

# A Reexamination of Aluminum in Alzheimer's Disease: Analysis by Energy Dispersive X-ray Microprobe and Flameless Atomic Absorption Spectrophotometry

Roland W. Jacobs, Taihung Duong, Robert E. Jones, George A. Trapp and Arnold B. Scheibel

**ABSTRACT:** We have attempted to verify the presence of increased aluminum (Al) levels in Alzheimer's disease (AD) brains by energy dispersive X-ray microanalysis (EDX) and flameless atomic absorption spectrophotometry (AAS). Tissue from seven AD brains, mounted on carbon polymerized coverslips, were stained with Congo-red or treated immunohistochemically to allow optical localization of AD-associated lesions during EDX. Despite a demonstrated sensitivity of 20-25 ppm, we were unable to detect Al in either plaque cores or neurons containing neurofibrillary tangles. For AAS, wet weight samples (ranging from 48-144 mg) from six of the seven AD brains and four controls were selected from regions similar to those studied under EDX, i.e., Brodmann areas 9, 11, 28, 46, 47, and the hippocampus. The tissue surrounding each sample site was sectioned and stained for thioflavin S. Both controls and AD samples revealed similar levels of Al ranging from undetectable to 1.80 ng/mg wet wt. (mean AD:  $0.28 \pm 0.39$  (SD), control:  $0.54 \pm 0.58$  (SD)), independent of degree of histopathology or age of the case. We conclude that the combined strengths of these two techniques on similar tissue specimens demonstrate that abnormal Al levels are not required to produce the histologic findings of AD and that this element may not accumulate in the aging brain. It is unlikely, therefore, that Al is essential in the etiology of pathogenesis of plaques and tangles in AD. Al's role as a primary or secondarily associated event, when present, needs further delineation.

**RÉSUMÉ:** Réévaluation du rôle de l'aluminium dans la maladie d'Alzheimer: micro-analyse par rayons X à dispersion d'énergie et spectrophotométrie à absorption atomique sans flamme Nous avons tenté de vérifier la présence de niveaux augmentés d'aluminium dans le cerveau de patients atteints de la maladie d'Alzheimer (MA) au moyen de la micro-analyse par rayons X à dispersion d'énergie (EDX) et de la spectrophotométrie par absorption atomique sans flamme (AAS). Des tissus provenant de cerveaux atteints de la MA, montés sur des lamelles de carbone polymérisé, ont été colorés au rouge Congo ou traités par immunohistochimie afin de permettre une localisation optique des lésions associées à la MA au cours de l'EDX. Malgré une sensibilité de 20-25 ppm, nous avons été incapables de détecter l'Al au coeur des plaques ou dans les neurones contenant des amas neurofibrillaires. Pour l'AAS, le poids brut des échantillons (intervalle de 48 à 144 mg) provenant des cerveaux de 6 des 7 patients atteints de MA et de 4 contrôles ont été choisis dans des régions similaires par rapport à ceux qui ont été étudiés par EDX, i.e., dans les zones 9, 11, 28, 46, 47 de Brodmann et dans l'hippocampe. Le tissu autour de chaque site d'échantillonnage a été coupé et coloré pour la thioflavine S. Dans les échantillons provenant des contrôles et des patients atteints de MA, nous avons détecté des niveaux d'Al allant de non détectables à 1.80 ng/mg du poids brut (moyenne de  $0.28 \pm 0.39$  (DS) pour la MA,  $0.54 \pm 0.58$  (DS) pour les contrôles), indépendamment du degré d'atteinte histopathologique ou de l'âge des cas. Nous concluons que la puissance combinée de ces deux techniques appliquées à des échantillons de tissus semblables démontre que des niveaux anormaux d'Al ne sont pas nécessaires pour produire les manifestations histologiques de la MA et que cet élément ne s'accumule peut-être pas dans le cerveau avec l'âge. Il est donc peu probable que l'Al soit essentiel à l'étiologie ou à la pathogénèse des plaques et des amas neurofibrillaires dans la MA. Le rôle de l'Al comme événement primaire ou associé secondairement, quand il est présent, doit être mieux défini.

*Can. J. Neurol. Sci. 1989; 16: 498-503*

For nearly two decades the presence and role of aluminum (Al) in Alzheimer's disease (AD) has been a topic of debate. The possibility of Al as a causative agent has spurred consider-

able research effort and public concern. In a review of the literature, the total number of studies determining the actual presence of this element in AD is relatively few and often contradictory.<sup>1-16</sup>

From the Departments of Psychiatry (RWJ and ABS), Anatomy (TD and ABS) and Earth and Space Sciences (REJ) and the Brain Research Institute (RMJ and ABS), UCLA; the Valley Hospital Medical Center, Van Nuys, CA (RWJ); and the VA Medical Center and Dept. of Psychiatry, Univ. of Texas Southwestern Med. School, Dallas (GAT)

Reprint requests to: Roland W. Jacobs, M.D., c/o Arnold B. Scheibel, M.D., Department of Anatomy, UCLA Center for Health Sciences, Los Angeles, CA, U.S.A. 90024

The induction of neuronal cytoplasmic "tangles" by Al in rabbits<sup>17,18</sup> and reports of increases in brain Al in AD in the early 1970's<sup>3,4</sup> initiated the current debate. These early findings were countered by subsequent studies which argued that: 1) brain Al content increases as a function of age irrespective of diagnosis; 2) the impact of atrophy and localized cell death were not considered in the AD group;<sup>16</sup> 3) the ultrastructure of induced rabbit neurofibrillary tangles (NFT) is significantly different from those of AD<sup>18,19</sup> and they are formed from antigenically distinct cytoplasmic substrates.<sup>20</sup> Further controversy ensued with the demonstrated toxicity of Al in dialysis dementia<sup>21,22</sup> and reports of Al in NFT-bearing hippocampal neurons in the amyotrophic lateral sclerosis-Parkinsonism dementia complex (ALS/PD) of the Pacific basin rim.<sup>23-25</sup> Although the presence of Al in these disorders could indicate a common pathogenic process, the presence of early motor impairment in animal NFT-induction models, dialysis dementia, and ALS/PD<sup>17,21,26</sup> argues against a pathogenic similarity with AD. By contrast, AD is characterized clinically and neuropathologically by relative sparing of motor systems (e.g. primary motor cortex, basal ganglia, and anterior horn motoneurons) until the latter stages.<sup>27,28</sup>

In recent years, sophisticated probing techniques have been employed to counter the claim that gross Al brain content is solely a function of age. Perl and Brody presented evidence of modest but significant Al levels in hippocampal NFT-bearing neurons by energy dispersive X-ray microanalysis (EDX).<sup>11-13</sup> Crapper et al demonstrated Al in chromatin<sup>29</sup> and faulted prior controversial brain Al studies<sup>6,9</sup> on selection of excessively large sample sizes, improperly diagnosed AD cases, and analytical methodology.<sup>30</sup> Candy et al, using EDX and nuclear magnetic resonance, found aluminosilicates in amyloid plaque cores in AD brain tissue.<sup>1,2</sup> Using similar techniques, however, Selkoe et al<sup>15</sup> and Moretz et al<sup>10</sup> were unable to demonstrate a significant presence of Al in the AD-associated lesions tested.

With access to an EDX, the Cameca Camebax, and a Varian AA-275 atomic absorption spectrophotometer (AAS), we set out to detect the presence of Al in unfixed, ultrafrozen AD diagnosed brains and in neurologically unimpaired controls. The protocol for the AAS includes a recently developed matrix modifier that significantly enhanced sensitivity and accuracy.<sup>31</sup> Similarly, with the technique described below for the EDX, we were able to improve its ability to detect trace elements. Thus, by combining the strength of site specificity of the EDX with the sensitivity of AAS between comparable brain regions, we demonstrated no increased presence of Al in AD. Our earlier results with EDX indicated the same, revealing a total of only one Al positive probe in 243 probes of 146 NFT, one in 667 probes of 282 nuclei, and one in 194 probes of 63 senile plaque-cores.<sup>32</sup>

## MATERIALS AND METHODS

Freshly frozen ( $-70^{\circ}\text{C}$ ; mean autolysis time of 13 hrs) human brain specimens from seven clinically diagnosed late stage *probable* AD cases (mean 80 yrs; range 68-102) and four controls (mean 66 yrs; range 58-78) were obtained from the National Neurological Research Bank, VA Wadsworth Medical Center. The AD cases were confirmed neuropathologically. Medical records were reviewed to insure that the controls were neurologically intact and maintained an unimpaired mental status until time of death.

## EDX Analysis

Tissue blocks from the frontal cortex (Brodmann areas 8, 11, 46, and 47) and temporal lobe (area 28), including the hippocampus, were fixed by immersion in 4% buffered paraformaldehyde at  $4^{\circ}\text{C}$  for 24-48 hours and rinsed in 0.12 M Millonig's phosphate buffer and 30% sucrose (pH 7.4) with gentle agitation for an additional 24 hours. The tissue was cut at  $40\ \mu\text{m}$  on a cryostat and collected in 0.1 M Tris buffer (pH 7.4). Alternative sections were stained with Congo red/toluidine blue and Bielchowski's reduced silver technique to identify selectively the plaques and tangles characteristic of AD. In addition, we introduced into the sequence a novel immunocytochemical label, amyloid P-component (AP-C), as recently described by Scheibel et al.<sup>33</sup> The advantage of this stain is its strong demonstration of senile plaques and NFT under standard light microscopy. During EDX analysis, both AP-C and Congo red were free from artifact and did not significantly alter the elemental profile as found with Bielchowski and other silver-containing, staining techniques. The prepared sections were mounted on glass slides or carbon polymerized plastic slides (Thermanox tissue culture cover slips: 5410, Lab-Tek Division, Miles Laboratories, Inc., Naperville, IL 60540).

We employed a Cameca Camebax scanning electron microscope (SEM) and electron microprobe in tandem with a Kevex 8000 energy detector (energy dispersive spectroscopy) with a standard beryllium window and a "take-off" angle of 40 degrees. The Cameca allows for simultaneous optical visualization of the tissue sample with a 400x binocular transmitted incandescent light microscope with polarizing capability.

Mounted sections, lightly carbon-coated to avoid electron charging, were placed within the Cameca's vacuum chamber and viewed by optical microscopy. Specific sites on identified neurons, plaques, and tangles were then selected under SEM for EDX. To ensure that "ghost cells" were not included in the sampling, only neurons with an identifiable nucleus were chosen. The microprobe data were obtained by narrowing an electron beam of specific current and voltage over the probe site for a specified length of time (acquisition time) during which X-ray counts were acquired.

Microprobe sensitivity was determined by preparing serial standard dilutions of Al (from reagent grade  $\text{AlCl}_3$ ) and placing a known quantity over a weighed, predetermined area of a  $40\ \mu\text{m}$  thick cortical "control" tissue section mounted on a plastic slide. Sensitivity in parts per million (ppm) was obtained by determining the least amount of Al required to illicit consistently an unequivocal peak (Figure 1). In addition, homogenized pine needles from the National Bureau of Standards (standard reference material 1575) were microprobed (Figure 2a). The certified value of Al in the standard reference material is 545 ppm. Al peaks as determined by the various microprobe parameters utilized during this study are shown in Figure 1.

Initially, we began by utilizing the EDX technique as described by prior investigators:<sup>2,11,12</sup>  $1\ \mu\text{m}$  diameter probe site at 10-15 KeV and 1.0 nanoamperes (nA) for 100-300 sec. A demonstrated sensitivity of approximately 400 ppm was achieved. Sensitivity was enhanced, without significant tissue damage to 50 ppm by increasing the current to 60 nA and prolonging the acquisition time to 900 sec. Most of these probes were performed on glass mounted tissue. As a result, minimal to

modest sized peaks of silicon were obtained. Under plastic slides nearly all probes were negative for silicon.

Our final parameter (5 KeV, 40 nA, 400 sec) achieved a sensitivity of 20-25 ppm. The lower voltage allowed the energy detector to focus on low energy X-rays, including Al, by blocking out a significant amount of extraneous, competing signals. Thus, when present, Al was proportionately stronger and rose more quickly above background noise. Sampling by a  $6.25 \mu\text{m}^2$ /probe site was selected to search for Al's presence adequately in identified structures without extending into adjacent locations. In addition, the risk of possible elemental migra-

tion during data acquisition was avoided. One probe was performed per structure identified, i.e., NFT, nuclei of NFT-bearing neurons, and senile plaque cores. All probes were performed on tissue mounted on plastic slides.

**AAS Analysis**

Freshly frozen grey matter samples, weighing 48-144 mg wet wt., were taken from Brodmann areas 8, 11, 28, 45, 46, and hippocampus from six of the original seven AD specimens and from all four controls. Extreme care was exercised to minimize possible Al contamination during handling of the tissue, includ-

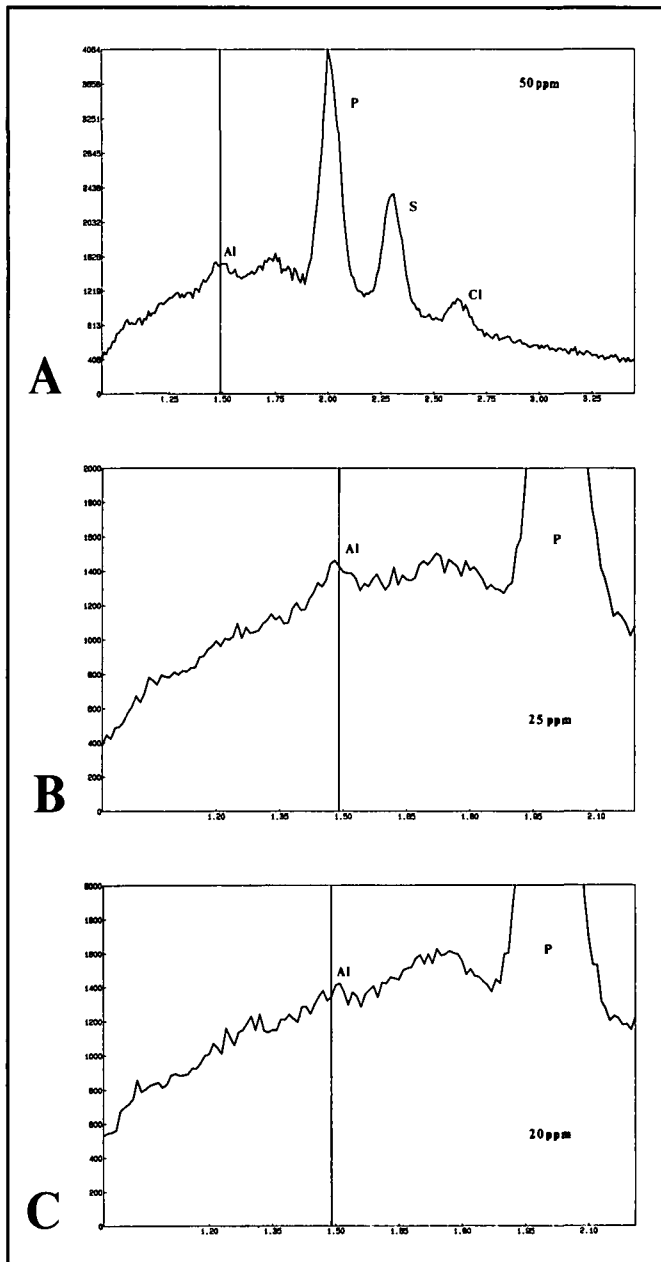


Figure 1 — Sensitivity determination by serial dilutions of  $\text{AlCl}_3$  on control tissue using parameters: 5 KeV, 40 nA, 400 sec. A. Equivalent of 50 ppm Al. Elemental profile of human tissue with a strong Al peak. B. 25 ppm. Computer enhanced image revealing significant Al. C. 20 ppm. Marginally significant Al peak. Note the suggestion of endogeneous magnesium at 1.25 KeV.

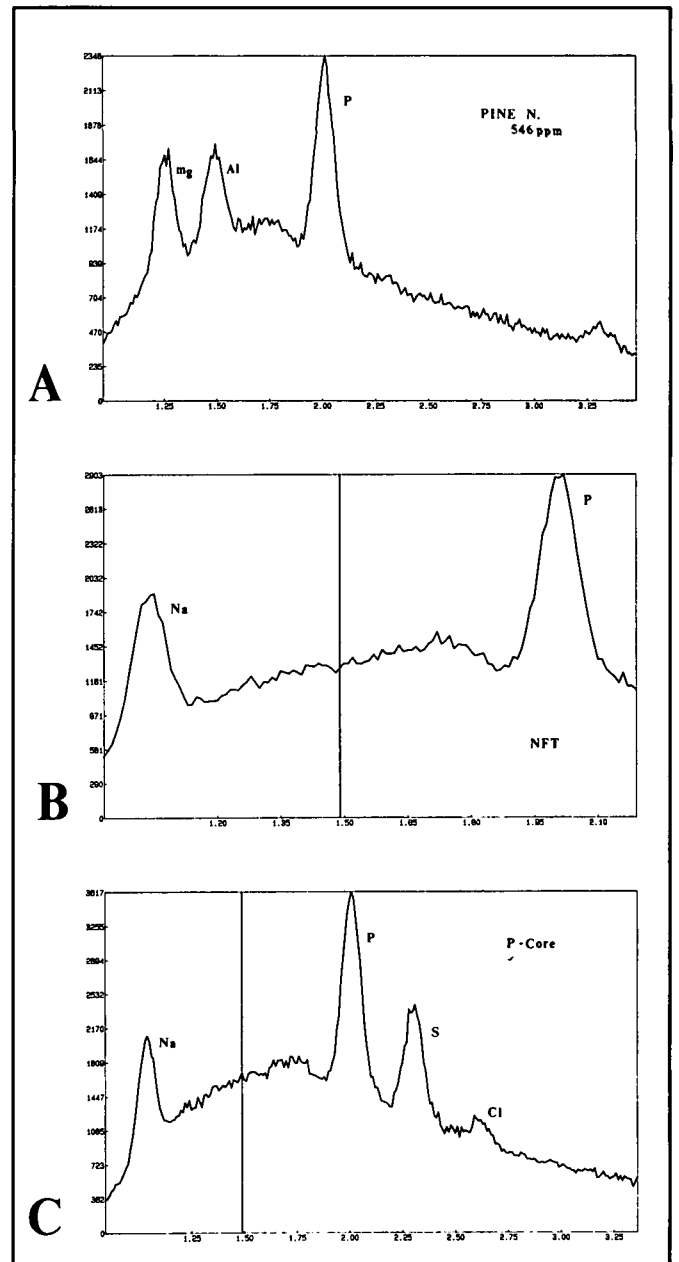


Figure 2 — Elemental profiles generated at 20 - 25 ppm sensitivity. A.) National Bureau of Standards reference material 1575 (pine needles) with an Al peak at 546 ppm. B.) Computer enhanced profile of NFT from AD neocortex. Line indicates where Al should be; no peak noted. C.) Senile plaque core from AD neocortex. No Al signal above background noise.

ing use of non-Al containing utensils, storage of deionized distilled water in nalgene containers, observing precautions against dust contamination, and washing of quartzware and plasticware in 6N HNO<sub>3</sub> and 0.01M EDTA solution. All solutions were stored in teflon bottles and reagents were either of analytical reagent grade or purified by a technique appropriate for that solution.<sup>31</sup> Nitric acid was redistilled from quartz. Sulfuric and perchloric acids were trace metal grade purchased from GFS Chemicals, Columbus, Ohio.

The remaining tissue blocks from which the various samples were drawn were fixed in 10% buffered formalin. Selected 40 µm sections were stained and analyzed with thioflavin S for density of plaques and NFT. Each AD sample was then categorized, based on surrounding histopathology, as "light", "moderate", or "heavy" (Figure 3). All control samples were similarly analyzed and found to be free of pathology. This index allowed us to compare Al levels not only across diagnostic categories but by intensity of pathology.

Each sample was digested in a quartz test tube containing 250 µl of a mixture of HNO<sub>3</sub>/HClO<sub>4</sub>/H<sub>2</sub>SO<sub>4</sub> (10/0.5/0.25). Temperature was slowly raised to 170° C to avoid foaming and to remove HClO<sub>4</sub>. The digest was diluted with 200 µl of a matrix modifier composed of 5.0M NH<sub>4</sub>NO<sub>3</sub>, 0.2M Ca(NO<sub>3</sub>)<sub>2</sub>,

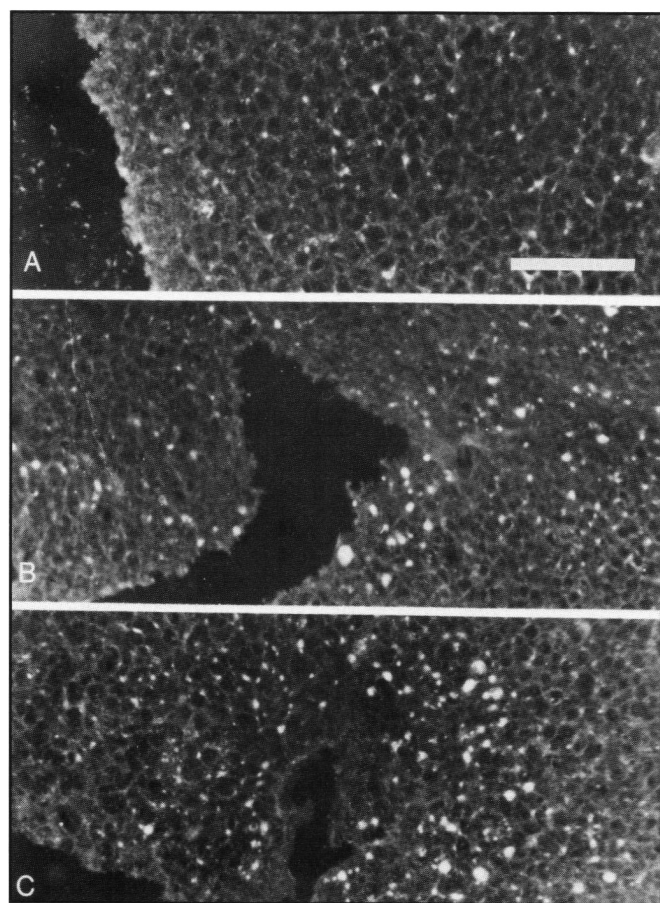


Figure 3 — Fluorescence microscopy of thioflavin-S stained AD neocortical tissue. Bright spots are plaque cores and/or NFT. Calibration bar equals approximately 1 mm. A. Light pathology: minimal plaques and tangles. B. Moderate pathology. C. Heavy pathology.

0.3M H<sub>3</sub>PO<sub>4</sub>, and LiNO<sub>3</sub>.<sup>31</sup> This matrix modifier has been shown to obviate the need for standard additions methodology.

Al was measured by atomic absorption spectrophotometry at 309.3 nm with a Varian AA-275 equipped for flameless technique with carbon rod atomizer CRA-90. The instrument was operated in the absorbance peak height mode and output peaks were recorded by a Varian 9176 strip chart recorder. Deuterium background correction was used. Sample size was 2 µl. Purge gas was argon and small amounts of hydrogen were added during the ash and atomize cycles. The dry cycle was 120° C X 40 sec, ash was 1500° C X 30 sec, and atomization was at 2500° C. The acid digestion blank was 5.8 ng Al ± 1.8 ng and was subtracted from experimental values. The sensitivity was 10 ng Al per tissue sample or two times the blank. Recovery from acid digests was 95-105%; analysis of NBS SRM 1575 (pine needles) yielded 95.8% ± 7.8% of expected value. Details of analysis of tissue for Al will be published elsewhere.

## RESULTS

Brain specimens from severely demented AD patients and normal controls were analyzed for the presence of Al by two sensitive and complementary techniques. Energy dispersive X-ray microanalysis with an Al determination as sensitive as 20-25 ppm was employed in site specific analysis of the neuron and senile plaque. Flameless atomic absorption spectrophotometry of histopathologically characterized, low weight samples resulted in detection levels as sensitive as 10 ng Al per tissue sample or two times the blank.

No Al was detected by EDX (sensitivity: 20-25 ppm) in 105 NFT, 70 nuclei, and 56 senile plaque cores sampled from seven AD brain specimens mounted on plastic slides, representing Brodmann areas 8, 11, 28, 46, 47, and the hippocampus (Figures 2b, 2c). Minimal levels of silicon were occasionally found. With the 50 ppm sensitivity technique, probes of 45 NFT, 58 nuclei, and 14 plaques cores were also negative for Al. AAS failed to find any elevation of Al in samples taken from these areas in the six analyzed AD brains as compared to controls (Table 1). There was no correlation between density of plaques and/or tangles and Al levels (Table 2). Similarly, no correlation was demonstrated between brain Al and age (Table 1).

Table 1: AAS: Sample Comparison of AD to Control  
Al amounts in ng/mg wet weight ± SD

Dx	Case #	Age	Sex	# of samples	Mean Al
AD	1	102	F	8	0.40 ± 0.56
AD	2	74	F	7	0.32 ± 0.32
AD	3	81	F	11	0.15 ± 0.22
AD	4	68	M	8	0.16 ± 0.25
AD	5	80	F	4	0.12 ± 0.15
AD	6	75	M	10	0.25 ± 0.34
TOTALS				48	0.28 ± 0.39
C	1	58	M	9	0.45 ± 0.52
C	2†	61	M	2	0.68 ± 0.75
C	3	71	M	6	0.44 ± 0.68
C	4	74	M	5	0.75 ± 0.66
TOTALS				22	0.54 ± 0.58

†Occasional plaque cores were present in Area 28. Remaining samples exhibited no pathology.

**Table 2: AAS: Sample Comparison by Degree of Pathology**  
Al amounts in ng/mg wet weight  $\pm$  SD

DEGREE OF PLAQUE & TANGLE FORMATION				
Case #		Light [range: 0-1.76]	Moderate [range: 0.004-1.11]	Heavy [range: 0-0.74]
AD 1	(7)	0.43 $\pm$ 0.60		(1*) 0.22
AD 2	(7)	0.32 $\pm$ 0.32		
AD 3	(6)	0.18 $\pm$ 0.28	(4) 0.09 $\pm$ 0.15	(1*) 0.24
AD 4			(5) 0.09 $\pm$ 0.12	(3) 0.29 $\pm$ 0.39
AD 5			(1) 0.03	(3) 0.14 $\pm$ 0.17
AD 6			(1) 1.11	(9*) 0.15 $\pm$ 0.17
TOTALS	(20)	0.32 $\pm$ 0.42	(11) 0.18 $\pm$ 0.33	(11) 0.18 $\pm$ 0.33

KEY: ( ) denotes number of samples analyzed from each category  
\*Includes hippocampus

## DISCUSSION

Our data fail to demonstrate the presence of elevated amounts of Al in AD brain tissue. Careful in situ analysis of neurons and senile plaque cores by a site-specified technique has not supported prior findings.<sup>1,2,11-13</sup> Tandem investigation by a sensitive bulk analysis technique has also failed to support the literature.<sup>3,4,30</sup> There was also no tendency towards increased brain Al due to aging in our sample of ten with an age range of 58 to 102 yrs.

One possible explanation for these unexpected results is that brain Al may vary among AD patients and that we obtained seven that did not have abnormal amounts. We are led by our current findings to conclude that Al may not play an etiologic or pathogenic role in the formation of the characteristic "plaques and tangles" of Alzheimer's disease and, when present, may only be an associated phenomenon.

## ACKNOWLEDGEMENTS

Tissue for this research was obtained from W.W. Tourtellotte M.D., Ph.D., director of the National Neurological Research Bank, VA Wadsworth Medical Center, Los Angeles, CA, U.S.A. 90073, which is sponsored by NINCDS/NIMH, NMSS, HD Foundation, and Veterans Administration. Sincere thanks to Iris Rosario R.N. and Randel Thomsen. Special thanks to Bob Jacobs for his helpful suggestions in preparation of the manuscript and to Enesto Pommier for superior technical assistance. Personal support for Roland Jacobs was provided by Valley Hospital Medical Center. Investigational support provided, in part, by the Eleanor Leslie Fund.

## REFERENCES

- Candy JM, Edwardson JA, Klinowski J, et al. Co-localization of aluminium and silicon in senile plaques: Implications for the neurochemical pathology of Alzheimer's disease. *In*: Traber J, Gispens WH, eds. *Senile Dementia of the Alzheimer Type*. Berlin: Springer-Verlag, 1985: 183-197.
- Candy JM, Klinowski J, Perry RH, et al. Aluminosilicates and senile plaque formation in Alzheimer's disease. *Lancet* 1986; 1: 354-356.
- Crapper DR, Krishnan SS, Dalton AJ. Brain aluminum distribution in Alzheimer's disease and experimental neurofibrillary degeneration. *Science* 1973; 180: 511-513.
- Crapper DR, Krishnan SS, Quittkat S. Aluminum, neurofibrillary degeneration and Alzheimer's disease. *Brain* 1976; 99: 67-80.

- Duckett S, Galle P. Mise en évidence de l'aluminium dans les plaques de la maladie d'Alzheimer: étude à la microsonde de Castaing. *C R Seances Acad Sci [D]* 1976; 282: 393-395.
- Markesbery WR, Ehmann WD, Hossain TIM, et al. Instrumental neutron activation analysis of brain aluminum in Alzheimer disease and aging. *Ann Neurol* 1981; 10: 511-516.
- Masters CL, Multhaup G, Simms G, et al. Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessel. *EMBO J* 1985; 4: 2757-2763.
- McDermott JR, Smith AI, Iqbal K, et al. Aluminum and Alzheimer's disease. *Lancet* 1977; 2: 710-711.
- McDermott JR, Smith AI, Iqbal K, et al. Brain aluminum in aging and Alzheimer disease. *Neurology* 1979; 29: 809-814.
- Moretz RC, Iqbal K, Wisniewski HM. Microanalysis of Alzheimer disease NFT and plaques. *Envir Geochem Health* (in press).
- Perl DP, Brody A. Alzheimer's disease: X-ray spectrometric evidence of aluminum accumulation in neurofibrillary tangle-bearing neurons. *Science* 1980; 208: 297-299.
- Perl DP, Pendlebury WW. Aluminum accumulation in neurofibrillary tangle-bearing neurons of senile dementia, Alzheimer's type (SDAT)-detection by intraneuronal X-ray spectrometry studies of unstained tissue sections. *J Neuropath Exp Neurol* 1984; 43: 349.
- Perl DP, Good PF. Laser Microprobe Mass (LAMMA) Analysis evidence that aluminum (Al) selectively accumulates in the neurofibrillary tangle (NFT). *J Neuropath Exp Neurol* 1988; 47: 318.
- Iqbal K, Alauddin M. Trace metal analysis of plaques and tangles in Alzheimer's disease. *Alzheimer Dis Assoc Disord* 1988; 2: 316.
- Stern AJ, Perl DP, Munoz-Garcia D, et al. Investigation of silicon and aluminum content in isolated senile plaque cores by laser microprobe mass analysis (LAMMA). *J Neuropath Exp Neurol* 1987; 45: 361.
- Trapp GA, Miner GD, Zimmerman RL. Aluminum levels in brain in Alzheimer's disease. *Biol Psychiatry* 1978; 13: 709-718.
- Klatzo I, Wisniewski H, Streicher E. Experimental production of neurofibrillary degeneration: 1. Light microscopic observation. *J Neuropathol Exp Neurol* 1965; 24: 187-199.
- Terry RD, Pena C. Experimental production of neurofibrillary degeneration: 2. Electron microscopy, phosphatase histochemistry and electron probe analysis. *J Neuropathol Exp Neurol* 1965; 24: 200-210.
- Terry RD. The fine structure of neurofibrillary tangles in Alzheimer's disease. *J Neuropathol Exp Neurol* 1963; 22: 629-642.
- Ihara Y, Abraham C, Selkoe DJ. Antibodies to paired helical filaments in Alzheimer's disease do not recognize normal brain proteins. *Nature* 1983; 304: 727-730.
- Alfrey AC, LeGendre G, Kaehny WD. The dialysis encephalopathy syndrome. *N Engl J Med* 1976; 294: 184-188.
- Arief AI, Cooper JD, Armstrong D, et al. Dementia, renal failure, and brain aluminum. *Ann Intern Med* 1979; 90: 741-747.
- Perl DP, Gajdusek DC, Garruto RM, et al. Intraneuronal aluminum accumulation in amyotrophic lateral sclerosis and Parkinsonism dementia of Guam. *Science* 1982; 217: 1053.
- Perl DP, Munoz-Garcia D, Good P, et al. Laser Microprobe Mass Analyzer (LAMMA) — A new approach to the study of the association of aluminum and neurofibrillary tangle formation. *In*: Fisher F, Hanin I, Lachman C, eds. *Alzheimer's and Parkinson's Diseases: Strategies for Research and Development*. New York: Plenum Press, 1986: 241-248.
- Garruto RM, Fukatsu R, Yanagihara R, et al. Imaging of calcium and aluminum in neurofibrillary tangle-bearing neurons in parkinsonism-dementia of Guam. *Proc Natl Acad Sci* 1984; 81: 1875-1879.
- Garruto RM, Yase Y. Neurodegenerative disorders of the western Pacific: The search for mechanisms of pathogenesis. *TINS* 1986; 9: 368-374.

27. Cummings JL, Benson DF. Cortical dementias: Alzheimer and Pick diseases. *In: Dementia: A Clinical Approach*. Boston: Butterworths, 1983: 35-72.
28. Katzman R. Alzheimer's disease. *N Engl J Med* 1986; 314: 964-973.
29. Crapper DR, Quittkat S, Krishnan SS, et al. Intranuclear aluminum content in Alzheimer's disease, dialysis encephalopathy and experimental aluminum encephalopathy. *Acta Neuropathol (Berl)* 1980; 50: 19-24.
30. Krishnan SS, Harrison JE, Crapper McLachlan DR. Origin and resolution of the aluminum controversy concerning Alzheimer's neurofibrillary degeneration. *Bio Trace Elem Res* 1987; 13: 35-42.
31. Trapp GA. Elimination of chloride interference in electrothermal atomic absorption analysis of serum aluminum. *Anal Biochem* (in press).
32. Jacobs RW, Duong T, Scheibel AB. A reexamination of aluminum in Alzheimer's disease. *Soc Neurosci Abstr* 1988; 14: 1083.
33. Scheibel AB, Pommier E, Duong T. Immunodetection of human serum amyloid P-component in Alzheimer's disease. *Soc Neurosci Abstr* 1987; 13: 1152.