concentrations according to Currie, is sufficient to deal with the actual concentrations [62].

From our results it can be concluded that both ICP-AES and INAA methods were adequate for the determination of Al (using our correction method) and Mg in human brain samples. The results of human brain samples obtained by different techniques were in good agreement (Tables 4 and 5). Time requirement of the two techniques is comparable if necessary digestion step for ICP-AES analysis is taken into account. Costs and equipment requirements (reactor), together with radiation hazards inevitably associated with the technique are major disadvantages of the INAA method, not nessesarily compensated by the simplicity and low contamination risk of direct solid analysis. For P determination ICP-AES is a well applicable technique.

A close relationship between the concentrations of essential trace elements in the right and left sides of the brain was also shown by other investigators (Ta-

ble 6) [23,44]. From the data of Table 4 it can be concluded that this statement is not true for nonessential Al.

The values obtained in this study (Table 7) are in fairly good agreement with the currently obtainable literature, although the data available regarding elemental levels in human brain is rather scarce [30,48,53,54, 75,83]. It can be established that Mg and P are nearly homogeneously distributed within the control human brain, while distribution of Al is heterogeneous. An enrichment of Al is observed in the cortex frontalis basalis. The lowest Al concentration is seen in Ammon's horn. The high interpersonal variability of Al is probably the result of the fact that in general the amount of Al present in diet and in drinking water (and consequently absorbed in the body) depends heavily on the geological characteristics of the area where the individual lives [56,60].

Al has been found in increased concentrations in the hippocampus and the cerebral cortex of AD subjects compared to controls [48,83]. Al imbalance has not been observed in another studies of senile dementia subjects and controls [30,53,75]. The possibility to produce memory and learning impairment by intracranial Al injection in different animal species, and the increased prevalence of AD cases in areas with a high Al level in drinking water support the assumed relevance of Al accumulation in the human brain for the dementia of the Alzheimer's type [56,60]. Recently several studies have been undertaken to investigate the effects of some chelators in AD [18]. Other investigations should be undertaken, to get more concrete knowledge about the role of chelators and their possible relevance concerning therapeutical aspects in this disease.

The reason for the differences in literature AD data are not clear. Perhaps differences exist in the occupational and environmental exposure to Al for the individuals studied. If an accumulation of brain Al is merely a secondary effect triggered by AD, the long-term availability of Al in the diet and environment may explain the differing data reported in AD brains. Alums (aluminium sulphates) are commonly used as coagulating agents in metropolitan water supplies. Although the Al in diet can come from many sources, alum-treated water make a substantial contribution to tissue levels of this element. This relationship has been postulated by others [59].

Sampling and sample handling procedures may also be involved, though means for control brain regions were similar in our study and in those of others [30, 53,75,83]. It has been suggested that the brain atrophy might explain elevated Al levels in AD and the sporadic high values found by other groups might be due to analysis of samples containing regions of localized cell death and atrophy [16,17].

A variety of techniques have been used to look at the brain Al of autopsied AD patients. There are several possible reasons for the discrepancies. One major problem affecting quantification by all technique is the lack of matrix-matched certified standards with a low Al concentration. In addition, aside from the different limitations associated with each technique (such as matrix effects and calibration), the sample preparation step is likely a major source of variation. Indeed, any elemental accumulation is unlikely to be uniform within the brain. Therefore, sample varying amounts and/or regions of brain tissues may result in contradictory results. Too small or too large area could correspond to a miss or a dilution, either of which could result in no detectable accumulation. Furthermore, the sources of contamination are numerous since sample preparation generally involves a number of steps, with the addition of a variety of chemicals, and is usually not carried out in cleanrooms. Finally, no blanks or controls are really available e.g. in the case of NPs since these are only present in AD brains.

Perhaps age-related alterations in neuronal membrane allow for selective accumulation in perikaya, where up to half of cortical Al has been found, or in nuclei, the deposition site described by others [17,59]. In addition to Al several studies have found silicon to be localized in the brain plaques of AD patients [65]. The observation that NPs contain aluminosilicate deposition within the central amyloid core has provided further impetus to the Al role. Although bulk data or when regions are compared do not support unambiguously the concept that Al has a role in AD, it is possible that major differences in its concentration are at the submicroscopic level in the disease, as suggested by the X-ray and inductively coupled plasma mass spectrometric studies [10,65]. Further investigation of the submicroscopic distribution of Al, its quantification, and its importance are warranted.

While current data would suggest the lack of a causative role, alterations in the brain caused by AD might increase the penetration of Al as well as other metals into the brain and lead to their contribution to such pathological features as NFs. It appears reasonable that some time during the progresse of the disease metal transport is affected so that more Al (or other metal) can get unintended places such as the brain. A number of investigators have noted changes in the

blood-brain barrier in line with this hypothesis [21]. The question can be asked why Al would be the only metal whose transport and passage through the blood-brain barrier is affected. Actually other metals have been implicated. A work that indicates no Al effect in AD patients did provide evidence that Hg was elevated in AD [84]. Both Al and Fe increase in ferritin during AD [33,34]. Such studies indicate that Al may not be unique, and that changed metal transport may affect other metals also. Perhaps the metal selected depends on environmental conditions that may frequently favour Al. Part of the reason for the controversy about the involvement of Al could be that metals other than Al may sometimes be encountered, and testing for Al will produce a negative result. It is assumed that a balance in trace elements is present in the brain, and perhaps an increase in Al levels over time takes place at the expense of other essential elements (e.g. Mg). At present the effects of accumulation or imbalance of trace elements in AD are not known.

It seems very important to discriminate between two types of Mg deficit: Mg deficiency and Mg depletion. In case of Mg deficiency, the disorder corresponds to insufficient Mg intake: it requires oral physiological Mg supplementation to cure the patient. In the case of Mg depletion, the disorder which induces Mg deficit is related to disregulation of control mechanism of Mg metabolism. Mg depletion requires more or less specific correction of its causal disregulation [25].

More recent studies do not support the view that Mg decreases in brain with age [26,42]. In literature it has not been found Mg imbalance in brain regions of AD patients compared to the control subjects [75,83]. The investigation of Mg status in AD patients disagrees with the hypothesis of Mg deficiency, but it stresses the possible role of some types of Mg depletion, which might be for instance due to alterations of albumin [24, 38]. It has been suggested that AD involves a defective transport process characterized by both an abnormally low Mg incorporation and an abnormally high Al incorporation in brain neurons [38]. The origin of this disturbance rests on an alteration of serum albumin, forming a species which has greater affinity for Al than for Mg, in contrast to the normal protein which binds Mg better than Al. The altered albumin crosses the blood-brain barrier more efficiently than the normal protein and competes with it in binding to brain neurons. Binding of altered albumin to the target neurons would both impede Mg uptake and facilitate Al uptake. Low level of Mg overexcites the brain's neurons and results in less coherence.

Mg plays an important role in the activity of Ca – Mg ATP-ase which helps to maintain the stability of cell membrane. In our previous study we have found significantly increased Ca concentrations in the investigated brain areas of AD patients [6]. This observation fits in with the theory concerning the interaction between Ca and Mg [24]. This Ca and Mg concentration changes may also affect metalloenzymes which are involved in DNA repair.

It was hypothetised that the Mg depletion by increasing Ca/Mg ratio in the CNS tissues, further accelerates the uptake of Al into the CNS, which promotes the neurodegenerative processes [61]. Al intoxication, combined with Ca-Mg deficiences, is not reversible by oral physiological Mg supplementation. Therefore it constitutes a type of experimental Mg depletion model [25].

It has been suggested that, since Al binds to both transferrin and ferritin, the Fe uptake system might be involved in Al absorption, and some evidence exists that Fe competes with Al for uptake [34,82]. These possibilities do not preclude via other, as yet unidentified system also. For example, there are no studies on the interaction between Al and Mg uptake.

The decline in P with AD may reflect a loss of myelin phospholipids over time. Al shows a significant negative correlation with P. It has been previously shown that high Al intake decreases P absorption and increases P excretion suggesting that they are antagonistic to each other [64]. The increase in Al we have observed in the brain might be at the expense of P, but this seems unlikely since their absolute concentrations are extremely different (Table 7). It is difficult to envision an alteration in P as a cause of AD. If anything, the change could be more likely to be a result of the disease processes.

Both the interanalytical method comparisons and results of biological SRM measurements underline the reliability of our data. In summary, it can be concluded that the analytical approach is adequate to goals. The concentration range for the investigated elements turned out to be similar to those reported in literature [30,53,75,80,83,88]. The main differences between control and AD brains (overall values) are shown by the ratio (R) of control and AD values ($R = 0.28$ for Al, 1.12 for Mg and 1.17 for P). Overall these variations are strongly related to the possibility of a multielemental involvement in AD. Alterations in mineral metabolism can lead to an accumulation or a reduction of other trace elements. Neurotoxic metals (such as Al) deposited in the brain parts of AD patients in combination with imbalanced essential element con-

centration (Mg and P) are likely to produce nerve cell damage via inhibition of ATP-ase, adenylate cyclase, neurotransmitters and neuroregulators. Only studies in larger number of patients will elucidate this question.

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