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Aluminum Metabolism in Health and Disease

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ALUMINUM METABOLISM IN HEALTH AND DISEASE

by

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VITA

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(1) Contamination-free serum samples for trace analyses, January, 1978; and

(2) Determination of aluminum in biological samples by atomic absorption spectrophotometry with a graphite furnace, September, 1978.

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CHAPTER I

INTRODUCTION

This dissertation is concerned with the establishment of an accurate and clinically useful method for the determination of aluminum in biological samples, and the application of the method to the study of the element's metabolism in humans. The problem is of interest in that it is claimed that aluminum is a factor in the progressive encephalopathy seen in some patients on maintenance hemodialysis. Also, there is a need to establish the role of aluminum-containing antacids in phosphate utilization.

Because of its ubiquitous distribution, aluminum enters people's lives daily in many forms from a variety of sources (1). In most cases this is adventitious, but in a few instances its introduction is for specific purposes. The most common route of designed aluminum intake in man occurs through the use of antacids (2), but the metal has generally been considered to be non-essential, non-toxic, and not absorbed through the gut (3). Small amounts are present in many foodstuffs.

A procedure for the analysis of aluminum in biological specimens, which requires no sample preparation for serum and urine, is sensitive to the appropriate levels, and is relatively simple and rapid to perform, is described, using graphite furnace atomic absorption spectrophotometry. The samples are pipetted to the interior of a graphite tube, where they are sequentially dried, charred, and atomized. Precautions for sample handling are discussed, and instrument settings are defined. Statistical analyses of the precision and accuracy of the method are

presented, as are analyses of the effects of salts, protein content of serum, and specific gravity of urine. Reference values for serum and urine are established for persons not consuming aluminum-containing antacids.

Metabolic balances are presented to evaluate the fate of ingested aluminum under normal dietary conditions, and while supplementary aluminum-containing antacids are given. The aluminum content of the diets, drinking water, medications, and complete urine and stool collections, as well as the plasma at various intervals, is included for several patients, during both control periods and antacid supplementation.

In the special case of chronic renal failure patients maintained on hemodialysis, large amounts of aluminum-containing compounds are consumed in an attempt to limit the absorption and prevent the accumulation of phosphate (4). Serum aluminum levels in these patients, and the effects of hemodialysis and other allied treatments on these levels, are reported. Recent work suggests that aluminum may accumulate in toxic amounts in the brain (5), and it is highly suspect in the etiology of "dialysis dementia."

CHAPTER II

REVIEW OF RELATED LITERATURE

A. Aluminum and its Determination.

In spite of the fact that aluminum is the most abundant metallic element in natural waters and the third most abundant element in the earth's crust, biological systems have evolved containing only trace levels of this metal. There is no evidence that aluminum has an essential biological function, or is required for life (6). This may be related to its abundance, in that, a deficiency, if possible, would be extremely rare. Two short reviews of aluminum's role in human nutrition have appeared (7, 8). Sorenson et al. (1) have published an extensive survey of 818 references covering the biology, toxicology, and uses of aluminum.

Many analytical methods have been used to measure aluminum. The procedures include: colorimetry, utilizing aluminon (9), Eriochrome Cyanine R (10), Alizarin Red S (11), and hematoxylin (12); fluorimetry, utilizing morin (13, 14) and oxine sulfonic acid (15); titrimetry (16, 17); emission spectrography (18, 19); paper chromatography (20); ion-specific electrodes (21, 22); neutron activation analysis (23-26); x-ray fluorescence (27); polarography (28); gas chromatography (29, 30); and several types of atomic absorption and emission. This last category includes: the carbon cup (31); flame atomic emission (32, 33); atomic emission with a graphite furnace (34); flame atomic absorption (35); and atomic absorption with a graphite furnace (36-39). Many of these methods have been applied to biological samples, but the results show tremendous variation (1), and more than

one investigator has commented that normal aluminum concentrations were too low to be estimated accurately.

The development of flameless atomic absorption instrumentation, particularly the graphite furnace, has provided the means for quantitating those elements whose biological concentrations were previously beyond the detection limits of conventional atomic absorption spectrophotometers. The graphite furnace is adaptable for all matrices, and for many elements provides the best detection limits (40). The advantages for biological samples include the small sample size requirement, ability to use samples with no prior preparation, and its use of temperature stages, which permits the pyrolyzation of organic components before atomization (41). Several papers have been published regarding the theoretical factors governing atomization with a graphite furnace (42-44).

B. Uses of Aluminum in Medications and its Effects on Various Biological Systems.

Aluminum-containing antacids are widely used in medicinal preparations, available without prescription, and their actions have been the subject of many investigations. The effects of these and related drugs have been reviewed by Morrissey and Barreras (45). In general, these compounds are more effective when administered as liquid suspensions, rather than in solid (tablet) form (46). The most common form of aluminum in these preparations is the hydroxide, and its use in the management of peptic ulcer began in 1924. In 1943, Kirsner (47) evaluated aluminum phosphate and aluminum hydroxide in peptic ulcer treatment, and found no alteration of acid-base balance or electrolyte levels by these two "nonabsorbable compounds."

Another major use of aluminum antacids is as phosphate binding agents, particularly in renal failure. Aluminum oxide (48) and dihydroxyhydroxodiaquo - ▲ - glucono calcium aluminate (calcium glucaldrate) (49) have been proposed as effective agents, also.

Aluminum also finds uses as adjuvants for vaccines and toxoids (50) and in penicillin preparations. Other therapeutic uses of aluminum compounds include: the treatment of hyperkalemia in renal failure (51), metastatic calcinosis cutis (52), and choleraic bile-salt diarrhea (53); the management of renal phosphatic calculi (54); and the improvement of metabolic renal acidosis (55).

McCaffrey and Lilly (56) suggest that complications of aluminum-containing antacid therapy are insignificant, and consist primarily of constipation, although they do advise caution in patients with existing bone disease. However, these medications may not be as innocuous, as was once believed. Due to their

constipatory nature, intestinal obstruction and perforation have been reported (57-60). On the other hand, Arora et al. (61) have reported four deaths, due to an inability to tolerate aluminum hydroxide gel with consequent calcific cardiomyopathy in renal failure.

Several studies have focused on the interactions between aluminum compounds, particularly antacids, and other drugs in combination therapy. The results show: an increased absorption for pseudoephedrine (62); a decreased absorption for ethambutol (63), isoniazid (64), propranolol (65), digoxin (66), sulfadiazine (67), and quinine (67); and no effect on cimetidine (68) or iron (69). In the cases of a decreased absorption of a drug given with an aluminum compound, the consensus is that the decrease is due to delayed gastric emptying.

Aluminum chlorhydrate and zirconium aluminum glycine complex are the active ingredients of most antiperspirants, and aluminum chloride hexahydrate solution has been reported to be the treatment of choice for axillary hyperhidrosis (70). Since Turk and Parker (71) showed granuloma formation after intradermal injection of these compounds, much research has been done to determine the extent of their toxicity. The above compounds were found to be hemolytic and damaging to macrophages and fibroblasts, in vitro (72), but were not found to be granulomagenic on intratracheal inoculation (73), although there was a dose-related acute inflammatory respiratory bronchiolitis.

Aluminum levels have been reported in association with various disease states, including: decreases in blood and increases in urine with rheumotoid arthritis (74), increases in plasma with chronic pneumonia (75), increases in the lung with silicosis (76), and accumulations in lymph nodes in non-filarial elephantiasis (77).

Many metabolic effects of aluminum have been noted in the literature, including: decreased blood and tissue iron (78), cytochrome oxidase activity in liver, kidney, heart, muscle, brain, and spleen (79), and blood glycogen levels (80), but increased blood sugar levels (80), in rats and rabbits injected subcutaneously with aluminum sulfate. Aluminum was found to induce experimental porphyria in rats (81), which the authors concluded was caused by aluminum entering the hepatocyte nucleus and there combining with the DNA. Aluminum salts depressed acetylcholinesterase activity in cultured neuroblastoma cells (82), and a complex of aluminum and alginic acid produced a hypocholesterolemic effect (83), by binding bile acids and increasing their excretion. Hematological effects reported after occupational exposure to aluminum by workers in aluminum-producing plants include decreased hemoglobin and erythrocyte count and increased leucocyte count (84). Increased prothrombin time in workers producing alumina (85), and accelerated coagulation in a patient given intravenous kaolin (86) have been reported.

Physiological effects reported for aluminum include: the relaxing effect of aluminum chloride on gastric smooth muscle (87), the increase in lower esophageal sphincter tone by aluminum hydroxide (88), the increase in fluoride uptake by molars with topical aluminum chloride (89), and skeletal defects and growth retardation in the offspring of rats treated with intraperitoneal aluminum chloride (90).

C. Dialysis Dementia.

In 1942, Kopeloff et al. (91) produced convulsive seizures in monkeys by the application of alumina cream to cortical motor areas. Since then the application of aluminum compounds to the brain in several species has become the standard approach to the production of experimental convulsions and epileptic seizures (92 - 100). These papers detail the histology and pathology of the lesions produced and the behavioral modifications caused by the neuronal degeneration. Clinical symptoms of this encephalopathy include paresis, ataxia, muscular hypertonia, and grand mal epileptic convulsions. Blinova et al. (101) cite the direct action of aluminum on the neurons' metabolism as the cause of the epilepsy. De Boni et al. (102) advanced this research by inducing neurofibrillary degeneration in rabbits with subcutaneous injections of aluminum lactate or tartrate. From this work the authors conclude that systemic aluminum is able to cross the intact blood-brain barrier and produce results identical to those from direct cortical application.

In recent years much discussion has centered around the retention and toxicity of aluminum from the antacids consumed by patients with renal failure, and primarily those on maintenance hemodialysis. Some investigators have claimed that there is no systemic toxicity from aluminum antacids (2, 4, 45, 103), while others insist that it is harmful (104-5), and suggest that its use be discontinued pending further studies (106). Elevated serum aluminum levels have been reported in renal failure patients (107-8).

Increasing the significance of this debate was the recognition of a syndrome of progressive dementia (109-10), leading to seizures and death in some patients undergoing maintenance hemodialysis. The clinical symptoms included:

language disorders, characterized by slow speech with stuttering progressing to mutism; loss of coordinated movements, associated with myoclonus and seizures; dementia, characterized by confusion, disorientation, and impairment of memory; and behavioral disturbances, manifested as agitation, delirium, paranoia, and hallucinations. Electroencephalograms in affected patients showed distinctive and similar, but undefinable, abnormalities, including generalized slowing, bisynchronous delta waves, and epileptic-type spikes, while the post-mortem neuropathological examinations were unremarkable.

Many diverse causes for the progressive dialytic encephalopathy have been proposed, including: tin toxicity (109); deficiencies of rubidium (111), dopamine (112), and asparagine (113); and a uremic encephalopathy in slow-motion (114). No substantiating evidence has been found for these mechanisms. However, it has been reported that brain aluminum concentrations are higher in patients dying with dialysis dementia than in controls and non-dialyzed uremics (5, 115), with grey matter containing three times more aluminum than white matter. In an extensive trace metal survey, Tipton and Cook (116) found by emission spectroscopy that the body tissues having the highest concentrations of aluminum were lung, omentum, and skin. The concentration in the lung varied directly with the age of the subject, which the authors conclude was environmental contamination. The lowest aluminum concentration was found in brain tissue.

One factor which hemodialysis patients have in common is the consumption of large amounts of aluminum-containing antacids to prevent the accumulation of phosphate. They are also dialyzed on a regular basis against a solution, the composition of which varies, as does the purity of the water used in its preparation. Some treatment centers have used tap water in which aluminum had been utilized in purification, others deionized water. Another controversy which exists in the literature is the source of the aluminum, if indeed this is

producing the toxic effects. Some investigators argue that the absorption of aluminum in the gut is too small, and that the intravenous administration of aluminum contaminating the dialysate is at fault (115, 117-19). In support of this view is the report by Alfrey et al. (109) that the EEG tracings worsened after dialysis. On the other hand, the syndrome has occurred in treatment centers using deionized water to prepare the dialysate (120), and it has been hypothesized that an altered intestinal permeability exists in uremia.

There have been only two reports of reversals of the encephalopathy, one by discontinuing the aluminum antacids (121), and the other by a successful transplantation (122).

Several editorial reviews have been published concerning dialysis dementia (123-5).

Two other reports exist of an encephalopathy associated with aluminum resulting in death. A 49 year old man, who worked in an aluminum powder factory, developed aluminum fibrosis of the lung, and an encephalopathy resembling that seen in dialysis patients (126). The second case of mental deterioration was in a 27 year old man with no history of exposure to aluminum, but with deposits of the metal found in the brain at autopsy (127).

Abnormally high concentrations of aluminum in the brains of patients dying with Alzheimer's disease, a progressive dementia of unknown etiology occurring after the age of 40, have been found by some workers (128-9). This has been refuted by others (130), who were unable to demonstrate a significant difference in aluminum concentration between normal and Alzheimer brains. Further, it has been pointed out that the lesion in Alzheimer's disease is different from that produced experimentally in animals by aluminum compounds (131).

CHAPTER III

MATERIALS AND METHODS (132)

A. Equipment.

The primary instrumentation for the aluminum determinations consisted of a Perkin-Elmer system (Norwalk, CT, 06852), including the Model 306 atomic absorption spectrophotometer, equipped with the Model HGA-2000 graphite furnace, a deuterium arc background correction system, the Model 56 chart recorder, the Model PRS-7A printer interface and sequencer, an Intensitron aluminum hollow cathode lamp, and standard graphite tubes and cones. A voltage stabilizer (Raytheon Manufacturing Company, Model VR3) was used between the current source and the spectrophotometer, background corrector, and chart recorder. Air was used as the purge gas for the deuterium arc background corrector with a Carborundum Fulflo Filter (Model B3A) in the line. High purity argon (99.995%) was used as the purge gas for the graphite furnace.

B. Instrumental Parameters.

1. The voltage stabilizer output was set to 115 volts with a voltmeter, and this smoothed out any fluctuations in line voltage, which would have interfered with the absorption signals. In addition, all connections were grounded.
2. The argon gas flow was adjusted to 1.1 liter/minute, 3.5 units on the furnace controller unit. Argon proved to be the purge gas of choice for the determination of aluminum, as it gave expected increase in sensitivity over that observed for nitrogen (133). The absorbance signals were 4.25 times greater with argon than with nitrogen.
3. The automatic gas flow interrupt was activated, so that the atom cloud remained in the light beam longer.
4. The water for cooling the furnace ran at a rate of 2.7 liter/minute.
5. The hollow cathode beam and the deuterium arc beam were balanced in intensity at a gain setting of midline on the energy meter. Because of the much greater intensity of the aluminum lamp compared to the deuterium lamp, the current to the former was reduced to accomplish the matching.
6. The signal peak mode was selected at slit width setting #4, providing a spectral band width of 0.7 nm.
7. The monochromator was tuned to the peak of the emission line of the aluminum hollow cathode lamp at 309.3 nm.
8. A graphite tube was inserted into the furnace and aligned using a glass rod with well-fired ends to prevent damage. The aluminum rod included with the instrument was not used. The tubes were aligned so that the

sample hole was centered in the injection port. This allowed for the easiest introduction of the samples. The graphite tubes were noted to be asymmetric with respect to the placement of the gas inlet holes around the sample hole, but tube insertion either way produced the same absorption signal. The tubes could be placed so that the gas inlet holes were either in a horizontal or a vertical plane. Carbon residue from serum and plasma samples seemed more prone to accumulate, when the tubes were placed with the holes in the vertical position, probably due to the cooling effect of the purge gas. Consequently, the tubes were always placed with the holes in the horizontal position. The sample hole of the standard tubes was found to be too small to allow for reproducible placement of serum and urine samples. The samples tended to cover the opening, and to be pulled out of the tube, upon withdrawal of the micropipettor tip used for sample delivery. Carefully increasing the size of the sample hole from its original 2.08 mm to 2.78 mm with a drill bit solved the problem. This allowed repetitive placement of the entire sample at a single site on the floor of the tube with no sample loss. New tubes were conditioned by heating them to a temperature of 2600 ^{o}C, until the absorbance returned to the baseline on the chart recorder.

9. A deuterium arc background corrector was used for all measurements with the air flow for purging set at 3 units on the power supply flowmeter.

10. The chart recorder was operated in the automatic mode with the pen actuator leads shorted, so that the pen responded at all times, but the chart only began to move during the final seconds of the charring stage. This allowed observation of the absorbance reading throughout the entire

program without recording all stages. The recorder was zeroed to the chart baseline with both the recorder zero and the spectrophotometer auto-zero plus peak-start combination. The recorder was operated in the servo position at a speed of 10 mm/minute, and usually at 10 mV.

11. The printer-sequencer was connected to its own power source to eliminate the drain that occurred when the unit was activated, which interfered with small absorption signals.

These instrument settings are summarized in Table 1.

Table 1: Summary of Instrument Parameters.

Atomic Absorption Spectrophotometer

Aluminum hollow cathode lamp current: 10 ma

Wavelength setting: 309.3 nm

Filter: out

Emission chopper: off

Phase: normal

Signal: peak

Gain: set to midline on energy meter

Slit: 0.7 nm (#4)

Function: absorption

Range: uv

Curve correction onset: 0

Curve correction magnitude: 0

Concentration: 0

Decimal point: off

Mode: absorption

Graphite Furnace Programmer

Argon gas flowmeter: 3.5 units (1.1 liter/min)

Recorder: automatic

Gas interrupt: automatic

Deuterium Arc Background Corrector

Air Flowmeter: 4 units

Reference energy: set to exceed Al lamp intensity by 1 unit on the energy meter

Chart Recorder

Power: servo

Polarity: +

Chart speed: 10 mm/min

Range: 10 mV full-scale

Accessories

Voltage stabilizer: output set to 115 V

Water flow rate: 2.7 liter/min

Graphite tube sample hole: increased to 2.78 mm

C. Sample Delivery.

Oxford pipettors were employed to deliver samples to the interior of the graphite tube. Some of the attendant tips were found to be irregularly contaminated with aluminum. This contamination could not be removed by washing with nitric acid, and Karin et al. (134) reported that aluminum could not be leached from the plastic matrix by acid washing. The tips were washed for 75 minutes with continuous stirring in a solution of 6 g of Na_2EDTA per liter. They were rinsed with two changes of water and stirred for 60 minutes in a large volume of water. The tips were air dried and stored in the container in which they had been washed. Aluminum could not be detected when the tips were used to add pure water to the furnace.

The pipettor was used in the "to deliver" mode and the tip was replaced for each new sample. Blotting of the tips was avoided to eliminate contamination by the lint of the tissues. To prevent sample from clinging to the outside of the tip during pickup, the sample tube was tilted, so that the tip was minimally immersed.

The actual sample delivery was accomplished by inserting the tip into the graphite tube sample hole until it just touched the lower section of the opposite side. When the sample was expelled while in contact with the tube, it formed a reproducible droplet at the point of contact, and due to the increased size of the sample hole, was not distorted upon withdrawal of the tip.

To determine the optimal sample volume, an aqueous standard was introduced into the graphite tube with pipettors delivering different sample volumes. Figure 1 shows that absorbance increased linearly to a sample volume of 25 µl, which volume was used for most analyses.

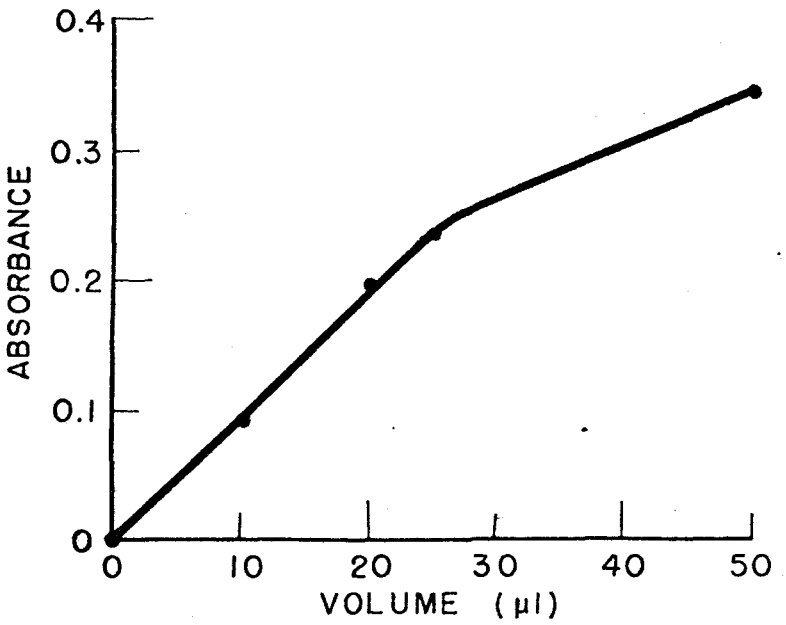

Figure 1: Curve obtained using pipettors of various volumes. A standard aluminum sulfate solution, 100 ug of Al per liter, was used.

D. Graphite Furnace Programs.

1. Drying. Drying parameters for the furnace program were determined by directly observing the sample within the graphite tube. With a 25µl volume a temperature of 100 °C for 60 seconds was sufficient to dry aqueous and urine samples. Any further increase in the drying temperature caused the aqueous and urine samples to splatter through the tube. However, this temperature was found to be too low for serum and plasma. For thorough drying of serum and plasma a temperature of 350 °C for 60 seconds was necessary. If the sample was not completely dried when the charring stage began, a portion of it was blown to the end or out of the tube, due to the sudden increase in temperature and the argon gas flow.

After the analysis of serum or plasma specimens at the lower temperature, some carbon residue remained inside the graphite tube. If the residue was not removed, it interfered with subsequent analyses, as shown in Figure 2. This interference was caused by physical blockage of the light beams by the residue, and not by carry-over to the next determination. Adequate drying reduced this problem to a minimum. The residue was removed by loosening it with a quartz rod with well-fired ends, and blowing it from the tube with a 1 oz. rubber bulb. No contamination from this procedure was observed in subsequent determinations, as checked by blank firings. Drying serum or plasma at either temperature gave the same absorbance values, when proper care was used to see that the graphite tube was clear after each firing, and that no losses had occurred when using the lower temperature.

2. Atomizing. With 25 µl aqueous aluminum standard, the absorbance increased linearly to an atomizing temperature of 2600 °C (Figure 3). Beyond this

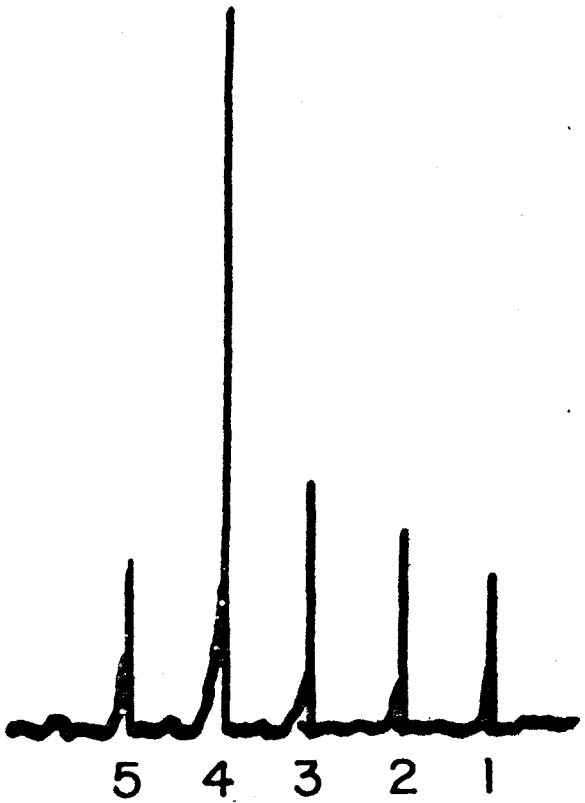

Figure 2: Chart recorder tracing demonstrating the need for removal of the residue from the graphite tube between analyses of serum and plasma. During five consecutive analyses of a sample, the residue was not removed until after the fourth determination. This was especially necessary if drying was at 100 ^{o}C.

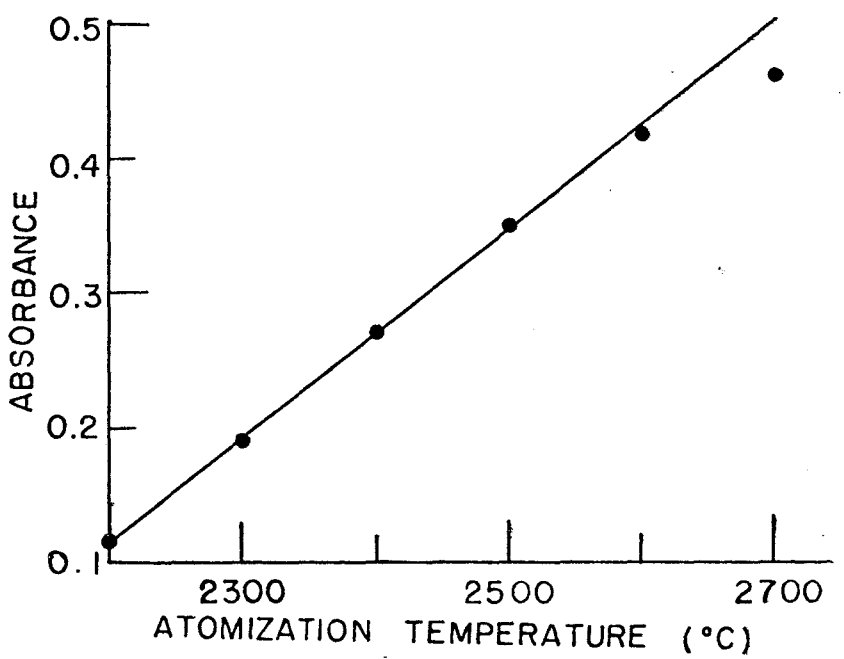

Figure 3: Optimal atomization temperature. The aluminum sulfate standard had a concentration of 150 ug of Al per liter.

temperature, the rate of absorbance increase was less, so that 2600 ^{o}C was chosen as optimal.

The minimum atomizing time at 2600 ^{o}C needed for the absorbance to return to the baseline and avoid carry-over was 12 s. This time also was adequate for serum and urine samples.

3. Charring. To optimize the charring conditions, the minimum temperature and time to eliminate non-specific background absorption were determined at 307 nm, a non-absorbing wavelength for aluminum. As shown in Figure 4, when charring 25 µl samples for 60 s, the minimum charring temperature is 1300 ^{o}C and is the same for both serum and urine. The minimum charring time for 25 µl samples of serum and urine at 1300 ^{o}C is 60 s, as illustrated in Figure 5.

The optimal charring temperature for 25 µl samples of serum and urine as determined at the primary wavelength for aluminum, 309.3 nm, is shown in Figure 6. It is 1500 ^{o}C.

A summary of the furnace programs is presented in Table 2.

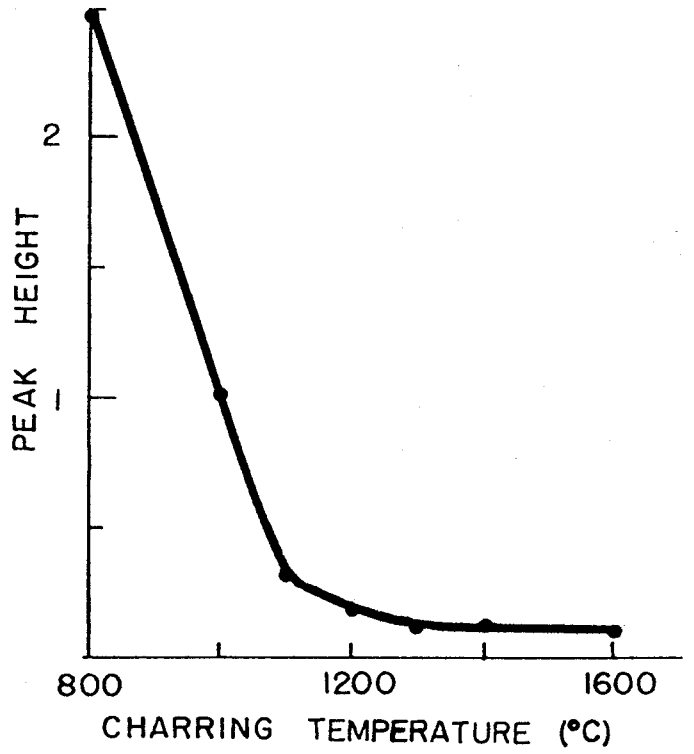

Figure 4: Minimum charring temperature for serum and urine.

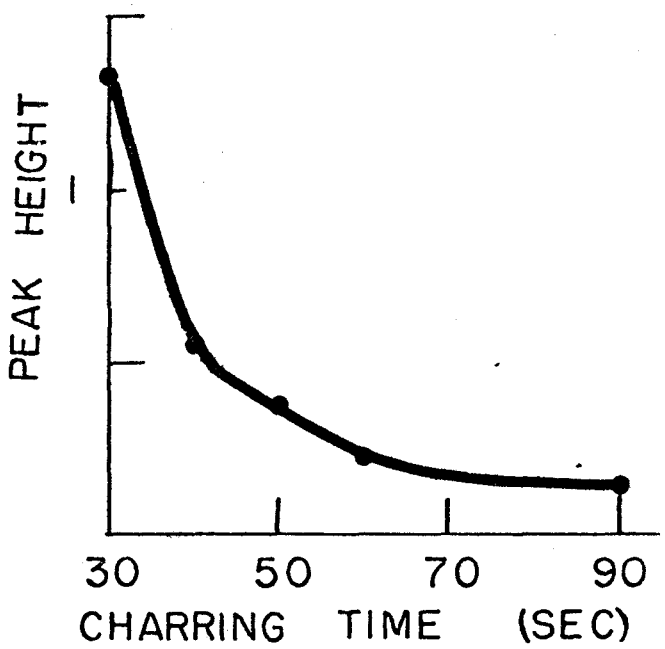

Figure 5: Minimum charring time for serum and urine.

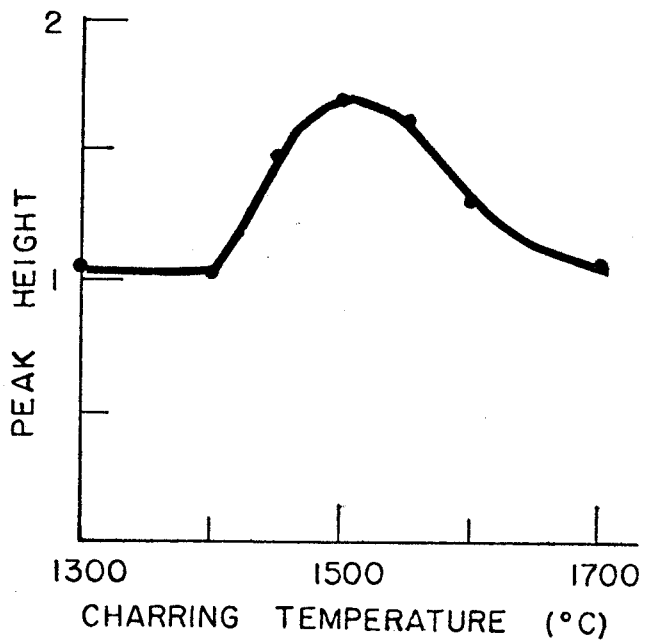

Figure 6: Optimal charring temperature for serum and urine.

Table 2: Summary of Graphite Furnace Programs.

Drying stage: serum - 60 s at 350 °C

urine and aqueous - 60 s at 100 °C

Charring stage: 60 s at 1500 °C

Atomizing stage: 12 s at 2600 °C

E. Standards and Reagents.

Standard aluminum stock solutions were prepared in several ways and compared. They were stored in polyethylene bottles at 4 °C.

1. From salts. Solutions of $Al_2(SO_4)_3 \cdot 18\ H_2O$ were found more stable than those of $K_2Al_2(SO_4)_4 \cdot 24\ H_2O$. For a concentration of 1 mg Al per liter, 12.26 mg $Al_2(SO_4)_3 \cdot 18\ H_2O$ was dissolved to make 1 liter of solution. This concentration sufficed to make acidification for stability unnecessary, eliminating one source of contamination. Dilutions for use were prepared daily from the stock.

2. Aluminum metal. Aluminum foil of a 99.997% purity (Alfa Division, Ventron Corporation, Danvers, MA, 01923) was weighed on a Cahn electrobalance (Model 4400) and 1.921 mg dissolved in 5.6 ml concentrated H_2SO_4. Solution was facilitated by the addition of 6 drops 30% H_2O_2. The final solution contained 0.9605 mg of aluminum and 0.09 mol of H_2SO_4 per liter. To show contamination did not result from the glassware or reagents, a blank acid solution was first prepared and assayed, and the standard prepared in the same 2 liter volumetric flask.

A standard was also prepared from the foil with HCl, which required gentle heating to affect solution. The final concentration was 1.0155 mg of Al and 0.04 mol of HCl per liter.

3. Commercial Standard. A solution of $Al_2(SO_4)_3$, containing 1000 ppm Al, was obtained from Alfa Division, Ventron Corporation, Danvers, MA, 01923.

The water was purified by reverse osmosis and passed through a mixed-bed resin exchanger (Continental Water, Melrose Park, IL, 60160). It was run for a few minutes before collection, and the metal could not be detected in it.

A gelatin multicomponent trace element reference material (TEG-50-B) was purchased from Eastman Kodak Company (Rochester, NY, 14650, Cat. No. 15087) for use as a control in the aluminum analyses. 245 mg was dissolved in about 150 ml water, with gentle heating, and this was diluted to 250 ml in a volumetric flask. The solution then contained 0.98 g gelatin per liter.

F. Subjects.

1. Reference Studies. The only criterion used for including samples to determine reference values for serum and urine was that the donor was not using aluminum-containing antacids.

2. Metabolic Studies. These studies were carried out in cooperation with the Metabolic Research Unit of Hines V.A. Hospital. The patients on this ward were volunteers admitted for evaluation and observation of various medical problems. They were required to be fully ambulatory, free of severe gastrointestinal and renal impairment, and not taking medications, which were not part of the study protocol. Generally, the hospital stay was a lengthy one, as the Metabolic Year ran from September through July each year. There was continual nursing care and daily physician visits.

The patients were carefully instructed as to the strict regulations which of necessity were required for the intake of food and fluid and the complete urine and stool collections. The Metabolic Unit maintained its own dietary kitchen, and the patients were allowed to eat only that food which was specially prepared for them. Due to the nature of metabolic studies, the diets were constant and had little variety, but individual tastes were considered whenever possible, using the food items of the constant diets.

Timetables were established for each patient as to administration of the drugs under investigation, in this case aluminum-containing antacids, and for adequate control periods before or after such administration, or both.

These studies were performed under strictly controlled conditions (135), beginning with the establishment of an isolated, self-sufficient hospital ward, in which the temperature and humidity remained at a constant level. This ensured

that losses of electrolytes, minerals, and water via the skin related to urine volume and body weight changes were minimized. In this regard, physical exercise was standardized and limited to prevent losses through perspiration. Total fluid intake and drinking water volume, after being individualized, were kept constant throughout the studies.

3. Dialysis Studies. These studies were carried out with the cooperation of the Renal Dialysis Unit of Hines V.A. Hospital. The patients in the hemodialysis studies were selected at random, but were all on a schedule of four or five hours of maintenance hemodialysis three times weekly. The patients in the hemofiltration studies were selected because of intractable hypertension or large weight gain due to fluid retention.

G. Collection of Specimens.

1. Blood. Many systems for obtaining specimens by venipuncture were tested for their possible contribution to aluminum contamination. The best results were obtained with a Monoject (Sherwood Medical Company, St. Louis, MO, 63105) 3 ml disposable syringe (Cat. No. 158150) and a Monoject 20 G x 1½" disposable needle (Cat. No. 250). The following technique was employed (136): after blood collection, "air is drawn into the syringe, clearing the needle and connector, until the plunger is within about 5 mm from the end, allowing for its easy removal later. The needle is capped, and the specimen is permitted to clot in the syringe with the needle up (Figure 7, A). The needle is then removed and replaced with a serum-bottle stopper of the sleeve type (B), which has been modified (C). The unit is inverted, and the plunger is carefully removed (D). The specimen is then centrifuged (E), and the serum is used directly or decanted into a previously prepared plastic tube." Freedom from contamination was verified with blank solutions.

Several types of Vacutainer brand evacuated blood collection tubes (Becton-Dickinson, Rutherford, NJ, 07070) were tested for suitability. Certain lots of tubes with no additives (Cat. Nos. 4736 and 4504) were found to be free of detectable aluminum, but other batches were found to have aluminum.

Plasma was obtained by transferring the whole blood to previously prepared tubes containing 18 µl of 30% sodium citrate per milliliter of whole blood and centrifuging. The sodium citrate was free of detectable aluminum. Serum and plasma were stored frozen at -7 ^{o}C until analyzed.

All glassware and containers used were tested for possible contamination. Except for the preparation of standards and stool ashes, all laboratory ware

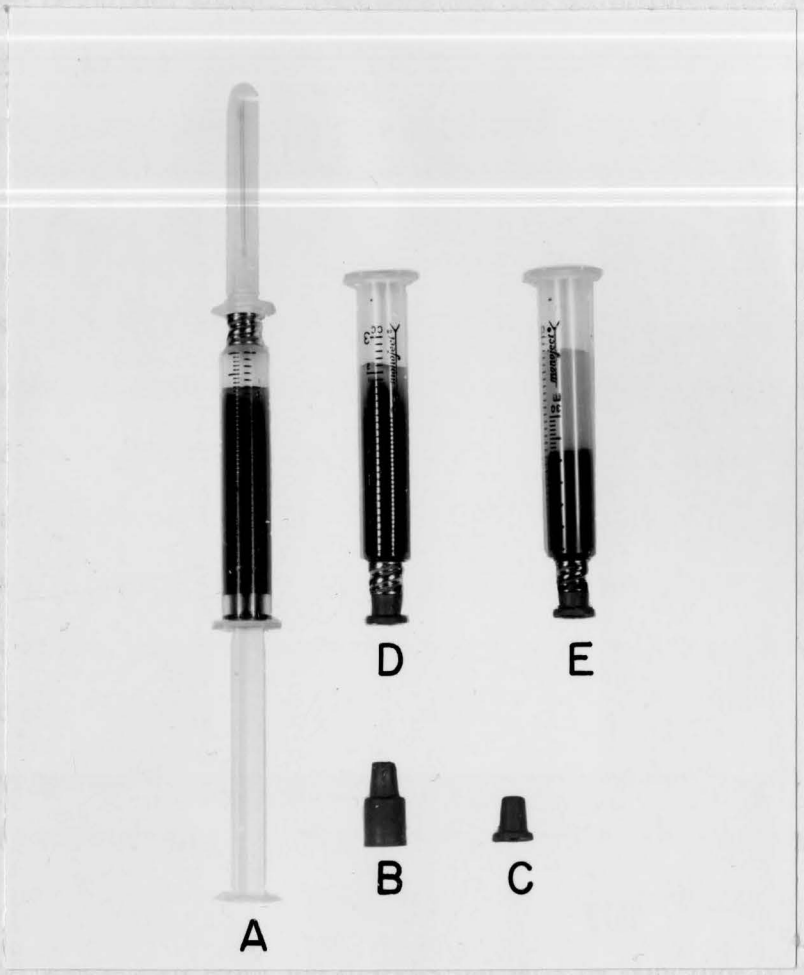

Figure 7: Various stages in the collection of a blood sample to minimize aluminum contamination (136).

used was plastic, chiefly polypropylene. Glassware was prepared by soaking it in a solution containing 3 mol HCl/L for one hour, followed by copious rinsing with aluminum-free deionized water. This included 100 ml beakers for ashing of stool samples, and volumetric flasks for dilution of stool ashes and preparation of standards. The plastic ware was prepared initially in the same manner, and once free of contamination, the same containers were rinsed well and reused. Regular washing with nitric acid showed no advantage, because of its aluminum content (137). In consideration of the extremely low levels of aluminum encountered in the biological samples, addition of reagents and transferring from container to container was avoided whenever possible.

It had been reported that Parafilm (American Can Company, Greenwich, CT, 06830) was a source of contamination in some chemical studies (138). This material was tested, and no contamination was observed. Therefore, all sample tubes were covered with Parafilm until analyzed.

Another source of contamination was sweat from the hands, which is rich in trace metals (139); thus, handling of all associated materials was kept to a minimum.

Blood was drawn from the dialysis patients by puncture of the sleeve on the arterial line of the dialysis tubing with the Monoject unit.

2. Urine. Urine specimens were obtained as 24 hour collections in previously prepared containers without preservative. The volume and specific gravity were recorded for each. For the metabolic balance studies the complete daily urine collections were pooled to obtain six-day pools, by mixing 1% of each day's volume. The patients voided into individual stainless steel urinals, and after

the volume was measured, the specimens were stored in polypropylene jugs in a refrigerator until pooled. Urine specimens, including pools, were refrigerated at 4 ^{o}C until analyzed.

3. Stool. Stool samples for the metabolic balance studies were collected in individual polypropylene beakers inserted into a commode-chair. Each collection was homogenized, and 50% by weight of each was mixed to produce the pool for that metabolic period, unless the patient was constipated, in which case the entire collection was used.

Stool periods were demarcated by the use of markers, carmine and charcoal, administered in capsule form. A marker was taken with breakfast at the beginning of each six-day metabolic period, alternating between 310 mg carmine and 320 mg charcoal. The appearance of a marker in the stool indicated the beginning of the collection for that metabolic period, which continued until the next marker appeared. In other words, the passing of the second marker heralded the start of the next period. The length of each period (6 days) and the number of periods studied (8-23) were chosen to eliminate the irregularities of stool passage, especially in older, constipation-prone patients.

4. Food. The diets for the metabolic balance studies were constant and known, and consisted of two low calcium (200 mg) menus, served on alternate days. The food was purchased in large quantities from the same lots to eliminate variation in preparation. All foods were weighed, and all fluids were measured. An homogenate was prepared of both menus, mixing together foods and fluids exactly as served.

5. Dialysate and Hemofiltrate. Entire dialysates were collected from the dialyzer unit drain tube into a specially washed 32 gallon polyethylene bin (Cole-Parmer, Chicago, IL, 60648, Cat. No. 6742). Entire hemofiltrates were collected, under vacuum, from the membrane into a specially washed 20 liter Pyrex bottle, equipped with specially washed tubing and connections. Aliquots of these fluids were taken after thorough mixing.

H. Preparation of Specimens.

1. Stool and Food. Five grams of the stool pool homogenates or diet homogenates was weighed in duplicate into 100 ml glass beakers, which had been specially prepared, and marked with a heat-resistant china marker. These were dried overnight in an oven at 110 ^{o}C. It was important that the samples be thoroughly dried, so that no losses occurred in the muffle furnace. The samples were then ashed in a muffle furnace overnight at 550 ^{o}C. After cooling, approximately 5 ml of 3 mol H_2SO_4 per liter was added to each, and these were heated on a hot plate until they just began to boil. The samples were allowed to cool, quantitatively transferred to specially washed 100 ml volumetric flasks, and diluted with water. After mixing, the solutions were transferred to specially prepared polypropylene bottles. These samples were stored at room temperature until analyzed.

2. Other Samples. All other types of samples (serum, plasma, urine, dialysate, and hemofiltrate) were analyzed with no preparation.

CHAPTER IV

RESULTS

A. Methodology (132).

A comparison of the three aluminum sulfate standards gave identical response. When using 25 µl of standard, the sensitivity, as defined by the quantity of aluminum needed to produce a 1% absorption, was 30 pg (1.11 pmol). With a 25 µl sample, this required a concentration of aluminum of 1.2 µg/liter (44.5 nmol/liter).

The gelatin control material was certified by the manufacturer to contain 60 ± 3(SD) µg Al/g. Analysis of this material with each batch of samples produced a value of 58 ± 3(SD) µg Al/g (N=19), for a coefficient of variation of 5.2%.

The within-day precision for 10 consecutive analyses of a serum sample, containing 48 µg Al/L, gave a coefficient of variation of 2.9%. Results obtained for 16 samples by the method of additions and from standard curves did not differ significantly (mean difference = 2.11 (less than twice the sensitivity) ± 4.76 (SD) µg Al/L, or 78 ± 177 (SD) nmol Al/L; Table 3). These samples ranged in concentration from 13.3 µg Al/L (0.49 µmol Al/L) to 137.3 µg Al/L (5.09 µmol Al/L). The recovery of aluminum added to 10 samples of serum, ranging in concentration from 4.2 to 136.4 µg Al/L (0.16 to 5.06 µmol Al/L), was 101.3 ± 7.2 (SD)% (Table 4). The interday variation of the method was determined by analyzing 15 sera on separate days (Table 5). These samples were stored frozen at -7 ^{o}C and ranged from 12.1 to 131.1 µg Al/L (0.45 to 4.86 µmol Al/L). The mean difference between the two groups of data was 2.56 ± 5.46 (SD) µg Al/L (95.0 ± 202.5 (SD) nmol Al/L). No

Table 3. COMPARISON OF RESULTS OBTAINED BY METHOD OF ADDITIONS & STANDARD CURVES.

Sample No.	Method of Additions (μg Al/L)	Standard Curves (μg Al/L)	Difference* (μg Al/L)
1	68.6	79.0	-10.4
2	51.5	52.8	-1.3
3	32.8	35.9	-3.1
4	13.3	11.8	+1.5
5	34.3	30.9	+3.4
6	137.3	136.4	+0.9
7	120.5	126.2	-5.7
8	99.3	108.9	-9.6
9	111.3	111.5	-0.2
10	38.8	37.2	+1.6
11	54.3	60.4	-6.1
12	17.8	15.7	+2.1
13	43.0	49.5	-6.5
14	33.2	36.4	-3.2
15	27.0	30.6	-3.6
16	23.0	16.5	+6.5

* Method of additions minus standard curves

N = 16 Mean $\pm$ standard deviation = 2.11 $\pm$ 4.76 μg Al/L

Table 4. RECOVERY ANALYSIS FOR ALUMINUM METHOD.

Sample No.	Concentration of Al Before Addition (µg/liter)	Addition (µg Al/liter)	Concentration of Al After Addition (µg/liter)	Recovery (%)
1	136.4	50	187.8	102.8
2	126.2	50	180.7	109.0
3	111.5	50	163.8	104.6
4	36.4	30	67.6	104.0
5	4.2	50	49.6	90.8
6	36.4	50	89.1	105.4
7	53.2	50	97.6	88.8
8	35.5	25	62.6	108.4
9	52.8	50	108.9	104.4
10	55.0	20	74.0	95.0

Mean $\pm$ standard deviation = 101.3 $\pm$ 7.2%

Table 5. ANALYSIS OF INTERDAY VARIATION FOR ALUMINUM METHOD

Sample No.	1st Analysis (µg/liter)	2nd Analysis (µg/liter)	Difference (2nd-1st) (µg/liter)
1	34.7	33.3	-1.4
2	27.4	26.4	-1.0
3	37.1	39.5	2.4
4	25.8	25.6	-0.2
5	16.1	16.3	0.2
6	26.2	28.3	2.1
7	19.8	20.5	0.7
8	26.6	24.0	-2.6
9	18.5	24.0	5.5
10	46.0	41.9	-4.1
11	12.1	13.6	1.5
12	29.8	30.6	0.8
13	120.5	131.1	10.6
14	81.8	88.9	7.1
15	70.9	87.7	16.8

Mean $\pm$ standard deviation = 2.56 $\pm$ 5.46 µg/liter

t-test (paired observations) = 1.816 $P > 0.05$

significant statistical difference between these two groups was found, as the t-test for paired observations produced a $\underline{P} > 0.05$.

To test for effects of matrix upon the aluminum analyses, standards in serum, urine, and water, covering a wide range of concentration, were analyzed (Figure 8). The method of additions established the concentration of the original serum and urine samples, after which aqueous aluminum sulfate standards were added to increase the concentrations. The difference between the serum and urine lines was not of statistical significance, and pooling the results produced a line with slope = 2.37×10^{-3} and y-intercept = 11.70×10^{-3} absorbance units. This line differed significantly from the water line with $\underline{P} < 0.01$. Comparing the pooled serum and urine line with the regression forced through the origin, that is, a line with slope = 2.48×10^{-3}, yielded no significant difference, $\underline{P}$ was only slightly less than 0.1. No statistical difference was observed in the comparison of the water line with the regression forced through the origin, which had a slope = 2.9×10^{-3}. The correlation coefficients of all lines were significant at the 0.1% level. From this it was determined that standards should be made in a matrix corresponding to the samples to be analyzed.

A study was made of the possible cause of the lower absorbance values for standards in serum and urine than those in aqueous solutions of aluminum sulfate. It was found, as has been determined by other workers (140), that aluminum chloride standards gave lower absorbance values than those prepared from the sulfate. However, some investigators (33, 141-2) have based their results on standards prepared as the chloride. Since $AlCl_3$ sublimes at 178 ^{o}C, and since chloride is the predominant anion in serum and urine, lower absorbance values for these matrices would be expected. It was found that the addition of NaCl to any of the samples lowered the absorbance observed, while the addition of sulfate

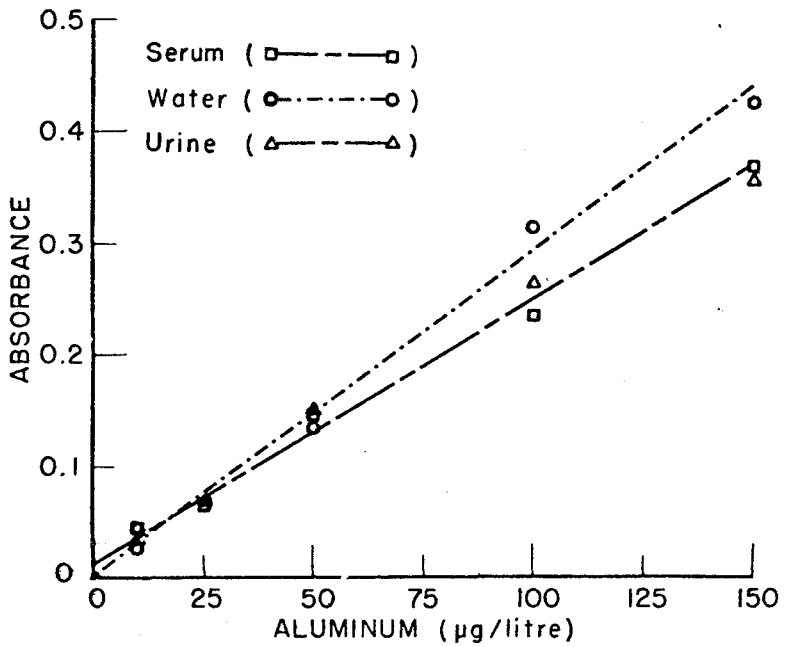

Figure 8: Standard curves showing the effects of different matrices.

Matrix	Slope $x10^3$	y-Intercept $x10^3$	Correlation coefficient
Serum	2.36	11.68	0.9953
Water	2.92	-1.31	0.9971
Urine	2.38	11.72	0.9948

enhanced the absorbance. Krishnan et al. (143) had reported no effect for chloride and an enhancement effect of 25% for sodium, but this was in the nitrous oxide/acetylene flame.

Initial experiments indicated that the addition of 13 mg Na_2SO_4 per ml serum gave maximal enhancement (Figure 9). Other compounds had a similar effect, most notably glucose, but Na_2SO_4 was found to be most convenient. Addition of sodium sulfate had no effect on standards prepared as aluminum sulfate, but the lower values observed with aluminum chloride standards were increased to those observed for aluminum sulfate standards by the addition of sodium sulfate. A small correction was necessary for the aluminum content of the sodium sulfate.

At this point, it was necessary to decide if it were essential to add sulfate to all samples. It would be advantageous if such an addition were not required. The concentration of aluminum in a serum pool was determined by the method of additions using either aluminum chloride or aluminum sulfate standard, and in the presence and absence of added sodium sulfate. The results are shown in Table 6, where the slopes of the lines and the calculated aluminum concentrations are given. When aluminum chloride was used in the method of additions, the value for the calculated aluminum content of the serum in the presence of Na_2SO_4 was significantly higher, but not so high as obtained with the aluminum sulfate standard. When sodium sulfate was added to the set prepared with added aluminum sulfate, higher absorbances were indeed obtained, but the slope of the method-of-additions line was also increased, so that the calculated aluminum concentration of the serum was the same. This suggested that the same analytical values can be obtained with and without the addition of sodium sulfate, provided the correct calibration line is used (Figure 10).

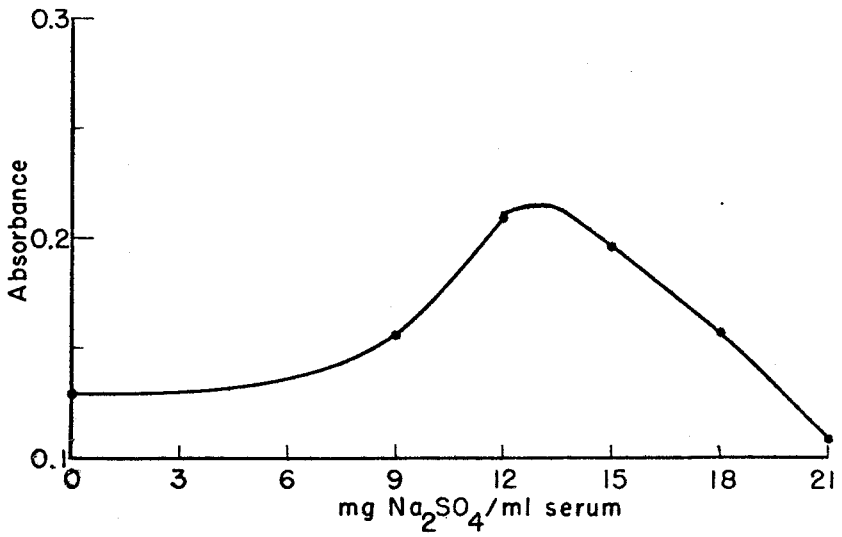

Figure 9: Effect of Na_2SO_4 on absorbance values. Maximal enhancement occurred with 13 mg Na_2SO_4/ml serum.

Table 6. EFFECT OF Na_2SO_4 ON ALUMINUM ASSAY OF SERUM

METHOD OF ADDITIONS IN THE ABSENCE AND PRESENCE OF Na_2SO_4

Na_2SO_4 Addition (13mg/ml serum)	Standard as Chloride		Standard as Sulfate	
	slope x 10^3	µg Al/L	slope x 10^3	µg Al/L
No	2.65	73.2	2.76	87.6
Yes	2.85	80.4	3.32	86.3

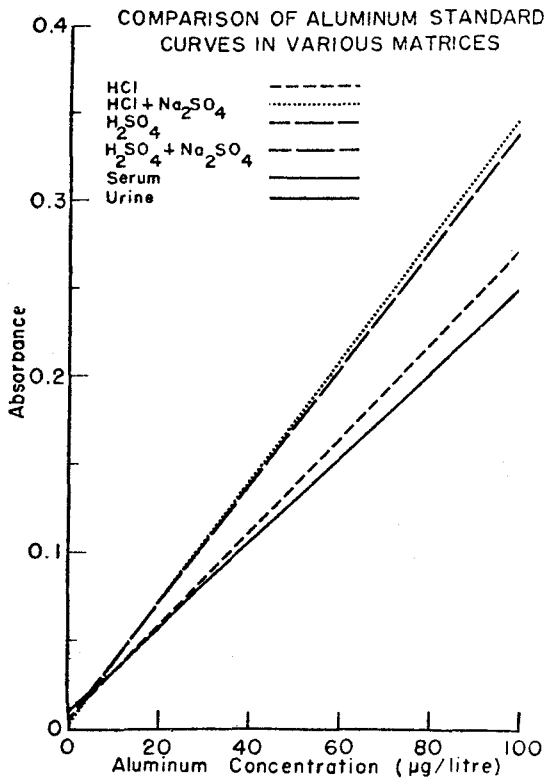

Figure 10: Comparison of aluminum standard curves in several different matrices.

To further determine the need for the addition of sodium sulfate, 15 serum (Table 7) and 25 urine (Table 8) specimens were analyzed with and without the addition of 13 mg of Na_2SO_4 per ml (92 mmol/liter). Calculations were made from the corresponding standard curves. In both cases the difference in concentration due to the sodium sulfate was insignificant, $P > 0.05$. The function of the sulfate is probably to lower the mol ratio Cl^-/SO_4^{2-}, to prevent the sublimation of $AlCl_3$. It may also furnish oxygen for the formation of aluminum oxide, as suggested by Campbell and Ottaway (144).

To study the effect of serum and urine components on the analytical results, a pool of each was assayed for aluminum by the method of additions. An aluminum sulfate standard of the same concentration was used to dilute each. The protein concentration, as determined by the Biuret method, had no significant effect on the aluminum concentration, as shown in Figure 11. The coefficient of variation of the absorbance values was 3.4%.

When the spectrophotometer response was compared with the specific gravity of the urine dilution, Figure 12, the coefficient of variation of the absorbance was 3.2%. Thus the concentration is not considered to have had a significant effect on the results.

The reference value for 24 sera by the method described is 27 ± 9 (SD) µg Al/liter (range = 12 - 46), or 1.00 ± 0.33 µmol Al/liter (range = 0.045 ± 1.71) (Table 9). The only criterion used for including samples was that the donor was not using aluminum-containing antacids. The range of values is lower than found by many investigators (Table 10), and this is thought to be due to the elimination of many techniques and reagents which act as sources of contamination. For this sample size, sex and age did not contribute significantly.

Table 7. SERUM ALUMINUM WITH AND WITHOUT ADDED Na_2SO_4

Sample No.	µg Al/liter without Na_2SO_4	µg Al/liter with 13 mg Na_2SO_4/ml serum
1	50.4	42.7
2	17.0	20.6
3	24.9	28.4
4	10.1	16.5
5	21.7	25.6
6	9.0	16.1
7	9.0	16.9
8	110.1	124.8
9	47.5	53.6
10	22.8	26.3
11	35.9	33.5
12	29.4	23.1
13	16.4	21.8
14	15.4	11.8
15	49.7	51.8

$t_d = 1.947$ $\quad$ $P > 0.05$

Table 8. URINARY ALUMINUM WITH AND WITHOUT ADDED Na_2SO_4

Sample No.	µg Al/liter without Na_2SO_4	µg Al/liter with 13 mg Na_2SO_4/ml urine
1	20.0	20.3
2	91.7	89.2
3	8.3	8.6
4	71.3	72.0
5	99.3	98.4
6	34.3	31.3
7	33.5	34.0
8	23.9	24.6
9	57.1	59.4
10	18.4	16.8
11	97.7	100.9
12	20.9	21.6
13	40.6	38.7
14	11.1	12.7
15	10.5	11.7
16	7.3	7.6
17	69.2	70.8
18	19.0	20.2
19	37.0	39.3
20	11.3	11.8
21	31.5	32.7
22	9.3	5.6
23	9.5	10.2
24	7.2	4.6
25	9.8	13.8

$t_d = 0.7347$ $\quad$ $P > 0.4$

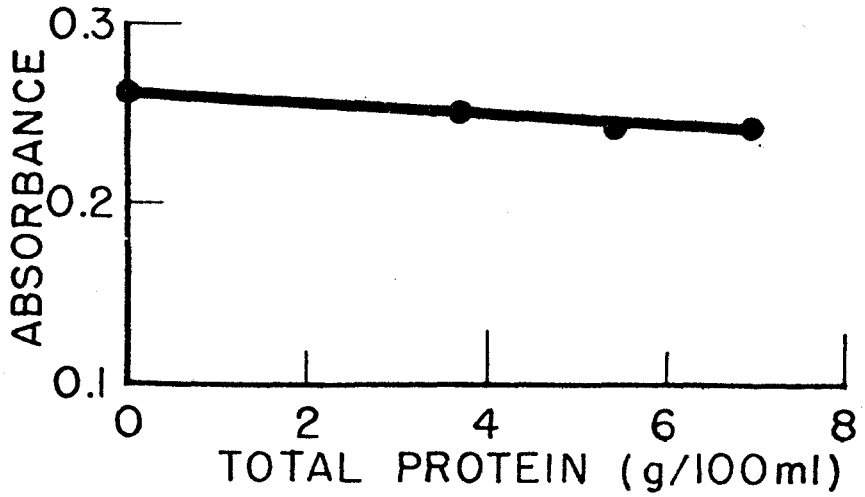

Figure 11: Graph demonstrating the effect of protein content on serum aluminum determinations. Total protein determined by the Biuret method.

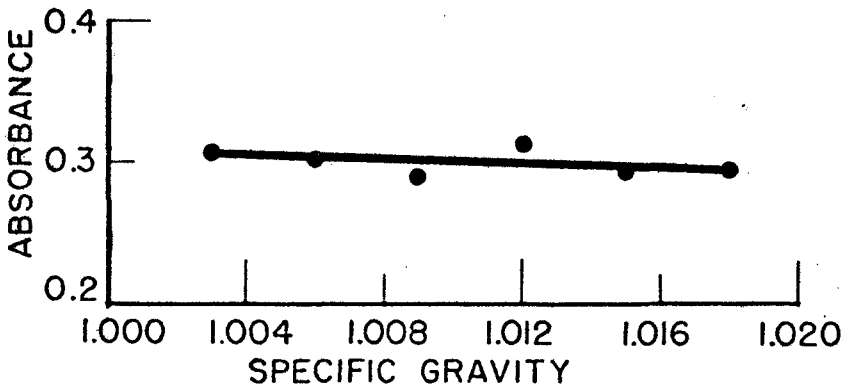

Figure 12: Graph demonstrating the effect of specific gravity on urinary aluminum determinations. Specific gravity determined from refractive index.

Table 9. REFERENCE VALUES FOR SERUM ALUMINUM:

Specimen No.	µg Al/L	Sex	Age (yrs)
1	32.4	M	65
2	34.7	M	27
3	27.4	F	30
4	37.1	M	22
5	25.8	F	24
6	16.1	M	25
7	26.2	F	37
8	19.8	M	25
9	26.6	M	26
10	18.5	M	28
11	46.0	M	30
12	12.1	F	26
13	29.8	F	22
14	26.0	F	41
15	22.7	F	29
16	20.1	M	23
17	29.6	F	25
18	39.5	M	50
19	22.6	F	27
20	36.0	M	28
21	16.3	M	26
22	40.7	M	25
23	24.9	M	26
24	13.5	M	42

Mean $\pm$ Standard Deviation = 26.9 $\pm$ 9.0 µg Al/liter

Sex shows no significance - $P > 0.3$

Age shows no significance - $P > 0.2$

Table 10: SERUM OR PLASMA ALUMINUM - REPORTED VALUES

(Numbers in parentheses indicate number of people tested)

Method	µg/liter	Reference
Spectrographic	240 ± 120 (30)	145
	450 ± 20 (122)	146
	551 ± 172 (63)	18
Atomic absorption	37 ± 25 (29)	147
	24 ± 5 (20)	148
	340 ± 190 (21)	85
	240 ± 55 (5)	107
	27 ± 9 (24)	This work
Neutron Activation	1460 ± 261 (5)	107
	72 ± 70 (10)	149

In the analysis of urine from 11 normal males not receiving aluminum-containing antacids, the excretion was 45 $\pm$ 32 (SD) µg Al/24 h (range = 6 - 92), or 1.67 $\pm$ 1.19 µmol Al/24h (range = 0.22 - 3.41) (Table 11).

Table II. URINARY ALUMINUM LEVELS - NORMALS

Specimen No.	µg Al/liter	24h volume (ml)	µg Al/24h
1	21	2,912	61
2	27	3,460	92
3	2	4,012	8
4	27	3,270	89
5	3	4,284	14
6	2	3,270	6
7	6	3,462	22
8	13	4,600	60
9	8	3,412	26
10	28	1,580	45
11	22	3,180	69

Mean $\pm$ Standard Deviation = 45 $\pm$ 32 µg Al/24h

B. Metabolic Balance of Aluminum.

Tables 12 through 17 show the aluminum balances for the 6 male patients studied. Parts "a" of the tables display the total aluminum intakes, urine, stool, and plasma aluminum levels, and the overall aluminum balances. Parts "b" give factors used in calculating the balances, including the total aluminum contents of all medications given, the urine volumes, and the stool weights. The study lengths ranged from 7 to 23 six-day periods.

The medications which were found to contain aluminum included: the carmine and charcoal stool markers, the calcium gluconate, and the zinc sulfate (Table 18). Carmine is the aluminum lake of carminic acid. The source of the aluminum in the calcium and zinc supplements is not known, but it most likely enters as a contaminant of the talc used to keep the preparations dry. Fluctuations in the total aluminum intake were generally due to different combinations and dosages of these medications.

Subject 1 had 4 initial control periods with a normal calcium intake, followed by 3 periods of the same regimen plus 30 ml TID Amphojel. This 69 year old patient's major medical problem was chronic renal failure. There was a minor negative balance initially, probably owing to the prior history of the patient. During the periods with Amphojel, there was a large positive balance. The plasma levels showed only slight increases during these periods, with all values remaining within the reference range.

Subject 2 was 42 years old and had osteoporosis. When the study began, he was on a low calcium diet with 800 mg P/day and 30 ml TID Mylanta. The phosphorus was increased to 1200, then to 1600 mg/day, when the Mylanta was increased to 30 ml QID. The final periods reverted to 800 mg P/day without Mylanta. There were substantial positive balances during the three period groups

Table 12a. Subject 1.

Aluminum Balance, mg/day

Study Condition	Period[a]	Intake[b,c]	Urine	Stool	Balance	Plasma (μg/L)
800 mg Ca	1	5.42	0.078	20.22	-14.88	
	2	4.77	0.111	79.69	-75.03	
	3	4.12	0.056	9.36	-5.30	
	4	5.42	0.146	8.66	-3.39	36
Average		4.93	0.098	29.48	-24.65	
800 mg Ca + Amphojel	5[d]	1909	0.405	1237	+672	44
	6	1911	0.223	2130	-219	
	7	1909	0.218	1623	+286	47
Average		1910	0.282	1663	+246	

[a]Each period of 6 days duration, except for Period 2, which was 12 days.

[b]Diet aluminum constant at 1.86 mg/day.

[c]Drinking water aluminum constant at 0.348 mg/day.

[d]Transition period from low to high aluminum intake.

Table 12b. Subject 1.

Aluminum Balance Factors

Study Condition	Period[a]	Medications (mg Al/day)	Urine Volume (ml/day)	Stool Wt. (g/day)
800 mg Ca	1	3.21	3212	381
	2	2.56	3319	371
	3	1.91	3290	254
	4	3.21	3240	369
Average		2.72	3265	344
800 mg Ca + Amphojel	5	1907	3360	381
	6	1909	3390	468
	7	1907	3362	332
Average		1908	3371	394

[a]Each period of 6 days duration, except for Period 2, which was 12 days.

Table 13a. Subject 2.

Aluminum Balance, mg/day

Study Condition	Period[a]	Intake[b,c]	Urine	Stool	Balance	Plasma (µg/L)
200 mg Ca +	1	1249	0.478	1003	+246	
800 mg P +	2	1250	0.397	870	+380	
Mylanta	3	1249	0.290	987	+262	
	4	1250	0.226	982	+268	
	5	1249	0.301	1113	+136	
Average		1249	0.338	991	+258	
200 mg Ca +	6	1250	0.243	1076	+174	
1200 mg P +	7	1249	0.243	970	+279	
Mylanta	8	1250	0.194	1336	-86.40	
	9	1249	0.236	1250	-1.24	
Average		1250	0.229	1158	+91.34	
200 mg Ca +	10	1527	0.306	1699	-172	
1600 mg P + increased	11	1664	0.228	1126	+538	
Mylanta	12	1665	0.192	1226	+439	
	13	1664	0.210	1421	+243	
	14	1665	0.214	1461	+204	29
Average		1637	0.230	1387	+250	
200 mg Ca +	15[d]	2.82	0.112	18.35	-15.64	22
800 mg P	16[d]	4.12	0.206	22.43	-18.52	
	17	2.82	0.215	6.05	-3.45	
	18	4.12	0.232	6.52	-2.64	
	19	2.82	0.234	6.16	-3.58	
Average		3.34	0.200	11.90	-8.77	

[a]Each period was of 6 days duration.

[b]Diet aluminum constant at 2.45 mg/day.

[c]Drinking water aluminum constant at 0.348 mg/day.

[d]Transition period from high to low aluminum intake.

Table 13b. Subject 2.

Aluminum Balance Factors

Study Condition	Period[a]	Medications (mg Al/day)	Urine Volume (ml/day)	Stool Wt. (g/day)
200 mg Ca +	1	1246	3584	707
800 mg P +	2	1247	3420	617
Mylanta	3	1246	3779	700
	4	1247	3752	696
	5	1246	3431	789
Average		1246	3593	702
200 mg Ca +	6	1247	3761	644
1200 mg P +	7	1246	3519	579
Mylanta	8	1247	3355	675
	9	1246	3712	634
Average		1247	3587	633
200 mg Ca +	10	1524	4049	719
1600 mg P + increased	11	1661	3369	598
Mylanta	12	1662	3541	654
	13	1661	3755	634
	14	1662	3637	651
Average		1634	3670	651
200 mg Ca +	15	0.02	3387	532
800 mg P	16	1.32	3311	645
	17	0.02	3319	567
	18	1.32	3517	633
	19	0.02	3544	578
Average		0.54	3416	591

[a]Each period was of 6 days duration.

Table 14a. Subject 3.

Aluminum Balance, mg/day

Study Condition	Period[a]	Intake[b,c]	Urine	Stool	Balance	Plasma (µg/L)
800 mg Ca	1	5.80	0.046	8.42	-2.67	39
	2	4.50	0.061	8.38	-3.94	
	3	5.80	0.054	8.29	-2.54	
	4	4.50	0.032	9.27	-4.80	
	5	5.80	0.060	54.42	-48.68	
Average		5.15	0.051	8.59	-3.49	
800 mg Ca + Maalox	6[d]	1081	0.181	933	+148	
	7	1082	0.165	1147	-65.37	
	8	1081	0.151	781	+300	
	9	1082	0.182	974	+108	99
	10	1081	0.188	1128	-47.39	103
Average		1081	0.173	993	+88.65	
800 mg Ca	11[e]	5.80	0.078	27.85	-22.13	
	12	4.50	0.041	8.13	-3.67	
	13	5.80	0.051	6.75	-1.00	
	14	4.50	0.050	7.62	-3.17	125
	15	5.80	0.047	5.60	+0.15	
Average		5.28	0.053	11.19	-5.96	

[a]Each period was of 6 days duration.

[b]Diet aluminum constant at 2.45 mg/day.

[c]Drinking water aluminum constant at 0.348 mg/day.

[d]Transition period from low to high aluminum intake.

[e]Transition period from high to low aluminum intake.

Table 14b. Subject 3.

Aluminum Balance Factors

Study Condition	Period[a]	Medications (mg Al/day)	Urine Volume (ml/day)	Stool Wt. (g/day)
800 mg Ca	1	3.00	3627	293
	2	1.70	3679	253
	3	3.00	3652	266
	4	1.70	3534	284
	5	3.00	3389	332
Average		2.48	3576	286
800 mg Ca + Maalox	6	1078	3395	473
	7	1079	3307	502
	8	1078	3405	470
	9	1079	3445	552
	10	1078	3283	559
Average		1078	3367	511
800 mg Ca	11	3.00	3543	303
	12	1.70	3485	366
	13	3.00	3534	328
	14	1.70	3530	347
	15	3.00	3420	332
Average		2.48	3502	335

[a]Each period was of 6 days duration.

Table 15a. Subject 4.

Aluminum Balance, mg/day

Study Condition	Period[a]	Intake[b,c]	Urine	Stool	Balance	Plasma (µg/L)
200 mg Ca	1	2.76	0.054	3.13	-0.42	27
	2	4.06	0.084	3.05	+0.93	
	3	2.76	0.073	4.70	-2.01	10
	4	4.06	0.066	4.90	-0.91	
	5	2.76	0.051	3.70	-0.99	
Average		3.28	0.066	3.90	-0.68	
800 mg Ca	6	5.74	0.033	3.15	+2.56	42
	7	4.44	0.033	3.55	+0.86	
	8	5.74	0.021	6.06	-0.34	
	9	4.44	0.031	3.89	+0.52	
	10	5.74	0.048	7.95	-2.26	
Average		5.22	0.033	4.92	+0.27	
800 mg Ca + Maalox	11[d]	1081	0.195	645	+436	
	12	1082	0.255	1350	-268	
	13	1081	0.184	843	+238	
	14	1082	0.248	469	+613	117
Average		1082	0.221	827	+255	
800 mg Ca	15	4.44	0.053	3.99	+0.40	
	16	5.74	0.058	7.13	-1.45	
	17	4.44	0.096	4.30	+0.05	
	18	5.74	0.111	5.57	+0.06	77
Average		5.09	0.080	5.25	-0.24	

[a]Each period of 6 days duration.

[b]Diet aluminum constant at 2.45 mg/day.

[c]Drinking water aluminum constant at 0.290 mg/day.

[d]Transition period from low to high aluminum intake.

Table 15b. Subject 4.

Aluminum Balance Factors

Study Condition	Period[a]	Medications (mg Al/day)	Urine Volume (ml/day)	Stool Wt. (g/day)
200 mg Ca	1	0.02	3315	198
	2	1.32	3174	219
	3	0.02	3230	200
	4	1.32	3290	231
	5	0.02	3242	189
Average		0.54	3250	207
800 mg Ca	6	3.00	2972	180
	7	1.70	3118	192
	8	3.00	2932	196
	9	1.70	3002	176
	10	3.00	2880	230
Average		2.48	2981	195
800 mg Ca + Maalox	11	1078	2732	453
	12	1079	2610	520
	13	1078	2662	436
	14	1079	2702	541
Average		1079	2677	488
800 mg Ca	15	1.70	2864	211
	16	3.00	2760	241
	17	1.70	2859	240
	18	3.00	2728	199
Average		2.35	2803	223

[a]Each period of 6 days duration.

Table 16a. Subject 5.

Aluminum Balance, mg/day

Study Condition	Period[a]	Intake[b,c]	Urine	Stool	Balance	Plasma (µg/L)
800 mg Ca	1	4.19	0.076	5.94	-1.83	48
	2	2.89	0.038	6.67	-3.82	
	3	4.19	0.029	9.57	-5.41	
	4	2.89	0.039	10.47	-7.62	
	5	4.19	0.059	4.90	-0.77	
Average		3.67	0.048	7.51	-3.89	
800 mg Ca + Maalox	6[d]	1079	0.120	866	+213	
	7	1081	0.151	1114	-33.28	
	8	1079	0.131	1047	+31.74	
	9	1081	0.148	1184	-103	94
	10	1079	0.143	1072	+6.73	93
Average		1 080	0.139	1057	+23.04	
800 mg Ca	11[e]	4.19	0.120	132	-127	
	12	2.89	0.037	15.01	-12.16	
	13	4.19	0.069	16.59	-12.47	
	14	2.89	0.056	12.76	-9.93	
	15	4.19	0.140	11.52	-7.48	71
Average		3.54	0.076	13.97	-10.51	

[a]Each period was of 6 days duration.

[b]Diet aluminum constant at 2.45 mg/day.

[c]Drinking water aluminum constant at 0.418 mg/day.

[d]Transition period from low to high aluminum intake.

[e]Transition period from high to low aluminum intake.

Table 16b. Subject 5.

Aluminum Balance Factors

Study Condition	Period[a]	Medications (mg Al/day)	Urine Volume (ml/day)	Stool Wt. (g/day)
800 mg Ca	1	1.32	3988	519
	2	0.02	4032	547
	3	1.32	3989	653
	4	0.02	3975	648
	5	1.32	3837	526
Average		0.80	3964	579
800 mg Ca + Maalox	6	1076	3815	594
	7	1078	3802	545
	8	1076	3813	506
	9	1078	3806	587
	10	1076	3734	492
Average		1077	3794	545
800 mg Ca	11	1.32	3846	401
	12	0.02	3984	514
	13	1.32	3834	553
	14	0.02	3797	442
	15	1.32	3784	595
Average		0.80	3849	501

[a]Each period was of 6 days duration.

Table 17a. Subject 6.

Aluminum Balance, mg/day

Study Condition	Period[a]	Intake[b,c]	Urine	Stool	Balance	Plasma (µg/L)
200 mg Ca + $ZnSO_4$	1	4.18	0.047	8.79	-4.66	
	2	2.82	0.047	8.25	-5.48	
	3	4.12	0.059	10.46	-6.40	
	4	2.82	0.087	9.41	-6.68	
	5	4.12	0.083	9.81	-5.77	
	6	2.82	0.101	7.10	-4.38	
	7	4.12	0.046	6.85	-2.78	43
Average		3.57	0.067	8.67	-5.16	
200 mg Ca +	8[d]	4347	0.503	2488	+1858	60
$ZnSO_4$ +	9	2719	0.203	2662	+56.53	89
Amphojel & Maalox	10	2718	0.162	3031	-313	
	11	2719	0.127	2307	+412	
	12	2718	0.157	3006	-288	
	13	2719	0.206	2365	+354	86
	14	2718	0.209	2276	+442	
	15	2719	0.217	3008	-289	
	16	2718	0.221	2460	+258	
Average		2899	0.223	2623	+277	
200 mg Ca +	17	2719	0.226	2117	+602	
Amphojel & Maalox	18	2718	0.237	2621	+96.50	
	19	2719	0.210	2979	-260	
	20	2718	0.221	2074	+644	
	21	2719	0.159	2235	+484	82
Average		2719	0.211	2405	+313	
200 mg Ca +	22	4605	0.193	5335	-730	81
increased Amphojel	23	4606	0.190	4735	-129	78
& Maalox						
Average		4606	0.192	5035	-430	

[a]Each period of 6 days duration, except for Periods 8 and 9, which were 4 and 8 days, respectively.
[b]Diet aluminum constant at 2.45 mg/day.
[c]Drinking water aluminum constant at 0.284 mg/day, except in Period 1, when it was 0.344 mg/day.
[d]Transition period from low to high aluminum intake.

Table 17b. Subject 6.

Aluminum Balance Factors

Study Condition	Period[a]	Medications (mg Al/day)	Urine Volume (ml/day)	Stool Wt. (g/day)
200 mg Ca + $ZnSO_4$	1	1.38	2802	457
	2	0.09	2620	345
	3	1.38	2527	436
	4	0.09	2851	391
	5	1.38	2797	412
	6	0.09	2562	389
	7	1.38	2619	433
Average		0.83	2683	409
200 mg Ca + $ZnSO_4$ + Amphojel & Maalox	8	4344	2800	825
	9	2716	2749	568
	10	2715	2872	629
	11	2716	2934	485
	12	2715	2695	506
	13	2716	2845	531
	14	2715	2902	548
	15	2716	2862	644
	16	2715	2892	520
Average		2896	2839	584
200 mg Ca + Amphojel & Maalox	17	2716	2782	387
	18	2715	2845	344
	19	2716	2832	455
	20	2715	2820	345
	21	2716	2867	350
Average		2716	2829	376
200 mg Ca + increased Amphojel & Maalox	22	4602	2853	509
	23	4603	2894	481
Average		4603	2874	495

[a]Each period of 6 days duration, except for Periods 8 and 9, which were 4 and 8 days, respectively.

Table 18. Aluminum Content of Medications Used in Balance Studies.

Medication	Source	How Supplied	Al Content
Amphojel	Wyeth Laboratories	Liquid	21.2 mg/ml
Calcium gluconate	Parke-Davis	1 g tablets	210 µg/tablet
Carmine marker	HVAH Pharmacy	567 mg capsule	7.9 mg/capsule*
Charcoal marker	HVAH Pharmacy	259 mg capsule	64.7 µg/capsule*
Maalox	Rorer, Inc.	Liquid	12.0 mg/ml
Mylanta	Stuart Pharmaceuticals	Liquid	13.8 mg/ml
Zinc sulfate	Pharmex	200 mg tablets	32.4 µg/tablet

*Includes gelatin capsule.

with Mylanta. This was followed by two periods of further aluminum excretion in the stool, then very small negative balances. The plasma levels were within the reference range.

Subject 3 was 54 years old and had osteoporosis and cerebellar degeneration. He was maintained on an 800 mg Ca/day regimen, which consisted of 5 initial control periods, 5 periods with Maalox 30 ml TID, and 5 periods after antacid supplementation. He had a small negative balance during the control periods, followed by a positive balance during Maalox ingestion. Period 5 was omitted from the average values, because of the anomalous stool value, probably due to contamination during collection. In the post-antacid periods, period 11 shows a residual excretion, followed by a return to baseline levels. The plasma level, while initially normal, rose to over twice the upper limit of normal, and this concentration persisted into the post-antacid periods. There was a significant increase in stool weight during Maalox ingestion, related to the diarrhea-producing magnesium component. An immediate return to control stool weights was seen after discontinuation of the medication.

Subject 4 was 71 years old and had osteoporosis. His regimen began with a low calcium diet, which was then increased to 800 mg/day. This level was maintained during Maalox administration (30 ml TID), and during the post-antacid periods. The balances during the control periods were essentially zero, but during Maalox supplementation a large positive balance resulted. An immediate restoration of baseline levels was observed during the post-antacid periods. The plasma values were within the reference range during the control periods, but rose to over double the upper limit of normal when Maalox was added. Twenty-four days after withdrawal from antacids, a moderate decrease was observed. The same effect of Maalox on stool weight as in subject 3 was observed.

Subject 5 was 50 years of age and had a history of osteoporosis and alcoholism. There were 5 periods of Maalox consumption (30 ml TID) in his protocol between two groups of 5 control periods each before and after antacid use. The calcium intake was 800 mg/day throughout the study, and this was taken as the lactate, which was found to be free of detectable aluminum. There was a small negative balance during the pre-antacid periods, followed by a modest positive balance through the periods with Maalox. A gradual return to baseline balance levels was observed after discontinuation of the antacid. Period 11 was omitted from the average values, because of the exceptionally high stool aluminum value, which may have been residual excretion. The plasma aluminum level was initially slightly above the upper limit of normal, and more than doubled during antacid addition. During the fifth period after cessation of Maalox, a 25% reduction was noted.

Subject 6 was 58 years old and had a history of tumoral calcinosis. This patient received a low calcium diet throughout, and zinc sulfate supplementation, during the first 16 periods. A combination of antacids (Amphogel + Maalox) was given in varying dosages in periods 8 through 23. There was a small negative aluminum balance initially, followed by large positive balances during antacid intake, except for the last group of two periods. The stool weights show diarrhea at the beginning of antacid therapy, which was stabilized by adjusting the mixture of the two antacids. Later in the study, periods 20 and 21, constipation developed, which was relieved by another dosage change, that brought out the residual aluminum in the stool; hence, the large negative balances for the final two periods. The plasma aluminum level was within the reference range before the antacids were given, but once therapy was instituted plasma aluminum doubled and remained fairly constant throughout the study.

In general, the increases in the urine aluminum levels were similar in all the subjects, being approximately tripled during antacid supplementation. The highest level observed was 0.503 mg/day in subject 6. The urine volumes showed no significant changes with the study conditions.

C. Aluminum in Maintenance Hemodialysis Patients.

Blood was drawn from 21 male patients undergoing maintenance hemodialysis, both before and after treatment, on recirculating single pass dialyzers equipped with cellophane coils. The results are shown in Table 19, where the subjects are listed in ascending order of the predialysis serum aluminum level. The values ranged from 27 to 254 μg Al/liter (1.00 to 9.42 μmol/liter). Only one patient had serum aluminum levels within the reference range both before and after dialysis, and the highest level observed was 5.5 times the upper limit of normal. Eleven patients had lower serum aluminum levels after dialysis, and 10 patients had higher levels after treatment. For this group of patients there was no statistical difference in serum aluminum values before and after hemodialysis, $P > 0.3$. The patients ranged in age from 37 to 68 years, and the period of time on dialysis from 1 to 141 months. The serum creatinine at the time of this study ranged from 9.1 to 24.8 mg/dl, and there was great diversity in the underlying renal disease. None of these factors contributed significantly to the results of this study. All 21 patients were taking varying amounts of Amphojel.

In 3 male patients undergoing hemodialysis, as above, samples of the dialysate were obtained before and after treatment, in addition to the blood samples. These aluminum levels are shown in Table 20. The dialysate was a hypotonic solution of NaCl, KCl, $MgCl_2$, $CaCl_2$, and $NaC_2H_3O_2$, and its aluminum content varied with the lots of the salts used. The solution contained 236 mOsmol/kg water. Patients 1 and 2 showed an increase in serum aluminum following dialysis, and patient 3 showed a decrease. The dialysates of patients 1 and 2 decreased in aluminum content after dialysis to approximately the same level, while the aluminum in the dialysate of patient 3 increased. All three patients were taking varying amounts of Amphojel.

Table 19. Serum Aluminum Levels Before And After Hemodialysis.

Patient	Serum Al-predialysis (µg/liter)	Serum Al-postdialysis (µg/liter)	Difference (µg/liter)
1	30	27	-3
2	41	50	+9
3	47	69	+22
4	54	41	-13
5	56	46	-10
6	60	58	-2
7	68	52	-16
8	69	73	+4
9	73	103	+30
10	74	76	+2
11	98	88	-10
12	99	111	+12
13	101	72	-29
14	101	98	-3
15	108	105	-3
16	111	129	+18
17	113	147	+34
18	140	143	+3
19	156	205	+49
20	193	186	-7
21	254	250	-4
N=21		11 decreased / 10 increased after dialysis	

Table 20. Serum and Dialysate Aluminum Levels.

Patient	Serum Al predialysis (μg/liter)	Serum Al postdialysis (μg/liter)	Dialysate Al predialysis (μg/liter)	Dialysate Al postdialysis (μg/liter)
1	111	129	141	33
2	113	147	141	27
3	37	27	4	25

In one male patient with intractable hypertension and edema undergoing hemofiltration without replacement (dry-suction) after conventional dialysis, blood samples were taken pre- and post-dialysis, and post-hemofiltration, for analysis of aluminum and protein content. The hemofiltration system employed a negative pressure parallel flow membrane with a vacuum of approximately 300 mm Hg, depending on the patient's blood pressure. The results in Table 21 reflect analyses performed on samples from consecutive treatments two days apart. On day 2 of the study the hemofiltration was discontinued after 40 minutes because of hypotension (110/70 mm Hg), and approximately 300 ml Ringer's lactate was administered. Following conventional dialysis there were small increases in the serum aluminum, but this was due to a concentration effect, as reflected by the increased protein content and the constant aluminum to protein ratio, and not to a net gain of aluminum. Following hemofiltration, the protein content increased further, as approximately one liter of fluid was removed on each day. However, the aluminum content showed an actual loss, as evidenced by the decreased aluminum to protein ratios. These ratios were fairly constant from one treatment to the next; and, in addition, the serum aluminum level, after dropping to 114 from 140 μg/liter, had returned to 138 μg/liter by the next treatment day. This patient was taking Amphojel 30 ml QID.

Table 21. Changes in Aluminum Content of Serum During Treatment.

	Conditions	µg Al/liter	Total Protein, g/dl	µg Al/g Protein
Day 1	Predialysis	140	7.21	1.94
	Post 3 hours hemodialysis	143	7.39	1.94
	Post 1 hour dry-suction	114	7.57	1.51
Day 2	Predialysis	138	7.39	1.87
	Post 3 hours hemodialysis	148	7.81	1.90
	Post 40 min dry-suction	121	8.34	1.45

CHAPTER V

DISCUSSION

Campbell and Ottaway (144) point out the importance of the anion component of the standard solution. Many papers dealing with atomic absorption spectrophotometry with a graphite tube do not indicate the anion composition (39). Although $AlCl_3$ is known to sublime at 178 °C some workers (141) have used this salt as the standard. Independent of the anion, the furnace conditions are such that all compounds are converted to Al_2O_3 before the atomizing temperature is reached (133, 144). It then is questionable if some $AlCl_3$ vaporizes before being converted to the oxide. That this happens is suggested by the lower absorbance values obtained with standards in hydrochloric acid compared to those in sulfuric acid. This is of special concern for biological materials that are high in chloride. Absorption of such materials can be increased by the addition of sodium sulfate to change the proportion of anion present as the chloride. That this is not necessary in practice was found when serum and urine were analyzed in the presence and absence of added sodium sulfate. Since the same results were obtained in both cases, it was elected not to add the sulfate, and thereby avoid a source of contamination.

A variety of programs have been used for the graphite furnace. Agreement as to the charring and atomizing temperatures and times is uniform. Drying of aqueous standards and urine requires a temperature near 100 °C, but if serum is dried at this temperature, the charring leaves a mound of carbon at the sample site which interferes with subsequent measurements, unless it is removed. Fuchs et al. (147) avoided this by first drying for 60 s at 100 °C and then for 60 s at

300 °C. By observing the sample during the drying and charring stages, it was found that drying serum at 350 °C thoroughly dried the sample and permitted reproducible results. Early in the drying, the sample bubbled a little, it then formed a small mound and started to decompose. By the time the charring step was reached, the residue was flat in the graphite tube, and the carbon combusted completely. An occasional sample of serum would leave non-combustible carbon in the tube, so that it is advisable for the operator to observe the inside of the tube before adding a new sample of serum.

The method described offers advantages over existing techniques for the quantitation of aluminum in serum and urine. It is adequately sensitive and, as it requires no additional reagents, it is less prone to contamination. The sensitivity is greater than that reported by Blotcky et al. (137) for the analysis of aluminum in urine by neutron activation.

This allows for use of a very small, unadulterated sample, eliminating many contamination-prone steps. Once the instrument has been adjusted to the appropriate parameters, the analysis itself is exceedingly simple to perform and produces results rapidly. Perhaps the most appealing factor is that it is readily adapted to routine clinical use. Whereas, most clinical laboratories have no access to the instrumentation required for such techniques as neutron activation analysis, they do have atomic absorption equipment available.

There are two reports of short-term net aluminum absorption studies in man in the literature. The first (149) was done in patients with chronic renal failure, and the aluminum absorption was reported to be 100-568 mg/day during administration of aluminum antacids. In this study urine and stool aluminum were determined in perchloric acid digests with the nitrous oxide-acetylene flame. In addition, sodium chloride was added to all samples. These results may be low, due

to insufficient sensitivity of the method, as the authors related an inability to detect aluminum in unconcentrated urine samples. They found an increase in plasma aluminum during aluminum hydroxide administration of 13 μg/liter by neutron activation. The second study (150) was in "normal subjects" and in patients with chronic renal failure, but the subjects were not age-matched. The average age of the control group was 25 years, while the two patients with renal failure were 55 and 64 years of age. The methods used were the same as in the first study cited, and again the authors were unable to detect aluminum in the urine, so that this variable was excluded from the balances. In the control group the maximum absorption of aluminum was 97 mg/day, while one renal failure patient absorbed slightly less than this, and the 55 year-old 2.5 times as much. The aluminum intake was 2400 mg/day. These investigators concluded that during aluminum hydroxide administration normal persons absorb less aluminum than patients with renal failure, and may absorb none at all. They suggested that there may be an abnormality in the gut wall in these patients, making it more permeable to aluminum.

In our project many more metabolic periods were studied to provide a clearer picture of the fate of ingested aluminum. During antacid administration aluminum balances ranged from 23 to 313 mg/day, which is higher than reported by the study cited above (150). Aluminum was detectable in every urine sample, but did not contribute significantly during the periods of antacid supplementation. This supports the view of Szczekocki and Chmielewski (151), that the kidneys play only a small role in aluminum excretion, and that the main route of elimination is the alimentary tract. This is in contrast to the opinion of Recker et al. (152), who stated that the kidneys can effectively remove absorbed aluminum.

Plasma aluminum levels approximately doubled during antacid administration in every subject. The values for the aluminum content of the diets

averaged just over 2 mg/day, which is lower than that reported by the groups of researchers, whose results were summarized by Sorenson et al. (1), except for the studies by White and by Gormican. The finding of small negative aluminum balances during the control periods is consistent with the work of others (153-4). The prolonged absorption of these levels of aluminum in our studies, up to 0.3 g/day, must lead to deposition in some body store, and Recker et al. (152) have given evidence that the target organ is bone. The average aluminum excretion above baseline values after discontinuation of the antacids was 3.20 mg/day.

As stated previously, the role of the aluminum antacids in renal failure is to limit the accumulation of phosphate, and the mechanism by which this occurs was the subject of the work done in 1941 by Freeman and Freeman (155). They could not decide whether phosphate was reduced only by a decreased absorption, or whether there was also some removal of phosphate from the tissues by aluminum hydroxide. Fauley et al. (156) found that both mechanisms occurred in the intestine. In addition, aluminum phosphate can deposit in the tissues (157). Spencer et al. (158) found, using metabolic balance studies, that these antacids not only inhibit phosphorus absorption, but also increase the urinary and stool excretion of calcium. This loss of calcium via the intestine can lead to uremic bone disease, as can the phosphorus depletion. Too little control of serum phosphate can produce uremic osteodystrophy, while overcontrol can result in osteomalacia. Many reports have been published in this area (159-166). The hyperparathyroidism produced by the reduced clearance of phosphorus has been examined recently for the role it plays in aggravating the uremic bone disease (52), and because of the indications that hyperparathyroidism may promote an increased absorption of aluminum (167). Goldsmith and Johnson (168) have published a dialysis program, the goal of which is to monitor and control serum calcium and phosphorus through antacid therapy and dialysate composition.

Berlyne et al. (107, 169) have stated that there is no risk of aluminum toxicity in hemodialysis patients, because it is dialyzed out. The work presented here shows that dialysis has no effect on serum aluminum levels. To be removed by dialysis, a compound must display little tissue and protein binding, have a low molecular weight, and be primarily excreted by glomerular filtration (170). A major portion of serum aluminum has been found to be protein-bound (171). Our work tends to substantiate this claim, in that the aluminum was not dialyzable, and only a small amount could be removed by hemofiltration. DeBoni et al. (102) have proposed that there may be carrier molecules in blood for aluminum, as there are for other metals, and that if these become saturated, whether through antacids or dialysate, the aluminum may attach to another constituent that readily crosses the blood-brain barrier. Further aluminum load could then precipitate the metal into the neurons. That this may occur is evidenced by the plasma levels obtained in our metabolic balance studies, particularly in subject 6, which reached a plateau, and did not increase further with additional aluminum absorption.

The studies in which the dialysates were collected point to the fact that the contaminating aluminum content of these solutions may be infused into a patient, where presumably it is rendered non-dialyzable by binding to protein. The hemofiltration studies show that at least a portion of the aluminum is filtrable, and that through meticulous attention to the aluminum concentration of the dialysate before treatment this fraction may be dialyzable, as in patient 3 in Table 20. The data from these studies and from the metabolic balance studies demonstrate, though, that there are two sources contributing to aluminum loading in these patients, namely aluminum-containing antacids and aluminum in the dialysate. Whatever the source, the mounting circumstantial evidence strongly favors aluminum as the causative agent of dialysis dementia.

CHAPTER VI

SUMMARY

Aluminum, generally considered non-essential and non-toxic, may accumulate in toxic amounts in the brain in cases of chronic renal failure. The literature, relating to the determination of aluminum, its uses in medications, effects on various biological systems, and role in neurological disorders, is reviewed.

A procedure is described for the analysis of aluminum in biological samples, that requires no sample preparation for serum and urine, and is sensitive at the appropriate concentrations by atomic absorption spectrophotometry with a graphite furnace. Samples are pipetted into the interior of a graphite tube, where they are sequentially dried, charred, and atomized. Precautions for sample handling are discussed, and instrument settings are defined. Precision and accuracy of the method are evaluated, as are the effects of salts, protein content of serum, and specific gravity of urine. Serum (N=24) and urine (N=11) of persons not consuming aluminum-containing antacids contains 27 $\pm$ 9 (SD) µg Al/liter and 45 $\pm$ 32 (SD) µg Al/24h, respectively.

Metabolic balance studies are presented for patients given aluminum-containing antacids under strictly controlled conditions. The aluminum contents of the diets, drinking water, and medications are compared to those of complete urine and stool collections. During control periods small negative balances were obtained, while during antacid supplementation large positive balances occur, ranging from 23 to 313 mg Al/day. Plasma levels during the various study

conditions showed a marked increase to a plateau while aluminum antacids were given and a gradual decline after withdrawal of the medications.

Hemodialysis had no effect on serum aluminum levels, which ranged from 27 to 254 μg Al/liter. Hemofiltration was found to remove a portion of the serum aluminum, but the majority remained bound to a non-filtrable component. Aluminum in the dialysate was also found to contribute to aluminum loading in these patients. These results are evaluated in light of present knowledge concerning dialysis dementia.

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The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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