# The Biological Speciation and Toxicokinetics of Aluminum

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This review discusses recent literature on the chemical and physiological factors that influence the absorption, distribution, and excretion of aluminum in mammals, with particular regard to gastrointestinal absorption and speciation in plasma. Humans encounter aluminum, a ubiquitous yet highly insoluble element in most forms, in foods, drinking water, and pharmaceuticals. Exposure also occurs by inhalation of dust and aerosols, particularly in occupational settings. Absorption from the gut depends largely on pH and the presence of complexing ligands, particularly carboxylic acids, with which the metal can form absorbable neutral aluminum species. Uremic animals and humans experience higher than normal body burdens of aluminum despite increased urinary clearance of the metal. In plasma, 80–90% of aluminum binds to transferrin, an iron-transport protein for which receptors exist in many tissues. The remaining fraction of plasma aluminum takes the form of small-molecule hydroxy species and small complexes with carboxylic acids, phosphate, and, to a much lesser degree, amino acids. Most of these species have not been observed *in vivo* but are predicted from equilibrium models derived from potentiometric methods and NMR investigations. These models predict that the major small-molecule aluminum species under plasma conditions are charged and hence unavailable for uptake into tissues.
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Aluminum is one of the most abundant elements in the environment. Some human exposure is unavoidable — daily intake is largely oral and averages 30–50 mg (*1*). Of this, no more than about 7 mg is expected to come from water, based on the maximum reported concentration of aluminum in drinking water (*2*) and the average consumption of 2 l water/day. Inhalation exposure is generally negligible, though it can be significant in some occupational settings, as described below. The contrast between widespread occurrence and relatively low intake underscores the importance of speciation in determining the bioavailability of aluminum, as this metal is of limited solubility in its environmentally occurring forms. Despite generally low exposures, the toxicity of aluminum, particularly to the nervous system, is of concern. Much research on aluminum in recent years has focused on its role in the etiology of Alzheimer's disease (AD), but epidemiologic studies attempting to link aluminum with AD in drinking water have been inconclusive and contradictory. This review examines sources of aluminum and the factors determining its absorption, distribution, and elimination in the body, with particular reference to the literature and analytical techniques developed within the last 10 years.

The chemical forms, or species, of aluminum formed in the body have important implications for the balance between the metal's uptake into tissues and its excretion. The study of aluminum speciation has presented a number of major difficulties to researchers. First of all, tracer studies with the aluminum isotope $^{26}Al$ are very expensive due to the rarity and low activity of the nucleus and also because of the cost of using accelerator mass spectrometry. Second, because of aluminum's ubiquity in the environment, contamination of samples (from dust particles, for example) is difficult to avoid. Finally, aluminum metal, mineral forms, and some salts are very insoluble except at extremes of pH or in the presence of chelating ligands.

## Aluminum and Alzheimer's Disease

There has been considerable interest and controversy concerning the relationship between aluminum in drinking water and AD. A number of studies (*3–8*) found correlations between aluminum concentrations in drinking water and the incidence of AD, although the relative risk of AD for those exposed to the highest aluminum concentration, compared to those exposed to the lowest aluminum concentration, was less than 2 in each case. Michel et al. (*9*) found a relative risk of about 4 for those exposed to the highest aluminum concentration (100 µg/l) compared to the lowest (10 µg/l). Studies failing to find an association were reported by Wood et al. (*10*) and Wettstein et al. (*11*). Although drinking water aluminum constitutes only a small percentage of total daily aluminum intake, Martyn et al. (*5*) suggested that aluminum in drinking water "is either dissolved or readily brought into solution." The aluminum in drinking water may therefore exist as species that are more readily absorbed than those from other sources of aluminum. However, there are no reports on the oral bioavailability of various aluminum species in water compared to species found in food or other sources. Continuation of the Michel et al. (*9*) study failed to show an association between drinking water aluminum and cognitive impairment when water pH was not considered. They observed a positive association up to pH 7.3, and a negative association at higher pH (*12*), suggesting a pH-dependent change in the aluminum species composition in water. In a review of most of the studies of drinking water aluminum and AD, Doll (*13*) pointed out that low pH was reported in several of the studies showing a positive association between aluminum concentration and AD; one such study was that of Frecker (*14*). In contrast, the water had a high pH in one of the studies that failed to show an association (*11*).

Odds ratios calculated by Forbes and McAiney (*8*), using a logistic regression model for the association between water pH and impaired mental functioning, suggest that medium pH, and to a lesser extent high pH, are protective relative to low pH. However, the authors did not state what variables were being controlled for in the analysis. Forbes et al. (*15*) and Forbes and McAiney (*8*) reported results suggesting a protective effect of fluoride on aluminum-associated dementia. This was not substantiated by the results of Jacqmin et al. (*12*). A protective effect of fluoride might be explained by a fluoride-associated decrease in aluminum bioavailability, as suggested by the results of Ondreicka et al. (*16*).

A number of criticisms of the above studies can be made. First of all, most do not demonstrate a dose–response relationship. Aluminum exposure measured over the short-term may not be a good surrogate for lifetime exposure, which is especially important given that AD is a chronic disease that may have a long latency period. Consequently, it is also difficult to establish temporality in the exposure–disease relationship. Also at issue are the imprecision in AD diagnosis and the error

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in measurement and extrapolation of data on exposure to aluminum in drinking water. Studies with $^{26}Al$ to determine the influence of other chemical species and pH of drinking water on oral aluminum bioavailability from food and water might help determine whether enough aluminum is absorbed from water to impact on aluminum body burden. However, such studies will not directly address the role of aluminum in AD. Finally, only a small percentage of total oral daily aluminum intake comes from drinking water.

## Specific Sources of Exposure

The aluminum content of foods has been reviewed by Pennington (*17*) and Greger (*18*). Common food additives containing aluminum are acidic sodium aluminum phosphate, a leavening agent, and basic sodium aluminum phosphate, an emulsifier. Aluminum is also found in food colorings, and anticaking agents may contain aluminosilicates. Processed cheese (a 28-g serving, with 297 µg aluminum/g, has 8 mg) and cornbread (18 mg in a 45-g serving with 400 µg aluminum/g) are major contributors to high aluminum exposures in the American diet. The high aluminum content of these foods is largely due to additives. Another significant dietary source of aluminum is soy-based milk products, which contribute as much as 2.1 mg aluminum/day, based on the typical intake of an infant; this exposure is of particular concern for infants suffering from renal deficiency (*18*).

There has been concern in recent years about leaching of aluminum from beverage cans and cookware. Aluminum beverage cans are generally coated with a polymer that prevents leaching. The average concentration of aluminum in cola drinks was found to be only 0.1 µg/g. Aluminum cookware, however, may leach aluminum into highly basic or acidic foods. Tomato sauce cooked in aluminum pans was found to accumulate 3–6 mg aluminum per 100 g serving (*18*).

Duplicate portion studies in different populations, in which subjects submitted for analysis duplicates of the diets they consumed over a 24-hr period, estimated the following average daily dietary exposures to aluminum: 3.1 mg [Holland, men and women (*19*)], 2.2–8.1 mg [Japan, men (*20*)], 13.7 mg (USA, men aged 25–30 (*17*)].

The maximum reported concentration of aluminum in drinking water was 3.5 mg aluminum/l (*2*). Aluminum in U.S. groundwaters ranged from 0.014 to 0.29 mg/l and in untreated surface waters from 0.016 to 1.17 mg/l. Aluminum sulfate is often added as a flocculant to surface waters, resulting in aluminum concentrations in finished drinking water from 0.014 to 2.67 mg/l. Assuming daily consumption of 2 l of water with an average concentration of 0.1 mg/l, daily intake of aluminum from water would be 0.2 mg (*21*).

Aluminum in pharmaceuticals has been reviewed by Yokel (*22*). Some over-the-counter pharmaceuticals such as antacids and buffered aspirin contain sufficient aluminum to increase the daily dose significantly. Many antacids consist of a mixture of $Al(OH)_3$ and other hydroxides, such as magnesium. Maalox Extra Strength tablets, for instance, contain 400 mg $Al(OH)_3$ and 400 mg $Mg(OH)_2$. The recommended dose for relief from gastric discomfort is up to eight tablets per day; that is, 3.2 g $Al(OH)_3$, or 1.1 g aluminum, which is a 30-fold increase over the average exposure from food and drinking water alone. Patients with renal insufficiency often take large quantities of aluminum-containing antacids to bind excess phosphate. The resulting $AlPO_4$ is insoluble, making the phosphate more easily excretable via the feces. Other potentially significant exposures, though likely to be short term, can occur through use of intravenous solutions: 10% calcium gluconate and 3 M potassium phosphate were found to contain 5.1 mg aluminum/g and 17 mg/g, respectively. Diphtheria-pertussis-tetanus vaccine, administered widely in the United States to children and adults, contains an aluminum adjuvant (*18*).

Dialysis patients can be exposed to large amounts of aluminum via their dialysis fluid. This exposure has been responsible for notable episodes of neurotoxicity (*23,24*). Toxicity associated with dialysis fluid can be largely reduced by removing aluminum from the fluid. Winney et al. (*25*) found that treatment of dialysate water with reverse osmosis led to decreases in blood aluminum. No new cases of aluminum toxicity occurred in Scotland over 5 subsequent years of follow-up among dialysis patients.

Inhalation exposure of the general population to aluminum in dust is as high as 0.14 mg/day, based on an upper-bound measurement of 5000 ng/m$^3$ [in urban air (*2*)] and typical exposure estimates (1 l air/breath and 20 breaths/min). In contrast, miners, smelters, and other metal workers can be exposed to toxic levels of aluminum through dusts and aerosols. A group of aluminum welders (*26*), for example, was exposed to 2.4 mg/m$^3$ of aluminum (8-hr time-weighted average), which results in inhalation of 23 mg over an 8-hr shift.

Miners in northern Ontario between 1944 and 1979 were deliberately exposed via inhalation to aluminum dust as a prophylactic measure against silicosis (*27*). Before each shift, miners were exposed to 20,000 to 34,000 ppm aluminum dust in an enclosed area for 10 min, resulting in estimated average exposures of 375 mg/year. Exposed and unexposed miners did not differ significantly in incidence of neurological disorders, but the exposed miners achieved lower scores on cognitive examinations and were more likely to fall into the "impaired" range.

## Techniques for Analysis of Aluminum and Its Speciation in Biological Media

Understanding the toxicokinetic behavior of any chemical requires detailed knowledge of the species it forms in the body, which determine the extent to which it is absorbed, how it is transported in the blood, and its bioavailability to tissues susceptible to toxicity. The study of aluminum has depended largely on equilibrium modeling of small-molecule species expected to form under various physiological conditions and on filtration techniques combined with chromatography to determine the specificity and extent of aluminum's binding to protein. Nonetheless, it is still not known whether small aluminum complexes or protein-bound forms contribute more to aluminum's toxicity. For aluminum to be absorbed directly across cell membranes, it would have to be in the form of a neutral species. Alternatively, aluminum could be taken up in the form of a protein complex by receptor-mediated uptake, such as that occurring with transferrin (see discussion below). Finally, nonspecific uptake of aluminum in any form could occur via pinocytosis.

***Atomic absorption.*** Total aluminum concentration in biological media is typically determined using atomic absorption spectroscopy. This method alone, however, provides no information about speciation. Atomic absorption spectroscopy is subject to contamination because aluminum from dusts is difficult to avoid. Spectral interference from other metals can be minimized by judicious choice of absorption lines. The detection limit of flame atomic absorption spectroscopy for aluminum is 30 ng/ml (1.1 µM). The detection limit for electrothermal atomic absorption is considerably lower, [0.005 ng/ml (*28*)].

***High-energy accelerator mass spectrometry.*** Accelerator mass spectrometry can measure as few as a million atoms of $^{26}Al$ (*29*). $^{26}Al$ is a long-lived radioisotope (half-life of about $10^5$ years) that enables studies of very low doses of isotopic aluminum. Day and co-workers (*30*) and Priest et al. (*31*) described the use of $^{26}Al$ for tracer studies in humans. A number of investiga-

tors performed similar experiments in rats (*30,32,33*); each of these studies is described below.

***Filtration techniques.*** Gel filtration and ultrafiltration, combined with atomic absorption spectroscopy, have been used to distinguish between protein-bound and nonprotein-bound aluminum in serum or serumlike solutions and, to a degree, among different proteins binding to aluminum. "Ultrafiltrable" as used here means able to pass through an ultrafilter and refers to small-molecule species such as aluminum citrate. Non-ultrafiltrable species are primarily protein bound but may also include insoluble or colloidal complexes of, for example, aluminum hydroxide. It is important to recognize that, at high concentrations, aluminum may precipitate out as a solid or form a colloid that cannot be distinguished by ultrafiltration from protein-bound and otherwise non-ultrafiltrable aluminum.

A number of researchers have attempted to investigate the nature of aluminum's binding to serum proteins by gel filtration techniques (*34–39*). This approach has been problematic in a number of respects. Many columns avidly bind aluminum, making recovery difficult. Favarato et al. (*37*) recovered aluminum from different columns, by washing with buffer containing desferrioxamine, in the following amounts: TSK-GEL HW-5SS, 75%; Sephadex G-200, 36%; Sephacryl S-500, 30%; and Bio Gel P-2, 24%.

***$^{27}$Al-NMR.*** Recently, nuclear magnetic resonance (NMR) directed at the $^{27}$Al nucleus has been used to investigate aluminum species in solution. Unlike $^{13}$C or $^{1}$H, $^{27}$Al has a spin quantum number greater than 1, resulting in broad signals when bound to asymmetrically arranged ligands, which makes structural assignments difficult. Theory and applications of $^{27}$Al-NMR have been thoroughly reviewed by Akitt (*40*). Results of $^{27}$Al-NMR experiments using citrate are summarized in Table 1. Fatemi et al. (*41*) demonstrated aluminum binding to transferrin, albumin, and citrate. For an aluminum–citrate solution over the range of pH 6.0–8.4, these investigators found a double signal in the region from about 0 to 10 ppm shift. At the highest pH, $Al(OH)_4^-$ was detected, as well as aluminum–citrate complexes. Feng et al. (*42*) detected a trinuclear aluminum–citrate species in solution within a range of intermediate pH. The identity of this species was confirmed by X-ray crystallography.

***Potentiometric data and equilibrium models.*** Equilibrium models, based on potentiometric data, allow a much more complete (although theoretical) description of species formed in aqueous solutions of aluminum with its ligands, compared to the analytical techniques described in earlier sections. In this technique, formation (or equilibrium) constants are fit to data on change of pH as a function of acid or base added to a solution. These constants can then be used, usually with a computer program, to predict equilibrium concentrations of the postulated species under specified concentrations of aluminum and its ligands, at different pH values. A summary of the predominant aluminum species predicted by each model, in the presence of citrate and range of pH values, is presented in Table 2.

Berthon and Daydé (*43*) described a model of aluminum speciation in the intestine designed to compare the fraction of neutral aluminum species formed after administration of either aluminum phosphate or aluminum hydroxide. This model predicted that $Al(OH)_3$ would dissolve better than $AlPO_4$ in the presence of organic acids and would complex better with the acids. Aluminum administered orally as $Al(OH)_3$ would be better absorbed in the gut than $AlPO_4$ and thus lead to the observed increased toxicity. Furthermore, this dissolution, complexation, and absorption should be higher in the proximal jejunum (about pH 7) than in the duodenum (pH 3–4).

Öhman (*44*) performed a titration study of aluminum–citrate speciation over time in aqueous solution that followed pH as a function of added base. Speciation was calculated by curve fitting using published equilibrium constants. One species, $Al(OH)H_{-1}Cit^{2-}$ [$Al(CitH_{-1})(OH)^{2-}$, using Öhman's convention] was found at physiologic pH in fresh (that is, unaged) solutions. A second species, $Al_3(OH)(H_{-1}Cit)_3^{4-}$, predominated in somewhat older solutions.

On the basis of a similar pH–time study, Öhman (*44*) first fitted stability constants for $Al(H_{-1}Cit)^-$ and $AlOH(H_{-1}Cit)^{2-}$ at time 0 assuming that the concentration of $Al_3(OH)(H_{-1}Cit)_3^{4-}$ was 0. He then predicted corresponding concentrations of the trinuclear species, based on the amount of hydroxide taken up, as evidenced by the pH, then constructed distribution diagrams over time. In these models, the trinuclear aluminum species appeared after 1 min at around pH 6 and then came to predominate over a broader pH range after a few minutes.

The existence of this trinuclear species, $Al_3(OH)(H_{-1}Cit)_3^{4-}$, was confirmed by Feng et al. (*42*) and in our laboratory (*45*) through the use of $^{27}$Al-NMR. We found further that the trinuclear species indeed appeared at an aluminum:citrate ratio close to 1. We were unable to see this species in solutions where citrate was increased relative to aluminum, however. Furthermore, the equilibrium models described below do not support the existence of this species under high citrate:aluminum ratios such as those found in blood. The published model found to be in best agreement with the experimental data generated in our lab was that of Öhman (*44*). When extrapolated to physiologic aluminum and citrate concentrations, this model predicts the neutral species $AlCit^{\circ}$ only below pH 3.5; at higher pH the predominant aluminum species are $AlCitH^-$ (pH 3.5–6.5) and $AlOH(H_{-1}Cit)^{2-}$ (pH 6.5–8.5) (*45*).

Motekaitis and Martell (*46*) examined potentiometrically the equilibrium between aluminum and a number of organic ligands, including citric acid, at metal:ligand ratios of 1:1 and 1:2. Equilibration times between additions of acid or base varied but were not reported. Only three species were assumed, each of them a 1:1 aluminum:citrate species in a different protonation state.

Gregor and Powell (*47*) assumed that citrate's hydroxy proton was available to complex with aluminum, and used a 5.3- or 6.3-fold excess of ligand over metal for potentiometric determinations. $K^+$-$Cit^{3-}$ ion pairing was taken into account because KOH was used as a titrating base. Eight aluminum-citrate species were assumed, but no polynuclear species. $^{13}$C-NMR supported the possibility that the hydroxy proton was used in bridging.

Martin (*48*) used adjusted equilibrium constants from the published literature to model the binding of aluminum citrate (assuming no polynuclear species), at ionic strengths of 0.1 to 0.16 M, and over a pH

**Table 1.** Nuclear magnetic resonance shifts from published investigations of the aluminum–citrate system

| Species | Shift (ppm downfield from TMS) | Reference |
|---|---|---|
| $Al(H_2O)_6^{3+}$ | 0 | (*41*) |
| Aluminum–citrate solution (conditions unspecified) | 8, 10, 12 | (*40*) |
| $Al(OH)_4^-$ | 80 | (*46,107*) |
| $Al_3(OH)(H_{-1}Cit)_3^{4-}$ | 0, 10, 12 | (*42*) |
| Aluminum–citrate solution | 14, 21.1, 28.1 | (*108*) |
| Equimolar aluminum–citrate | pH 2: 0, 8 ppm<br>pH 3: 10 ppm | (*109*) |
| Equimolar aluminum–citrate (0.1 M each) | pH 2.7: -4 ppm<br>pH 11.4: -87 ppm | (*110*) |

[a]Conditions unspecified; no significant change noted between different mole ratios.

range of 1–9. At physiologic pH and a ratio of 100:1 citrate to aluminum, the species $Al(H_{-1}Cit)^-$ was predicted to predominate.

Venturini and Berthon (*49*) examined the aluminum–citrate equilibrium at physiologic ionic strength and devised a model containing seven citrate species, including a trinuclear and a binuclear aluminum species, and two hydroxy aluminum species. At physiologic pH and about 60:1 citrate:aluminum, $AlCit_2(OH)^{4-}$ [$ML_2H_{-1}$] by the authors' convention (*49*)]; $Al_3(OH)(H_{-1}Cit)_3^{4-}$ [$M_3L_3H_{-4}$]; and $AlCit_2(OH)_2^{5-}$ [$ML_2H_{-2}$] were major species, while the trinuclear aluminum species (stoichiometrically equivalent to that postulated by Öhman) predominated at physiological pH at citrate:aluminum ratios of about 5:3 and 6:1.

Two recent studies suggest that aluminum–phosphate species are the predominant small-molecule forms of aluminum in serum. Daydé et al. (*50*) titrated an acidic aluminum chloride/orthophosphoric acid mixture at different mole ratios over a wide range of pH to produce formation constants; the aluminum–phosphate species best fitting the model were $AlPO_4$, $AlHPO_4^+$, $AlH_2PO_4^{2+}$, $Al_2PO_4^{3+}$, $Al_2PO_4(OH)_2^+$, and $Al_2PO_4(OH)_3^o$. A model constructed from these formation constants and those previously determined for aluminum citrate and hydroxide predicted the predominant low-molecular-weight species, under physiologic conditions, to be $Al(OH)_3^o$ (51%), $AlPO_4^o$ (41.5%), and $Al_2PO_4(OH)_2^+$ (7.2%).

Harris (*51*) derived a largely different set of equilibrium constants for the aluminum-phosphate system from linear free-energy relationships. The two species judged to be most important in serum were $AlPO_4OH^-$ and $AlPO_4^o$. This study developed a speciation model containing all ligands found in serum that are expected to complex appreciably with aluminum, as well as metal ions ($Zn^{2+}$, $Ca^{2+}$, and $Mg^{2+}$) that compete with aluminum for binding sites on transferrin. The model was studied at aluminum and ligand concentrations typical of normal or uremic plasma and at pH 7.4. The following assumptions were made: 1) the N-terminal binding site on transferrin is 60% saturated with iron, which is not displaced by other metals; 2) aluminum does not bind to albumin; and 3) trinuclear aluminum citrate [$Al_3Cit_3(OH)_4^{4-}$] and aqueous $Al(OH)_3$ exist. Concentrations of organic ligands in normal serum were taken from the Geigy Scientific Tables, and formation constants (other than for phosphate) were adopted from a number of literature sources and averaged when more than one was available. The principal species predicted from the complete model were aluminum–transferrin (about 81%, which was supported by results obtained with $^{26}Al$ (*31*), $AlPO_4OH^-$ (about 16%), and a few hydrolyzed species. Harris's work is the first published so far to incorporate citrate and phosphate in the same equilibrium model and the first to suggest that phosphate might be a more significant binder of aluminum than citrate.

## Absorption of Inhaled Aluminum

Although inhalation exposure is not likely to be of concern to the general population, miners, smelters, and other metal workers can be exposed to toxic levels of aluminum through dusts and aerosols. Elinder et al. (*52*) found that two welders, each with about 20 years of exposure to 3.0–8.9 mg aluminum/$m^3$, excreted 107–351 µg/l aluminum in their urine and had 18–29 µg/g aluminum in their bones. A group of workers exposed to aluminum-flake powders experienced higher whole-blood aluminum concentrations (0.33 µM; 8.9 µg/l) than the comparison group (0.11 µM; 3.0 µg/l) (*53*). Welders exposed to aluminum fumes excreted aluminum in urine at a median concentration of 82 µg/l (3.0 µM) (*54*). Foundry workers exposed to <1 mg aluminum/$m^3$ (*55*) for a median of 7 years experienced increased serum aluminum concentrations compared to controls, but urinary excretion was unchanged. Exposure of rabbits to 0.56 mg aluminum/$m^3$ over 5 months led to an 15.8-fold increased accumulation of aluminum in lung (compared to controls), a 2.5-fold increase in brain, and a 1.65-fold increase in kidney (*56*). It has been estimated that about 3% of aluminum is absorbed into the blood from the lung (*2*).

## Absorption of Aluminum in the Gastrointestinal Tract

The commonly cited estimate of gastrointestinal aluminum absorption of 0.1–0.3% (*1,21*) was based in part on the assumption that urinary excretion represents absorption, which does not take into account tissue distribution, as discussed below. Other studies (*2,30*), one using $^{26}Al$ in a human, suggest that 1% is absorbed from the gastrointestinal tract. However, Schönholtzer et al. (*57*) found <0.1% of an oral dose of $^{26}Al$ (as hydroxide) in the urine of normal rats and rats with five-sixths of each kidney surgically removed ("5/6 nephrectomy") in the first 300 min after dosing. Considering the rapid urinary elimination of most aluminum (*31*), these results suggest <0.1% oral absorption of the $^{26}Al$. Jouhanneau et al. (*33*) found only 0.02% of an oral dose of $^{26}Al$, as the citrate, in urine and another 0.02% of the dose in the liver of rats, suggesting absorption of only 0.04% from the gastrointestinal tract.

Some absorption of aluminum may occur in the stomach (*58*); the majority of aluminum absorption, however, is expected to occur in the intestine. In general, the two-step absorption process in the gut is 1) lumen→mucosa and 2) mucosa→bloodstream. Aluminum must be in the form of neutral complexes in order to be absorbed by diffusion through the plasma membrane of cells. Ionic aluminum may be absorbed actively by specialized iron-absorption pathways in the gut, as discussed below.

The most important aspect of the gastrointestinal tract with regard to its uptake of aluminum is its change in pH, from 2 to 3 in the stomach to 3 to 8 in the small intestine. The low pH of the stomach allows for complete dissolution of, for example, $Al(OH)_3$ in antacids. This dissolution yields free aluminum ($Al^{3+}$) that is thus made available for complexation and possible absorption. Studies of intestinal uptake that fail to specify or control for pH must therefore be considered unreliable.

The contents of the gut obviously vary greatly between individuals and species and have an important impact on absorption of aluminum. The presence of citric acid and other carboxylic acids have the potential to form neutral species but may also serve simply to redistribute aluminum from insoluble to soluble forms, making the metal more available for active-transport pathways. Silicic acid, a form of silicon, on the other hand, ties aluminum up as insoluble complexes that make it less bioavailable, as discussed below.

***Animal Studies.*** Addition of citric acid (0.111 M) to drinking water containing 0, 100, or 500 mg aluminum/l as the chloride significantly increased tibial, plasma, urinary, and fecal aluminum over 12 weeks, suggesting that citrate increases aluminum absorption (*59*). The same study found that addition of 0.111 M ascorbic acid to the water increased urinary and fecal aluminum. Absorption of aluminum chloride by the *in situ* rat gut preparation was significantly increased by addition of equimolar citrate ($2.5 \times 10^{-3}$ M) to the perfusate (*60*).

Domingo et al. (*61*) compared the effect of organic acids (ascorbic, gluconic, lactic, malic, oxalic, and tartaric) on tissue and urinary concentrations of aluminum compared to the effect of citric acid in rats administered aluminum by gastric intubation. Each of these acids has fewer dissociable protons than citric acid, which can lose three protons in order to bind to $Al^{3+}$; for the most part, administration of these acids resulted in higher tissue concentrations than did citrate. Ascorbic acid's effect on tissue accumulation of aluminum after 5

weeks of exposure was similar to that of citric acid, except for kidney, which had a significantly higher accumulation of aluminum after ascorbic acid administration. Organic acid-induced urinary excretion of aluminum would have provided further evidence of increased gastrointestinal absorption, but no significant changes in urinary aluminum were found as a result of administration of the organic acids studied.

***Human studies.*** The effect of gastric pH on absorption of aluminum was examined by Rodger et al. (*58*). These investigators administered either placebos or ranitidine, a drug that suppresses production of stomach acids (and would thus elevate gastric pH), to healthy subjects and to patients with renal disease, followed by $Al(OH)_3$. The subjects who received placebos had higher levels of urinary excretion of aluminum than those who received ranitidine, implying that low gastrointestinal pH promotes absorption of aluminum.

It has been suggested that patients with AD might have a genetic tendency to absorb excessive amounts of aluminum. To test this hypothesis, Taylor et al. (*62*) administered an aluminum citrate-containing drink to 20 AD patients and 20 age- and sex-matched controls, measuring blood aluminum before and after consumption of the drink. The investigators found a greater increase in blood aluminum among younger AD subjects than controls, but not among the older AD subjects compared to their controls. This study could have been improved by using a study population large enough to allow sufficient statistical power for stratified analysis or randomization. Furthermore, as has been reported elsewhere, there was considerable variability among measured serum aluminum concentrations, which could have been due to a contamination problem or the result of confounding by unstratified exposure variables.

The measurement of total aluminum concentration in urine before and after exposure is the simplest, although probably not the most accurate, means of assessing absorption. Coburn et al. (*63*) administered 950 mg calcium citrate four times daily and/or 2.4 g $Al(OH)_3$ daily to eight normal men. Baseline aluminum excretion was 0.24 µg/mmol creatinine. Excretion increased somewhat upon ingestion of $Al(OH)_3$, but was much higher (by a factor of 5.3 to 11.1) when calcium citrate and $Al(OH)_3$ were ingested together, compared to $Al(OH)_3$ alone. Presumably, soluble citrate becomes available to complex with aluminum, making it more absorbable, which is consistent with other studies. Walker et al. (*64*) similarly found that sodium bicarbonate had a lower ability than sodium citrate to increase aluminum excretion, and Nolan et al. (*65*) found that calcium acetate increased urinary aluminum excretion much less than did calcium citrate. Maximum urinary excretion of aluminum, after coadministration with calcium citrate, was 176 ± 103 compared to 6 ± 3 µg/g creatinine/day when only $Al(OH)_3$ was taken (*65*).

House (*66*) examined risk factors for elevated serum aluminum in a group of 71 office workers. Higher serum aluminum was associated with antacid consumption and with the batch in which the sample was analyzed, which strongly suggests sample contamination problems. Consumption of cola drinks from aluminum cans appeared to be inversely related to serum aluminum, but this was believed, again, to be an artifactual result of the high variability due to contamination. Therefore, no conclusions can be drawn from this work with regard to absorption of aluminum from soda cans.

Edwardson et al. (*67*) measured uptake of $^{26}Al$ dissolved in orange juice containing no added silicon or 100 µM silicon and found that silicon reduced the amount of $^{26}Al$ measured in serum. It has been hypothesized that dissolved silicates in drinking water may protect against aluminum-associated AD by complexing with aluminum and making it less bioavailable.

## Mechanisms of Aluminum Absorption from the Gastrointestinal Tract

Absorption of aluminum from the gastrointestinal tract, if purely passive, would require that the metal be in some neutral form in order to diffuse across membranes. Equilibrium models suggest that this scenario requires very specific conditions of pH and concentrations of the appropriate complexing ions. If, on the other hand, active transport mechanisms predominate, it is possible that transport could occur under a broader range of chemical conditions, such as the wide variety of pH and chemical compositions found in the gastrointestinal tract.

***Role of active transport and iron absorption pathways.*** Feinroth et al. (*68*) examined absorption of aluminum using a rat everted gut-sac, maintained at pH 7.35–7.42. Absorption was inhibited by dinitrophenol and by the absence of glucose, both of which inhibit cellular respiration, suggesting that aluminum is actively transported out of the intestine. As aluminum behaves similarly to iron in many biological systems (for example, binding to transferrin, discussed below), $Al^{3+}$ may also be taken up via the specialized iron absorption pathways found in the gut. As evidence for this hypothesis, iron deficiency has been found to increase the absorption of aluminum in rats (*69*) and in renally impaired humans (*70*). Others, in contrast, found that administering extra iron orally to dialysis patients did not prevent absorption of aluminum from phosphate binders (*71*), which would have implied competition between aluminum and iron for absorption. Investigators using an excised gut-section model found that $Fe^{2+}$ enhanced disappearance of aluminum from the intestinal lumen but did not increase systemic or portal blood aluminum. $Fe^{3+}$ had no effect on uptake of aluminum from the intestinal lumen (*72*).

An iron-binding protein has recently been identified in the duodenal mucosa of rats and humans that may prove to bind to aluminum as well and may thereby account for part of the mechanism of aluminum's uptake at the gut. The protein, named mobilferrin after the Alabama city in which it was discovered, is biochemically and immunologically distinct from both ferritin and transferrin, the two major iron-binding proteins in blood, as assessed by molecular size (56,000 daltons), electrophoretic mobility, and amino acid composition. The dissociation constant for iron from mobilferrin was found to be $8.92 \times 10^5$ (*73*). The physiologic role of this protein is not yet known; to date it has only been isolated from duodenal homogenates.

***Role of citrate.*** Citrate has been shown in a number of experimental investigations to increase markedly the gastrointestinal absorption of aluminum. It is also a common component of human diets. The polyanion of triprotic citric acid, citrate forms stable complexes with $Al^{3+}$, possibly resulting in, among other species, a neutral molecule ($AlCi^{o}$) at low pH that can be absorbed into the blood or make aluminum available to iron-transport pathways. It is possible that the hydroxy proton of citrate may also be involved in the citrate–aluminum coordinate bond at physiologic pH, in a proposed trinuclear aluminum complex. Based on stability constants, Martin (*48*) predicted that the maximum concentration of the neutral aluminum–citrate complex, at 1 µM aluminum (about five times greater than normal), would occur at pH 3 (close to that of the upper intestine, where much absorption activity takes place) and 10 mM citrate. Other models predict considerably lower concentrations of this species, as discussed in the earlier section on equilibrium modeling.

Citrate-enhanced absorption of aluminum is, however, at least in part an energy-dependent process. Van der Voet et al. (*74*) based this conclusion on their observation that administration of dinitrophenol, which inhibits respiration by uncoupling oxidative phosphorylation, decreased the citrate-mediated disappear-

ance of aluminum from the intestinal lumen. As mentioned above, citrate increased absorption of aluminum as the chloride from the *in situ* rat gut preparation (*60*).

Froment et al. (*75*), in an examination of the mechanism of enhancement of aluminum uptake by citrate, tested the hypothesis that citrate increases the permeability of the tight junctions between intestinal epithelial cells. The investigators excised the duodena of rats, added a solution of ruthenium red, and filled each gut section with either aluminum chloride or aluminum citrate. Examination of the isolated duodenal sections by transmission electron microscopy, in the presence of aluminum citrate, revealed infiltration of ruthenium red through the tight junctions, supporting the hypothesis. In addition, aluminum citrate was found to decrease the transcellular electrical resistance of tight junctions. The investigators did not examine the effect of citrate alone, however.

Schönholtzer et al. (*32*) found that when $^{26}Al$ was administered to rats as aluminum citrate or aluminum maltolate, 0 to 300-min urinary $^{26}Al$ was 0.7 and 0.1% of the dose, respectively. After administration of the $^{26}Al$ citrate with 1 mmol/kg sodium citrate, 5.3% of the $^{26}Al$ was found in the urine in the first 300 minutes, suggesting that citrate increased intestinal permeability to aluminum citrate.

## Aluminum in Blood

Because of the high concentration of potential ligands relative to the concentration of the metal, aluminum is expected to be entirely soluble in blood at concentrations up to at least 100 µg/l. Based on more than 50 literature sources, Ganrot (*1*) reported that the most credible values for serum aluminum are in the range of 1–5 µg/l, or 0.037–0.185 µM; he judged that values much higher than these stem largely from contamination. More recently, Wang et al. (*76*) measured levels of aluminum in 63 Canadians by Zeeman atomic absorption spectroscopy and found an average of 0.06 ± 0.05 µM (1.62 µg/l) in serum and 0.20 ± 0.10 µM (5.4 µg/l) in urine.

Fulton and Jeffery (*59*) examined the effect of dietary citrate or ascorbate on the absorption of aluminum from drinking water and subsequent tissue concentrations and excretion. They found a dose-dependent increase in aluminum concentration in bone, stomach, intestine, and kidney; liver aluminum was 1.5-fold greater than controls, but there was no dose response, and no aluminum was observed in brain. Ascorbate and citrate increased the concentration of aluminum in tibia, plasma, urine, and feces. Plasma aluminum (about 0.7 µM) was less than 0.5% of total blood aluminum, which is inconsistent with other studies and particularly surprising given that transferrin is a plasma protein. Red blood cell aluminum did not vary with dose of aluminum, whereas plasma aluminum did, which is consistent with other work. Plasma aluminum concentrations were within the range of values reported by other studies (*1*).

***Nature and amounts of carrier molecules.*** Although many of the ligands found in the gut are also found in the blood (Table 3), the major plasma binder of aluminum is transferrin, an iron-transport protein that also binds other metals, including aluminum, at two specific binding sites. Some nonspecific aluminum binding may also take place with albumin, and at least two other aluminum-binding proteins, an 8 kDa component (*38,77*) and an 18 kDa component, albindin (*38*).

Transferrin, which appears to be the major ligand for aluminum in serum, is a protein of 76–80 kDa (*78*). It is similar in size and amino-acid sequence to albumin, but binds metals (particularly iron) with high specificity at two independent binding sites with similar metal-binding affinities. Binding of a metal ion to transferrin involves deprotonation of three tyrosine

**Table 2.** Equilibrium model predictions for the equimolar aluminum-citrate system: species predicted to predominate over a range of pH[a]

| Reference | Species | pH range of predominance |
|---|---|---|
| (*44*) | $Al^{3+}$ | <2.5 |
| | $AlCit^{0}$ | 2.5–2.8 |
| | $Al_3(OH)(H_{-1}Cit)_3^{4-}$ | 2.8–8.3 |
| | $Al_3(OH)_4(H_{-1}Cit)_3^{7-}$ | 8.3–9.7 |
| | $AlCitH^{+}$ | Minor, 2–2.75 |
| | $Al(CitH_{-1})(OH)^{2-}$ | Minor, >6 |
| | $Al(H_{-1}Cit)^{-}$ | Minor, 2.5–5 |
| (*46*) | $Al^{3+}$ | <2.9 |
| | $AlCitH^{+}$ | 2.9–3.1 |
| | $AlCit^{0}$ | 3.1–3.4 |
| | $AlCit(H_{-1})$ $(AlH_{-1}Cit)$ | 3.4–8.3 |
| | $Al(OH)_4^{-}$ | <8.3 |
| (*48*) | $Al^{3+}$ | <2 |
| | $AlCit^{0}$ | 2–2.6 |
| | $AlCit(H_{-1})^{-}$ | 2.6–6.5 |
| | $AlCit_2^{3-}$ | 6.5–7.5 |
| | $Al(OH)_4^{-}$ | >7.5 |
| (*47*) | $Al^{3+}$ | <1.6 |
| | $AlCitH^{+}$ [$AlHL^{+}$] | 1.6–2.6 |
| | $AlCit^{0}$ [AlL] | 2.6–6.3 |
| | $Al(OH)_4^{-}$ | >6.7 |
| | $AlCit_2OH$ [$AlL_2H_{-1}^{4-}$] | Minor, 5–7.2 |
| (*49*)[b] | $Al^{3+}$ [$M_{3+}$] | <1.75 |
| | $AlCitH^{+}$ [MLH] | 1.75–2.3 |
| | $AlCit^{0}$ [$ML^{0}$] | 2.3–2.6 |
| | $Al_2Cit_2(OH)_2^{2-}$ [$M_2L_2H_{-2}$] | Minor, 6.0 |
| | $Al_3(OH)(H_{-1}Cit)_3^{4-}$ [$M_3L_3H_{-4}$] | Minor, 6.0–10 |
| | $Al(OH)_4^{-}$ | >10 |
| | $AlCit_2(OH)_2^{3-}$ [$ML_2H_{-2}$] | Minor, 10–11+ |

[a]Formulas in brackets represent authors' original designations.
[b]Calculations based on published equilibrium constants.

**Table 3.** Concentrations of components in serum available to bind aluminum (*51*)

| Component | Binding capacity | Component | Binding capacity |
|---|---|---|---|
| Albumin | 630 µM | Histidine | 77.0 µM |
| Cystine | 10.9 µM | Lactate | 1.51 mM |
| Citrate | 99.0 µM | Oxalate | 9.20 µM |
| Cysteine | 33.0 µM | Phosphate | 1.10 mM |
| Glycine | 2.30 mM | Sulfate | 330 µM |
| Glutamate | 60.0 µM | Transferrin | 49 µM[a] |

[a]Actual aluminum binding capacity, because there are two sites per molecule, and 30% of sites are normally occupied by iron.

residues and concomitant binding of a bicarbonate ion (whose concentration is not limiting in serum). Transferrin is normally 30% saturated with iron (*79*). Although its usual concentration in serum is 37 µM (*51*), lower levels have been found in dialysis patients, about 30 µM (*79*).

Bertsch and Anderson (*80*) studied the speciation of the aluminum–citrate system using ion chromatography. They found that only two peaks were eluted, one containing hexaaquo (fully hydrated) aluminum and the other apparently containing neutral aluminum citrate and $AlHCit^{1+}$. Other singly charged species were not distinguishable from the second peak and, presumably, more highly charged species were undetectable. Citrate complexes were highly sensitive to changes in ionic strength.

***Binding to serum proteins.*** Perez Parajón et al. (*81*) evaluated two ultrafiltration methodologies: conventional ultrafiltration, using Amicon Diaflo YM10 and DDS GR61PP membranes, and ultramicrofiltration, using the Amicon MPS-1 system with a 30 kDa YMT membrane, for *in vitro* fractionation of aluminum. Contamination was a large problem in both systems. These investigators found, using the Amicon MPS-1 system, that 8.3% of serum aluminum from normal subjects and 13.3% of that from patients with lower than normal renal function (i.e., uremic) was ultrafiltrable.

Yokel and McNamara (*82*), using the MPS-1 system, found that a greater proportion of aluminum was ultrafiltrable in the serum of renally impaired rabbits than in serum from renally competent animals. These results were obtained after incubation of aluminum with rabbit serum *in vitro* and after an intravenous dose of 100 µmol/kg. These investigators also compared the ultrafiltrability of different aluminum salts in bicarbonate buffer and found that the citrate salt of aluminum remained completely ultrafiltrable up to a total aluminum concentration of 1 mg/ml, while aluminum chloride, nitrate, and lactate salts decreased in ultrafiltrability when total aluminum exceeded 0.01 mg/ml. The concentrations at which aluminum was not totally ultrafiltrable were above the high end of the expected range of serum concentrations. Two of the rabbits in this study died prematurely, possibly (according to the authors) from kidney stones, which could conceivably have consisted of precipitated aluminum.

The similar sizes of transferrin and albumin make it difficult to separate these proteins chromatographically, and failure to add bicarbonate to the elution buffer can decrease or prevent binding of aluminum to transferrin. Many authors have assumed that aluminum binds to both proteins, although evidence discussed below suggests that binding to albumin is insignificant. Martin et al. (*83*), based on the weak binding of aluminum to albumin *in vitro*, the high competition *in vivo* for albumin binding sites, and the generally low affinity of metal ions for albumin, argued against significant *in vivo* binding of aluminum to albumin. This would be particularly true under uremic conditions, where potential binding sites on albumin are likely to be occupied by other ligands that accumulate in serum.

Day et al. (*30*) administered an oral dose of 100 ng $^{26}Al$ and about 1 µg $^{27}Al$ (natural aluminum) in sodium citrate solution to a human volunteer. The highest plasma $^{26}Al$ concentration measured was 0.3 ng/l at 6 hr after ingestion, which suggests that at least 1% of the administered dose (assuming a plasma volume of 3 l) was absorbed (that is, this concentration does not account for aluminum that has already been distributed to tissues or eliminated). The study confirmed, by gel permeation chromatography and anion exchange chromatography at pH 7.4, that 80% of aluminum in plasma was associated with transferrin, 15% existed as other high molecular weight (>5 kDa) complexes (including albumin), and 5% as low molecular weight species. Ion-exchange chromatography, however, may cause redistribution of ionic aluminum among proteins, so the findings of protein-specific binding are not reliable.

Favarato et al. (*38*) compared the protein binding of aluminum in serum among renally competent workers exposed and unexposed to aluminum and detected a novel protein, dubbed "albindin." This protein picked up more than 40% of serum aluminum after treatment of the serum with desferrioxamine, a powerful chelator of trivalent metallic cations. The amino-acid composition of albindin was distinct from that of transferrin or albumin (*38*). The protein-binding profile of aluminum was most complex in the more highly exposed group of workers (classified on the basis of total serum aluminum content). Proteins were identified by polyacrylamide gel electrophoresis (SDS-PAGE), but transferrin and albumin could not be resolved chromatographically (*38*).

Cochran et al. (*39*) eluted plasma (which had been spiked with aluminum) from uremic patients on Sephacryl S-300 and assessed the reproducibility of the aluminum/transferrin and aluminum/albumin molar ratios in adjacent elution fractions. They found that the aluminum: transferrin ratio remained at about 0.12, whereas aluminum/albumin varied from 0.002 to 0.024. These results provided evidence for an association between aluminum and transferrin but not albumin. Transferrin and albumin concentrations were assessed by immunodiffusion, but the methods used were neither described nor referenced. The overall recovery of aluminum was not reported for this experiment, so the significance of the results is uncertain. Dialysis of purified albumin against aluminum solution resulted in the association of 10% of the aluminum with albumin, but when calcium and phosphate were added, no association of aluminum with albumin was detectable. There was no evidence of association of aluminum with either the Sephacryl S-300 or Sephadex G-50 gel used by Cochran et al. (*39*).

***Role of transferrin.*** Evidence for transferrin's role as an aluminum carrier is strengthened by a study by Cannata et al. (*69*) that showed increased aluminum levels in the urine and brain of rats depleted of iron and exposed orally to aluminum). This phenomenon may occur because reduced iron stores lead to increased production of transferrin (*84,85*), a central concept in iron homeostasis. Cannata et al. also found that iron-depleted rat intestinal epithelial cells *in vitro* contained significantly more aluminum when exposed to transferrin-bound aluminum than did normal cells (*69*). Although these results, which show aluminum retention, may reflect aluminum uptake into tissues in general, this evidence is not specifically relevant to the intestine because transferrin is not found in the intestinal lumen.

*In vitro* analytical chemical methods such as titration have been used to estimate the affinity of aluminum (and other metals) for transferrin and other potential chelators found in serum. Transferrin's affinity for iron is considerably higher than for aluminum [e.g., log $K_1$ = 22.7 and log $K_2$ = 22.1 for $Fe^{3+}$ (*83*) compared to log $K_1$ = 13.72 and log $K_2$ = 12.72 for $Al^{3+}$ (*51*). Because aluminum is present in small amounts in the blood relative to iron concentrations and the capacity of transferrin to bind aluminum (as discussed above), and because iron normally occupies only 30% of available sites on transferrin, aluminum binding to transferrin is not expected to be limited by either the concentration in blood of transferrin (37 µM) (*51*) or of iron.

## Kinetics of Aluminum Uptake by Cells

As in the gastrointestinal tract, aluminum may be taken up into cells by mechanisms similar to those used to take up iron. Foremost among such mechanisms is receptor-mediated uptake of transferrin-bound metal, which is saturable. Small-molecular forms of aluminum may be

taken up via diffusion of ions or via pinocytotic uptake of small amounts of extracellular fluid.

Several investigators have examined the uptake kinetics of transferrin and iron by cells. Cole and Glass (*86*) found binding of iron to transferrin was not saturated at iron concentrations up to 50 µM. Iron uptake by mouse hepatocytes increased with increasing transferrin concentrations, but nonspecific uptake (pinocytosis and/or diffusion) accounted for 10–20% of uptake. Dissociation constants of 0.081 µM (for suspended cells) and 0.29 µM (for plated cells) were reported for the dissociation of transferrin from cells. Page et al. (*87*) found similar results using cultured rat hepatocytes. Cochran et al. (*88*) examined the competition between aluminum and iron for transferrin binding and uptake in reticulocytes (immature red blood cells). Binding was significantly less strong in these cells ($K_d$ = 3 µM) than Cole and Glass (*86*) reported for hepatocytes. Furthermore, aluminum + radiolabeled transferrin (5 µM) was taken up 1.8 times faster than labeled iron–transferrin. Iron uptake reached a plateau by 40 min; aluminum uptake, in contrast, continued to increase after 40 min. In addition, uptake of radiolabeled iron–transferrin was inhibited to a greater extent by addition of unlabeled iron–transferrin than by addition of aluminum–transferrin. The authors postulated a post-uptake feedback mechanism to regulate uptake of iron, which does not appear to have an effect on uptake of aluminum (*88*). McGregor et al. (*89*) reported that aluminum in the form of an $AlCl_3$ solution became associated with cells (probably nonspecifically) more than did aluminum in a citrate solution. This may occur because, at physiologic pH, aluminum from $AlCl_3$ would be expected to be hydrolyzed to colloidal $Al(OH)_3^o$, in contrast to aluminum citrate, which forms only charged species at intermediate pH.

Interestingly, in contrast to the results of Cochran et al. (*88*), transferrin-bound aluminum downregulated the number of cell-surface transferrin receptors on human erythroleukemia K562 cells to a greater degree than iron did (*90*). It is possible that hepatocytes possess a regulatory mechanism for transferrin receptors that is different from that in cultured leukemia cells. Alternatively, there may be an interspecies difference, as Cochran et al. used rat hepatocytes, but the differences may also be due largely to the different study designs.

## Pharmacologic Behavior of Aluminum: Distribution and Excretion

After gastrointestinal absorption (or intraperitoneal injection), aluminum travels via the portal circulation into the liver, where it is thought to undergo a first-pass clearance; that is, much is removed from the bloodstream (*91*).

A 41-year-old male volunteer given $^{26}Al$ intravenously as aluminum citrate excreted all but 10–15% of the aluminum during the first day (*31*). However, 7% of the aluminum remained 170 days after the injection, at which time the authors estimated the clearance half-time to be >1 year. Analysis of their results by RSTRIP (*92*), a program for pharmacokinetic analysis, showed that the terminal elimination half-lives of $^{26}Al$ that could be estimated in blood and the whole body were about 7 and 300 days, respectively. However, the confidence in this estimate of a 300-day half-life, based on samples collected to only 170 days, is not great. Further analysis of these results suggests that the whole-body aluminum elimination half-life increases with time after exposure. This phenomenon might be explained by the retention of aluminum in a chemical species different from that administered. This species may represent a depot of aluminum that might serve as an aluminum source within the body as it is slowly eliminated from its site of distribution. The whole-body retention of 7% of the administered aluminum 6 months after dosing suggests the prolonged residence of a significant fraction of the administered aluminum. With continued exposure the aluminum depot would be expected to increase. The result would be an increase in aluminum over the human life span, as reported for brain aluminum (*93*).

Much of the work conducted thus far on aluminum speciation and distribution has looked at exposures other than ingestion, such as intravenous injection, which can bypass the first-pass effect of the liver. Exposure to dialysis fluid can be considered analogous to intravenous injection because the bloodstream is directly exposed, without being filtered first by the liver.

***Implications of speciation for aluminum excretion and aluminum in uremia.*** The ionic milieu and speciation of aluminum in various body fluids are important to the nature and rate of aluminum elimination by various routes. Aluminum is excreted primarily in the urine, as described above, and to a small degree in the feces (largely as insoluble aluminum phosphate) by way of the portal circulation, liver, and bile. Xu et al. (*94*) found that biliary excretion accounted for <1% of the aluminum administered as the sulfate to rats, whereas urinary elimination accounted for 9–17%. Greater than 80% of an intravenous dose of $^{26}Al$ citrate was eliminated in the urine of a human within 2 weeks, whereas <2% appeared in the feces (*31*). The relative contributions of each route of excretion depend in part on such factors as the solubility of aluminum species, presence of complexing ions in serum, renal competence, and aluminum dose. Under normal renal conditions, aluminum should be excreted in urine in the form of small, charged, and thus water-soluble ions or complexes with anions such as citrate and phosphate. Renal dysfunction could result in the excretion of protein-bound aluminum as well.

Uremic animals and humans seem to be more susceptible to aluminum accumulation and toxicity than renally competent subjects, even in the absence of dialysis. Alfrey et al. (*95*) found that nondialyzed uremic patients experienced aluminum burdens in liver 6.3 times, spleen 12 times, bone 6.2 times, and brain 1.7 times higher than renally competent patients. Dialyzed uremics and, in particular, dialyzed uremics suffering from encephalopathy, had tissue burdens many times higher than nondialyzed uremics.

Hosokawa and Yoshida (*96*) performed 5/6 nephrectomy on rats and collected serum 3 months later, without administering additional aluminum above the normal dietary exposure. They found that the average concentration of aluminum in serum was more than 10 times that seen in renally intact rats, and the concentration in kidneys was about 10 times higher. Arieff et al. (*97*) obtained results similar to Alfrey's human data for concentrations of aluminum in liver of nonuremic dogs and uremic rats but, in contrast, they found that brain aluminum was higher in nondialyzed than dialyzed chronic renal failure patients. No information was given on the aluminum content of the water used in dialysis. It is possible that, if water containing little or no aluminum was used to prepare the dialysis fluid, dialysis could have cleared the blood of small-molecule aluminum, although most aluminum is not in this form. Another explanation could be dietary or therapeutic differences between dialyzed and nondialyzed individuals that would result in the administration or gastrointestinal absorption of different total quantities of aluminum.

There are a number of possible reasons for the observation that uremia leads to an increased body burden of aluminum. The simplest explanation is that, in having lower excretory capacity, uremics might simply not be able to rid themselves of aluminum efficiently, allowing it more time to be distributed to peripheral tissues. Yokel and McNamara (*82*) found that the systemic clearance was significantly lower in renally impaired animals than renally intact (39 compared to 53 ml/kg-hr), whereas Meirav et al. (*29*) compared the

systemic clearance of aluminum in a renally impaired rat to that in two normal rats and found that the renal clearance was similar. Ittel et al. (*98*) found that urinary excretion of aluminum was greater in uremic than in control rats, but ascribed their results to greater absorption of aluminum rather than greater clearance. A mechanism postulated for an increase in absorption was secondary hyperparathyroidism.

The aluminum toxicity experienced by uremics may also be iatrogenic, through exposure to aluminum in dialysis fluid. This possibility has already been addressed in the clinical setting: dialysis fluid is now made from low-aluminum water, and patients appear to suffer less aluminum toxicity as a result (*1*).

An alternative explanation for Ittel's observation of increased excretion of aluminum in uremia is that the free, or ultrafiltrable, fraction is increased relative to the protein-bound fraction. One study found that a greater percentage of the aluminum added to serum from renally impaired rabbits was free than in serum from normal rabbits (*82*). However, Graf et al. (*99*) found a negative relationship between plasma aluminum concentration and percent ultrafilterable aluminum in 24 dialysis patients. Another study (*36*) found 46% of the aluminum in serum ultrafiltrate from 30 normal patients and 33% of the aluminum in the ultrafiltrate from 30 uremic patients, with an increase in non-ultrafiltrate aluminum as serum aluminum increased.

Alternatively, it is plausible that, in uremia, potential aluminum-binding sites on transferrin (and possibly albumin or some other protein) are occupied by competing ligand molecules that accumulate in the blood. Excretion of aluminum would thus be expected to increase with time after onset of uremia, as competing ligands accumulate, but this hypothesis has not been investigated. Cochran et al. (*79*) reported that the serum transferrin concentration in dialysis patients is about 30 µM. Given that serum aluminum concentrations are higher than normal in these patients, this reduction in aluminum-binding capacity may have implications for aluminum speciation.

As uremics also experience higher aluminum tissue burden and toxicity (*95*), it may be that this fraction is more available to tissues than the protein-bound fraction, although tissue burden may reflect selective retention rather than increased bioavailability. Another explanation that has not been examined is that tubular reabsorption of aluminum occurs to a greater degree in uremics.

## Distribution of Aluminum to the Central Nervous System

The brain appears to be one of the most important target sites for aluminum toxicity. The blood–brain barrier is normally permeable only to small molecules, or larger molecules, such as proteins, by active-transport mechanisms. Because of this low permeability, it is important to understand the mechanisms by which aluminum permeates the barrier. Aluminum–protein complexes are unlikely to permeate the blood–brain barrier directly because of their size, although transferrin-receptor-mediated uptake is a possible mechanism (*100*). Transferrin receptors are found in a number of CNS cell types, including neurons, oligodendrocytes, astrocytes, ependymal cells (found in the spinal cord), and choroid plexus cells (*101*). Transferrin is believed to play a role in the development of myelinated cells. In the presence of other, smaller complexes, however, aluminum could cross the blood–brain barrier. A case study was reported of several renal dialysis patients suffering from bone aluminum toxicity who were treated with desferrioxamine, an iron-chelating drug that also efficiently chelates aluminum. The subjects experienced neurological symptoms resembling dialysis dementia; several of them died. The investigators conjectured that the resulting desferrioxamine–aluminum complex, which is much smaller and more soluble than aluminum–protein complexes, had distributed into the brain (*102*). Yokel et al. (*103*) supported this hypothesis in a rat study using microdialysis probes to monitor aluminum–desferrioxamine distribution into various tissues, including the brain.

Allen and Yokel (*104*) used *in vivo* microdialysis to compare the permeabilities of aluminum and gallium through the blood–brain barrier with a view to using gallium as a model for aluminum. They found aluminum and gallium permeation to be dissimilar. Gallium, although attractive because it possesses usable radioisotopes, therefore does not seem an appropriate model for aluminum penetration into the brain. Specifically, these investigators found that aluminum permeates the brain primarily at the cerebral capillaries, whereas gallium permeates equally at the cerebral capillaries and at the choroid plexuses. The permeation of aluminum was nonsaturable, suggesting a nonspecific process such as diffusion or pinocytosis of small-molecule aluminum rather than binding of aluminum–transferrin to cell-surface receptors. The permeation of aluminum into the brain could, however, be a combination of specific and nonspecific processes and still appear nonsaturable overall, so there still may be a role for transferrin.

Farrar et al. (*105*) used HPLC and gel electrophoresis to study the binding of radioactive gallium (which they presumed to be analogous to aluminum) to transferrin. Binding was lower in people with either AD or Down's syndrome than in normal subjects. The authors postulate that reduced binding of gallium, and presumably aluminum, to transferrin could lead to increased uptake of the metal across the blood–brain barrier as a neutral citrate complex. As discussed above, aluminum appears to be taken up in a nonsaturable fashion (i.e., nonspecifically), at least at the cerebral capillaries (*104*).

Roskams and Connor (*100*) examined the binding of aluminum–transferrin and iron–transferrin to homogenized rat brain and found evidence of a receptor that binds both. Dissociation constants ($K_d$) were 5.7 nM for iron–transferrin and 13.1 nM for aluminum–transferrin. The affinity of this receptor for aluminum–transferrin, although lower than that for iron–transferrin, is still higher than the affinity of receptors in other cells (such as hepatocytes and lymphocytes) for iron–transferrin. Unfortunately, homogenized rat brain is probably not a good model for the blood–brain barrier. These authors suggested that aluminum's toxicity in the brain might involve, at least in part, disruption of normal iron homeostasis and iron-dependent cellular processes. Fleming and Joshi (*106*) found that aluminum can interfere with the rate of binding of iron to ferritin, an iron-storage protein, in support of Roskams and Connor's suggestion (*100*).

## Summary and Conclusions

A small fraction (0.1–1%) of aluminum appears to be absorbed gastrointestinally from the diet, possibly by iron-absorption pathways. Upon reaching the serum, 80–90% of aluminum binds to the iron-transport protein transferrin and, possibly, to albumin; another aluminum-binding protein that has not yet been well characterized has also been tentatively identified. The remaining 10–20% of aluminum forms soluble (operationally defined as ultrafiltrable), small-molecular complexes, particularly with citrate and phosphate, some of them hydrolyzed. Aluminum is expected to be entirely soluble in serum due to large excesses of complexing ligands. Existing analytical techniques are, however, inadequate to establish definitively the nature of the aluminum species present in biological fluids. Equilibrium modeling and nuclear magnetic resonance indicate that most of the major species at physiologic pH are charged. The only neutral

species postulated is $Al(OH)_3^{\circ}$ (aq), whose existence has not been established and which, in any case, appears, based on modeling data, not to constitute a large fraction of soluble aluminum species in serum.

The charged, soluble aluminum species are unavailable for diffusion into tissues, but are presumably readily excretable. Receptors for transferrin, on the other hand, exist in many cells, including at the blood–brain barrier, and provide a means for uptake of transferrin-bound aluminum into tissues. Radionuclides of aluminum are expensive, making tracer studies difficult; atomic absorption spectroscopy is the usual means of quantitating aluminum in biological samples. Radioactive gallium has been used as a model for aluminum uptake, but its behavior does not appear to be strictly comparable. Uptake of aluminum into brain and bone lead to toxicity. Aluminum toxicity is of particular concern to individuals with kidney impairment, in whom aluminum concentrations are higher.

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