Aluminum (^{26}Al) Metabolism in Rats (44159)

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Abstract. **Because of the lack of a suitable isotope and a sensitive technique of analysis, aluminum has been studied indirectly using analogs such as ^{67}Ga ($t_{1/2}$ = 78 hr). Recently, with the development of accelerator mass spectrometry (AMS), it has become possible to use the artificially produced radionuclide of aluminum, aluminum 26 (^{26}Al), ($t_{1/2}$ = 7.16 × 10^5 years). AMS is used for measuring long-lived and stable isotopes with the sensitivity of an attomole (10^{-17} mol). To study aluminum metabolism, $^{26}AlCl_3$ was administered to rats intraperitoneally (ip) by injection and orally by gavage (n = 3/group). Blood was collected periodically. On Day 8 following perfusion, blood, liver, kidney, femur, brain, and spleen were collected and analyzed for ^{26}Al. Of all the tissues studied, ^{26}Al accumulation was greatest in the bone. ^{26}Al accumulated in tissues as: bone > spleen > kidney ≃ liver > brain, but absorption was low (0.97% of dose). AMS offers great potential in Al research as it is the only technique available for tracer aluminum study.** [P.S.E.B.M. 1997 Vol 216]

Aluminum has been considered a toxic substance since it was first isolated from patients on dialysis treatment or patients suffering from Parkinson's disease, Huntington's disease, or Alzheimer's disease (1). In healthy individuals, its absorption is low and excretion efficient. Despite low intakes of 10–12 mg/day from food (2), the body burden of aluminum in healthy individuals is 30–50 mg (3). Due to renal malfunction, inefficient excretion of aluminum from the body results in high accumulation in brain and bones causing dialysis encephalopathy or osteomalacia (4).

Recently, use of accelerator mass spectrometry (AMS) for analyzing aluminum 26 (^{26}Al) has expanded the potential for aluminum research (5–7). ^{26}Al, which has a half-life of 716,000 years, is the only practically useful radioisotope of aluminum. An advantage of using AMS is that it measures individual atoms and not radioactivity. Therefore, a very small quantity of ^{26}Al is required in research, which reduces radiation exposure of the subjects. AMS has the capability of measuring as little as 10^{-11} μCi of ^{26}Al in biological samples with a precision of 5%–10%. Since no ^{26}Al is found in nature, contamination of the samples from background is zero.

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Techniques for quantifying total aluminum, including electron microprobe x-ray microanalysis or graphite furnace atomic absorption, are vulnerable to background contamination. The objective of this study was to determine the gastrointestinal absorption and tissue distribution of aluminum using AMS in a rat model.

Materials and Methods

Six male Sprague-Dawley rats (160–180 g; Harlan, Indianapolis, IN) were divided into two groups and housed individually in stainless steel cages in a room with 12-hr reversed day-night cycle. They had free access to a semipurified AIN 76A nutritionally adequate diet (8) and deionized water. Each rat in Group 1 was given an oral dose of 330 pCi ^{26}Al as $AlCl_3$ (2.6 mg total aluminum) (aluminum chloride; Radio Chemicals, ICN Irvine, CA) in 0.5 ml normal (0.9%) saline solution at pH 3.0 by gavage. Each rat in Group 2 was given 11 pCi ^{26}Al as $AlCl_3$ (86 μg total aluminum) in 0.5 ml normal (0.9%) saline solution at pH 5.0 injected in the peritoneal cavity. After dosing, 0.1–0.2 ml of blood was taken from the tail of the rats at 5, 24, and 48 hr. On Day 8, all the rats were anesthetized with an intraperitoneal (ip) injection of ketamine/xylene. Tissues were perfused with 0.9% saline by injection in the carotid artery, excised, and weighed. Liver, kidney, spleen, femur, brain, and blood were stored at −20°C for later analysis of ^{26}Al by AMS.

Except for bone and blood, all the tissues were well homogenized by mincing with a separate stainless stell scalpel blade. Carrier solution, prepared as 25 mg of ^{27}Al/ml in 50% nitric acid, was added to homogenized tissue samples (200–500 mg) to give an estimated ^{26}Al/^{27}Al ratio of 5 ×

10^{-11}. The aluminum content of the carrier solution was verified by Graphite Furnace Atomic Absorption Spectrometry (Model 170-70 polarized Zeeman; Hitachi, Tokyo, Japan). The tissue and carrier mixture was vortexed, freeze-dried, and ashed in a muffle furnace (Type 30400, Thermolyne-Sybron, Dubuque, Iowa) at 600°C for 48 hr. The remaining ash was redissolved in 25% nitric acid and re-dried in an oven where the temperature was gradually increased to 200°C. The white ash produced was then redissolved in 25% nitric acid. Tissue preparation methods and mineral analysis were verified by analyzing chicken liver and NBS standard bovine liver (9) to which a known quantity of zinc, copper, and gallium 67 (^{67}Ga) had been added. Recovery of the three elements was 99%–102%. The percent recovery of ^{26}Al is measured by the ratio of ^{27}Al added as carrier to the samples assuming ^{27}Al is well homogenized with ^{26}Al in the given samples. The addition of the carrier was tested to ensure that it was homogenized by the preparation of four tissues in triplicate. These replicate samples agreed within the 10% precision of AMS.

The femur and blood samples were combined with a known quantity of carrier solution and 2–3 ml of concentrated nitric acid. They were allowed to digest overnight, dried in an oven at 200°C, and redissolved in 25% nitric acid for ^{26}Al analysis. All the tissues, blood and femur samples were dried and baked at 800°C to convert aluminum to Al_2O_3. The ratio of ^{26}Al/^{27}Al in the samples was determined by AMS at PRIME Lab (Physics Department Purdue University, West Lafayette, IN) (10). ^{26}Al concentration was then calculated as the percentage of dose per gram in each sample. This method of calculation eliminates the concern of different dosages used for oral and ip routes of administration.

Percent absorption of ^{26}Al each rat was determined as the ratio of oral to ip dose in various tissues by the formula:

$$\% \text{ Absorption} = \frac{\% \text{ Oral Dose/g tissue}}{\% \text{ ip Dose/g Tissue}} \times 100$$

Because ip administration bypasses the gut, the fraction of an ip dose that reaches a given tissue provides a reference for 100% absorption.

Results

Disappearance of ^{26}Al from blood after oral and intraperitoneal administration is shown in Figure 1. Blood concentration of ^{26}Al was higher when administered as an intraperitoneal injection. Most of the aluminum is excreted rapidly from either an oral or ip dose.

Figure 2 shows tissue ^{26}Al as percent retention from

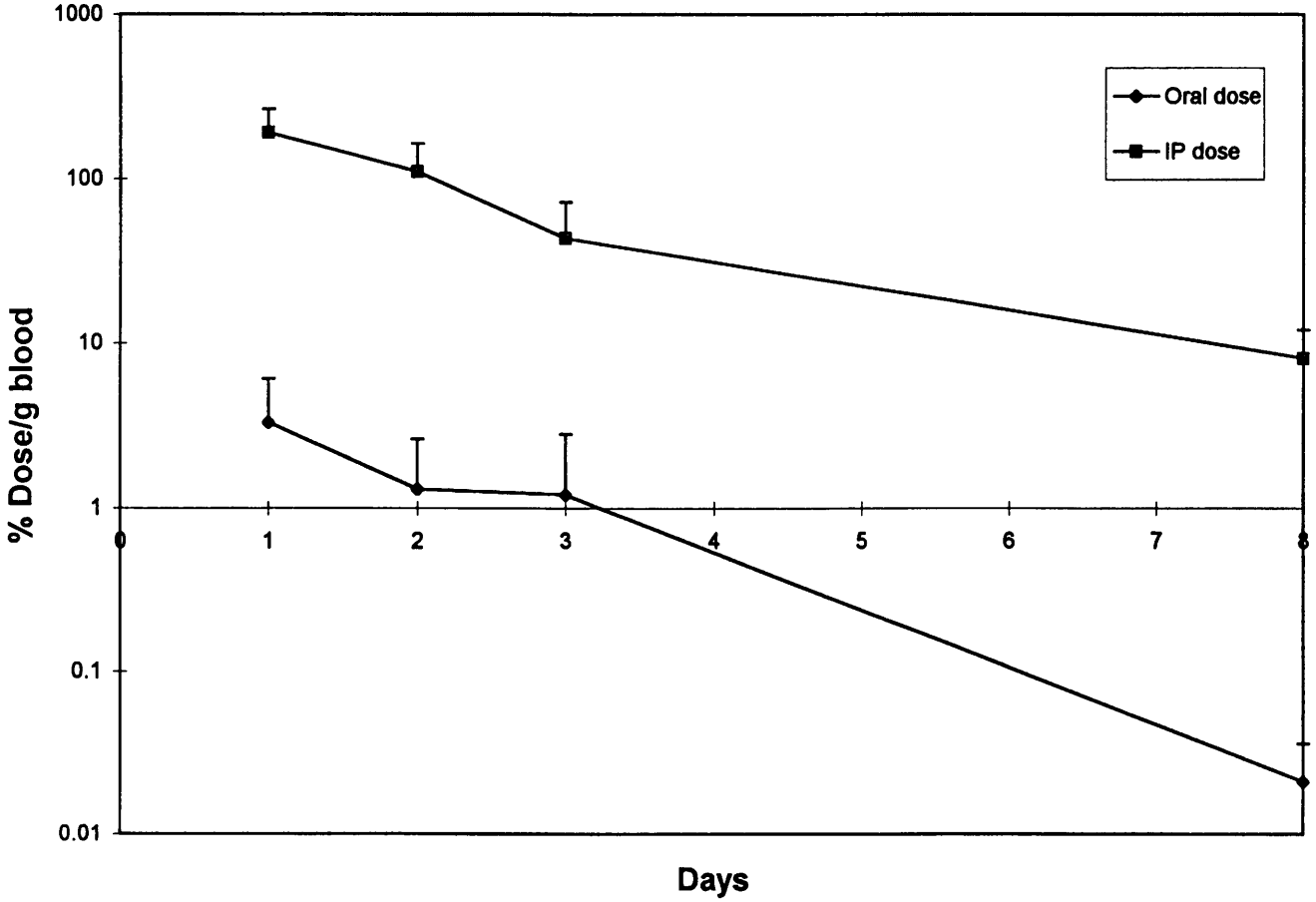

Figure 1. ^{26}Al in blood following oral and intraperitoneal administration in rats. Points represent mean ± SD (n = 3).

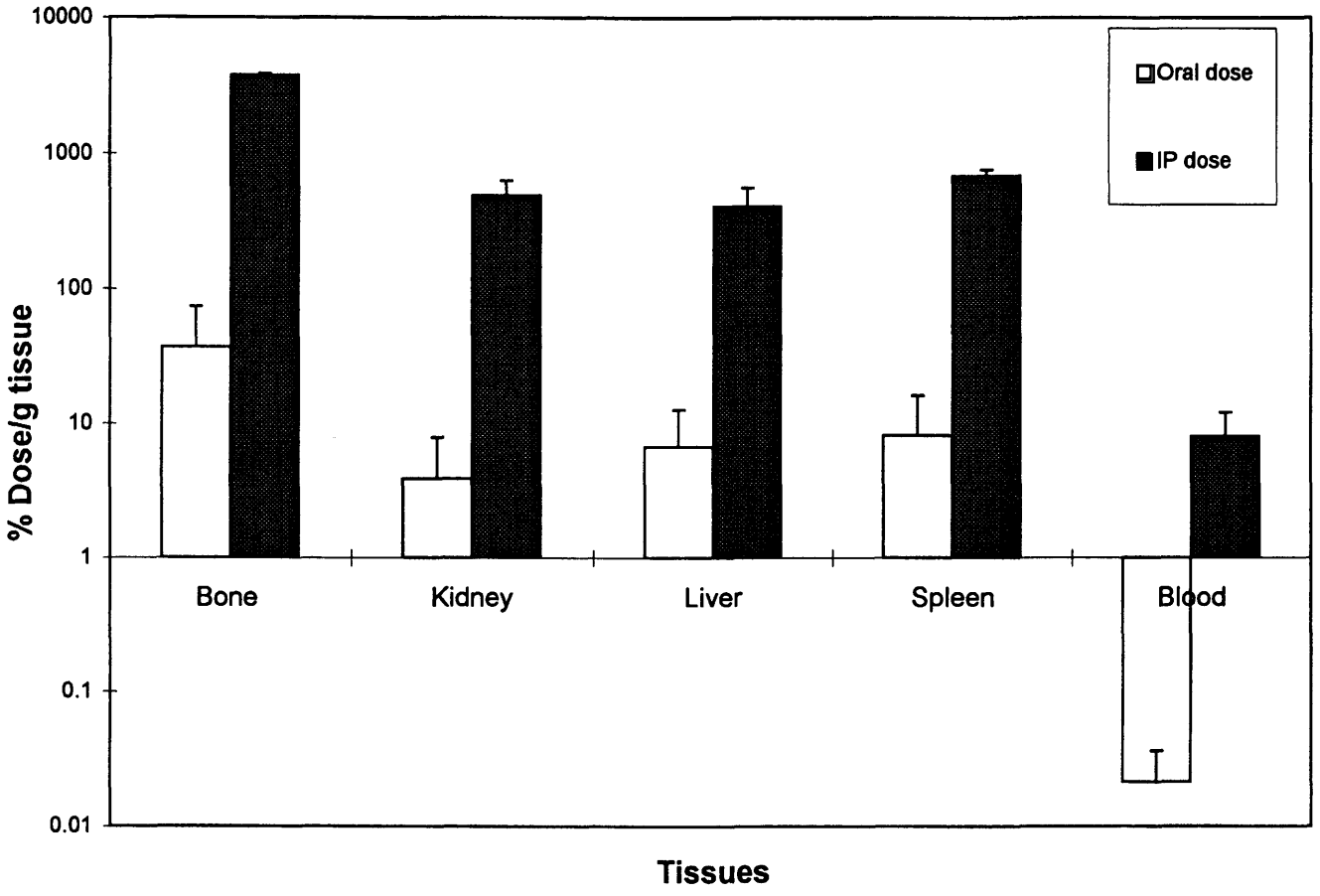

Figure 2. Mean ^{26}Al content in rat tissues at Day 8 after isotope administration (mean ± SD, n = 3).

both oral and ip administration on Day 8 following dosing. Most of the aluminum is excreted rapidly from either an oral or ip dose. Retention of ^{26}Al followed the order: bone > spleen > kidney ≃ liver > brain. Table I shows the percent intestinal absorption of ^{26}Al calculated by comparing retention of the oral versus the intraperitoneal dose in the various tissues. Absorption averaged from the five tissues was 0.97% ± 0.22% of the dose.

Discussion

Excessive quantities of aluminum have been implicated as a causative factor in dialysis dementia and osteomalacia. It is imperative to understand the absorption and retention of aluminum from usual daily intakes in healthy individuals to estimate accumulation with age. Recently, with AMS and ^{26}Al, it has become possible to administer low levels of aluminum to research subjects in order to study its metabolism without disturbing the physiological steady-state conditions.

Appearance of ^{26}Al in the blood and calculated absorption in rats following an oral dose was very small. Appearance of ^{26}Al in the blood (Fig. 1) was similar (0.003%/10 ml) to a previous report in rats where plasma uptake averaged 0.001%/10 ml (11). In one human subject, a 1% retention in plasma was reported after 6 hr of ^{26}Al ingestion assuming a total plasma volume of 3 liters (12). This value was two orders of magnitude higher than that calculated for the rat study. However, Day *et al.* (12) used excess citrate, which enhances absorption of aluminum (7, 13). Both of these investigators calculated absorption based on an assumed knowledge of plasma volume in rats or humans, and they assumed that plasma appearance after 6 or 8 hr represented absorption. Our approach did not require these assumptions because absorption was calculated after adjusting for the amount of isotopes in plasma or other tissues from an

Table I. Percent Absorption[a] of ^{26}Al determined from Relative Accumulation of Oral and ip Doses in Various Tissues

Tissue	^{26}Al
Bone	0.97 ± 0.57
Kidney	0.79 ± 0.46
Liver	1.64 ± 0.95
Spleen	1.19 ± 0.69
Blood (Day 8)	0.26 ± 0.15
Intestinal absorption[a]	0.97 ± 0.22

Note. Values are mean ± SEM (n = 3).
[a] Mean percent intestinal absorption derived from % dose retained in five tissues at Day 8 and calculated as: % Oral Dose ÷ % ip Dose in the Same Tissue × 100.

intraperitoneal dose at any given time point. This resulted in an estimate for ^{26}Al absorption of 0.97% of the dose. This standard approach has been used for determining absorption from other minerals (14) and is also useful for quantitating aluminum absorption.

^{26}Al clears from the blood quickly. After 5 hr, only 0.02% of the intraperitoneal dose per gram remained in the blood (Fig. 1). Approximately 75% of the dose was reported (15) to be excreted in the urine within 24 hr from an intravenous dose in one rat. ^{26}Al retention was highest in the bones and lowest in the brain. Figure 2 shows the descending order of tissue aluminum concentration as: bone > spleen > kidney ≃ liver > brain both from oral and ip doses on Day 8. A similar order of tissue aluminum content from an intravenous dose was reported in a single rat sacrificed 21 days after the dose (16).

Despite the low level of absorption and rapid excretion from normal adult individuals, aluminum accumulation may still lead to health problems due to chronic overexposure. Aluminum is a common ingredient in antiperspirants. Aluminum is added in food during preparation, processing, and cooking. It is found in many food additives including leavening agents, preservatives, stabilizers, emulsifiers, thickeners, curing agents, and anticaking compounds. It is present naturally in plants, animals, and water. Purified water is further clarified using aluminum salts, which contributes aluminum to the diet. Foods are wrapped in aluminum foils and cooked in aluminum pots, which also contributes some aluminum to the food we eat. According to the FDA Total Diet Study, commonly consumed foods contain from 1 mg to 26 mg aluminum per serving, and the minimum daily consumption is estimated to be almost 12 mg (2). Exposure is often more, depending on food choices, cooking, and food storage practices of individuals. According to the FDA Total Diet Survey report, this level of aluminum ingestion is not of a concern. However, an individual's vulnerability to low-level aluminum accumulation over time is not known. Low intakes of aluminum from drinking water and aluminum cooking utensils over time has been associated with dementia type of disorders (17, 18) and osteoporotic fractures (19) in epidemiological studies.

Infants are another vulnerable subset of the population because of their underdeveloped gastrointestinal barrier to aluminum absorption. In a study of infant formula aluminum content and absorption, soy-based formula was reported to be the highest in aluminum content, while human milk was the lowest (20). However, the most aluminum was absorbed by infants from mother's milk, suggesting it was the most bioavailable form. Rats' milk has been found to be high in aluminum when fed a high-aluminum diet (21). Thus, because of potential health risks, it is critical to develop sensitive techniques to characterize Al metabolism accurately.

To study low-level aluminum metabolism requires a tracer. Many investigators have previously used ^{67}Ga as a marker for aluminum. However, whole-body retention of ^{67}Ga was markedly higher than Al in one human subject after intravenous injection (22), thereby suggesting Ga does not mimic Al metabolism.

^{26}Al is a preferred tracer for the study of aluminum metabolism. The limitations of AMS are the limited availability of the instrument and the complexity of analysis, which limits the number of samples feasible for each study. However, despite these limitations, AMS is becoming more widely utilized and is unparalleled in its ability to be used in tracer studies for aluminum metabolism.

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1. Edwardson JA, Oakley AE, Pullen RGL, McArthur FK, Morris CM, Taylor GA, Candy JM. Aluminum and the pathogenesis of neurodegenerative disorders. In: Massey RC, Taylor D, Eds. Aluminum in Food and the Environment. London: Royal Society of Chemistry, Vol **73:**20–36, 1989.
2. Pennington ATJ, Schoen AS. Estimates of dietary exposure to aluminum. Food Addit Contam **12:**119–128, 1995.
3. Wills MR, Savory J. Aluminum poisoning: Dialysis encephalopathy, osteomalacia and anemia. Lancet **2:**29–34, 1982.
4. Scientific Review Committee. Nutrition Recommendations. (report) Bureau of Nutritional Sciences, Department of National Health and Welfare, Ottawa, Canada: Scientific Review Committee, 1990.
5. Middleton R, Klein J. ^{26}Al: Measurement and applications. Mol Trans R Soc Lond A **3233:**121–143, 1987.
6. Elmore D, Phillips FM. Accelerator mass spectrometry for measurement of long-lived radioisotopes. Science **236:**543–550, 1987.
7. Day JP, Barker J, King SJ, Miller RV, Templar J, Lilley JS, Drumm PV, Newton GWA, Fifield LK, Stone JOH, Allan GL, Edwardson JA, Moore PB, Ferrier LN, Priest ND, Newton D, Talbot RJ, Brock JH, Sanchez L, Dodson CB, Itzhaki RF, Radunovic A, Bradbury MWB. Biological chemistry of aluminum studied using ^{26}Al and accelerator mass spectrometry. Nuclear Instruments and Methods in Physics Research **B92:**463–468, 1994.
8. Bieri JG. Second Report of the Adhoc Committee on Standards for Nutrition Studies. J Nutr **110:**1726, 1981.
9. National Bureau of Standards (NBS). Bovine Liver. Standard Reference Material 1577a. Gaithersburg, MD: Office of Standard Reference Materials, February 1, 1985.
10. Elmore D, Dep L, Flack R, Hawksworth MJ, Knies DL, Ma XZ, Michlovich ES, Miller TE, Mueller KA, Richey FA, Sharma P, Simms PC, Woo JH. The Purdue rare isotope measurement laboratory. Nuclear Instruments and Methods in Physics Research **B92:**65–68, 1994.
11. Jouhanneau P, Lacour B, Raisbeck G, Yiou F, Brown E, Drueke T. Gastrointestinal absorption of aluminum in rats using ^{26}Al and accelerator mass spectrometry. Clin Nephrol **40:**244–248, 1993.
12. Day JP, Barker J, Evans LJA, Ackrill P, Lilly JS, Drumm PV, Newton GWA. Aluminum absorption studied by ^{26}Al tracer. Lancet **337:**1345, 1991.
13. Patridge NA, Regnier FE, Reed WM, White JL, Hem SL. Contribution of soluble aluminum species to absorption of aluminum from the rat gut in situ. Clin Sci **83:**425–430, 1992.
14. Koo JK, Weaver CM, Neylan MJ, Miller GD. Isotopic tracer techniques for assessing calcium absorption in rats. J Nutr Biochem **4:**72–76, 1993.
15. Meirav O, Sutton ALR, Fink D, Roy M, Klein J, Walker RV, Halabe A, Vetterli D, Johnson R. Accelerator mass spectrometry: Application to study of aluminum kinetics in the rat. Am J Physiol **260:**F466–F469, 1991.
16. Walker RV, Sutton ALR, Meirav O, Sossi V, Johnson R, Klein J, Fink

D, Middleton R. Tissue disposition of ^{26}Al in rats measured by accelerator mass spectrometry. Clin Invest Med **17:**420–425, 1994.
17. Martyn CN, Barker DJP, Osmond C, Harris EC, Edwardson JA, Lacey RF. Geographical relation between Alzheimer's disease and aluminum in drinking water. Lancet **1:**59–62, 1989.
18. Forbes WF, Gentlemen JF, Maxwell CJ. Concerning the role of aluminum in causing dementia. Exp Gerontol **30:**23–32, 1995.
19. Cummining GR, Klineberg JR. Aluminum in antacids and cooking pots and the risk of hip fractures in elderly people. Age Ageing **23:**468–472, 1994.
20. Hawkins MN, Coffey S, Lawson SM, Delves TH. Potential aluminum toxicity in infants fed special infant formula. J Pediatr Gastroenterol Nutr **19:**377–381, 1994.
21. Muller G, Hutin MF, Burnel D, Lehr P. Aluminum transfer through milk in female rats intoxicated by aluminum chloride. Biol Trace Elem Res **34:**79–87, 1992.
22. Priest ND, Newton D, Day JP, Talbot RJ, Warner AJ. Human metabolism of aluminum-26 and gallium-67 injected as citrates. Hum Exp Toxicol **14:**287–293, 1994.