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Influence of dietary constituents on intestinal absorption of aluminum

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Orally-ingested aluminum compounds have been implicated in the development of dialysis encephalopathy, osteomalacic dialysis osteodystrophy and other disorders in both hemodialyzed and nonhemodialyzed patients suffering from chronic renal failure [1–10]. Both dialysate aluminum content [7, 11, 12] and aluminum-containing phosphate binding agents [12–15] have been identified as contributing to hyperaluminemia in uremic patients. The health threat from dialysate fluids has been reduced by the recommendation that the dialysate contains less than 10 μg/liter of aluminum [16]. Alternative phosphate-binding agents which do not contain aluminum are available but these agents are not free of problems [17], and uremic patients continue to ingest significant doses of aluminum-containing phosphate binding agents.

Aluminum is the most common metal in the biosphere of humans but, aside from uremic patients, causes no widespread toxicity. This may be as a result of the extremely limited solubility of aluminum at the pH range of the small intestine and blood [18]. Advances in analytical chemistry have made it possible to measure picogram quantities of aluminum in body fluids, thus enabling accurate determination of plasma aluminum levels in the part per billion (μg/liter) range. These analytical techniques have shown that orally ingested aluminum-containing antacids elevate plasma aluminum levels in man [13]. Balance studies monitoring aluminum absorption and elimination revealed an average positive balance from 23 to 313 mg of aluminum per day when diets were supplemented with 1 to 3 g of aluminum per day [15]. These studies show that a small fraction of the ingested aluminum is absorbed. This absorption presents potential toxic effects to uremic patients whose ability to eliminate aluminum is impaired.

In addition, Slanina et al [19] have shown that addition of citric acid to aluminum-supplemented dietary regimens results in blood aluminum levels that are significantly higher than those found in subjects treated with aluminum-supplemented dietary regimens alone. This result suggests that dietary factors may contribute to aluminum absorption.

This study was undertaken to determine if the form of aluminum present in the intestinal lumen significantly affects the absorption of aluminum following oral ingestion.

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Methods

Perfusions of in situ rat gut preparations [20] were performed within a laminar flow hood in order to prevent contamination of samples. All glass and plasticware was soaked overnight in 50% concentrated nitric acid, washed with distilled water and oven dried before use. Perfusate solutions contained NaCl (140 mM/liter), KCl (4.56 mM/liter), and $CaCl_2$ (1.25 mM/liter), as well as 2.5×10^{-3} M aluminum chloride and equimolar sodium citrate, where indicated. The aluminum chloride concentration was selected to represent the midpoint between the amount of aluminum estimated to be in the diet in the absence of an aluminum-containing phosphate binding agent, 8 to 80 mg equivalent Al_2O_3 [21], and the 500 mg of equivalent Al_2O_3 in the normal dose of an aluminum-containing phosphate binding agent [22]. If it is assumed that the aluminum-containing compound is dispersed in 300 ml of gastric fluid [23], then the expected aluminum concentration range in the gastric fluid would be 2.5×10^{-4} to 2.5×10^{-2} M.

Male Wistar rats (275 to 375 g) were fasted for 12 hours and then anesthetized with pentobarbital, 50 mg/kg. Intestinal cannulas were placed below the ligament of Trietz and proximal to the ileocecal juncture. The intestinal segment was then carefully returned to the peritoneal cavity, and the abdominal opening closed with sutures.

Renal arteries were ligated in order to prevent elimination of absorbed aluminum.

A 25 ml volume of perfusate containing 2.5×10^{-2} M aluminum chloride at an initial pH of 3.2 was transferred to a jacketed beaker which was maintained at 37°C. The perfusate was stirred continuously and circulated (Polystaltic pump, Buchler, Fort Lee, New Jersey) through the entire small bowel segment. This aluminum concentration was selected to represent the maximum concentration of solubilized aluminum in the gastric contents following a dose of an aluminum-containing phosphate binding agent. During perfusion the pH increased to 5.5 and a precipitate formed. The perfusate was centrifuged and the supernatant discarded. A portion of the precipitate was washed with absolute methanol to minimize the development of crystallinity [24] and dried at room temperature over desiccant.

The dried precipitate was prepared for infrared spectroscopy by grinding with oven-dried potassium bromide. The resultant fine powder was then compressed into a pellet. Infrared spectra

(IR-33, Beckman Instruments, Fullerton, California, USA) were recorded from 4000 to 600 cm^{-1}.

In a separate series of experiments, 25 ml of perfusate containing 2.5×10^{-3} M $AlCl_3$ or 2.5×10^{-3} M $AlCl_3$ and 2.5×10^{-3} M sodium citrate were adjusted to pH 6.0 and circulated through the entire small bowel segment, as before. The pH of the perfusate within the beaker was continuously monitored and maintained within 0.1 units by a pH-stat titrator (PHM 62, ITT 60, ABU 12 [2.5 ml], TTA 60, REA 160, Radiometer, Copenhagen, Denmark).

Blood samples were collected via a cannula inserted into the right carotid artery of heparinized animals. Samples were collected before perfusion in order to establish baseline plasma aluminum values, then at intervals over the course of the perfusions. Samples were centrifuged, then diluted with an equal volume of solution containing 0.1% Triton X-100 (Sigma Chemical Company, St. Louis, Missouri, USA) and 1.4 g/liter magnesium nitrate. Plasma samples were analyzed in triplicate by electrothermal atomic absorption spectroscopy (Atomic absorption spectrophotometer model 560, programmer model HGA 500 and graphite furnace model HGA 500, Perkin Elmer, Norwalk, Connecticut, USA), using a method adapted from the literature [25].

A reference aluminum solution (1000 μg/ml, Fisher) was used to prepare fresh aluminum standards daily. A typical calibration curve was linear with a correlation coefficient (r^2) value of 0.99. The analytical method was validated by standard addition of aluminum to plasma. The measured aluminum concentration was linearly related to the theoretical aluminum content, having an r^2 value of 0.99.

The effect of selected dietary constituents on the pH of precipitation was determined by titrating a 0.1 M aluminum chloride solution containing 0.3 M dietary constituent of interest with 0.1 N NaOH using an automatic titrator (Radiometer). Titrant addition was continued until a precipitate was visually observed.

Results

Aluminum-containing phosphate binding agents are chemically a form of aluminum hydroxide. As seen in Figure 1, they exhibit a high solubility in gastric acid, producing soluble $Al(OH_2)_6^{3+}$ by equation 1. This soluble species is then transferred to the small intestine by gastric emptying.

$$Al(OH)_3 + 3\ H_3O^+ \rightarrow Al(OH_2)_6^{3+} \qquad \text{(eq. 1)}$$

Aluminum chloride was selected as a model for the form of aluminum which is produced in gastric acid because aluminum chloride dissociates in aqueous solution to produce $Al(OH_2)_6^{3+}$ by equation 2.

$$AlCl_3 + 6\ H_2O \rightleftharpoons Al(OH_2)_6^{3+} + 3Cl^- \qquad \text{(eq. 2)}$$

However, aluminum is particularly insoluble at neutral pH as a result of the formation of stable hydroxide complexes [26] which limit the solubility of aluminum to less than 1 μm/ml at pH 6 (Fig. 1). Thus, aluminum cations solubilized by gastric acid are expected to precipitate in the intestine as a result of the increased pH. This hypothesis was examined by perfusing 25 ml of a 2.5×10^{-2} M aluminum chloride solution through the in situ intestinal segment. The pH of the aluminum chloride solution initially was 3.2 and was not controlled during perfusion. The pH of the perfusate was monitored and, as expected, steadily increased. Perfusion was continued for 180 minutes, at which time the pH of the perfusate was 5.5. A precipitate was observed in the perfusate, the infrared spectrum of which is presented in Figure 2. The spectrum contains broad O-H stretching bands at 3400 cm^{-1}, which are attributed to amorphous aluminum hydroxide [27]. This broad band is consistent with the expected slow development of crystallinity in freshly precipitated materials. The prominent water band at 1635 cm^{-1} represents water strongly bound at the solid surface or adsorbed during sample preparation. The strong water band and the broad hydroxyl-stretching band both suggest that the precipitate has a high surface area. Broad bands at 965 and 1060 cm^{-1} indicate that phosphate is also a component of the precipitate. This observation is consistent with the use of aluminum hydroxide as a phosphate binding gel [22]. Likewise, the bands at 1420 and 1525 cm^{-1} are attributed to carbonate. The infrared spectrum identifies the precipitate as amorphous aluminum hydroxyphosphate carbonate. Thus, the phosphate and bicarbonate ions present in the intestinal lumen are incorporated into the amorphous aluminum hydroxide which precipitates in the intestine as a result of the elevated pH.

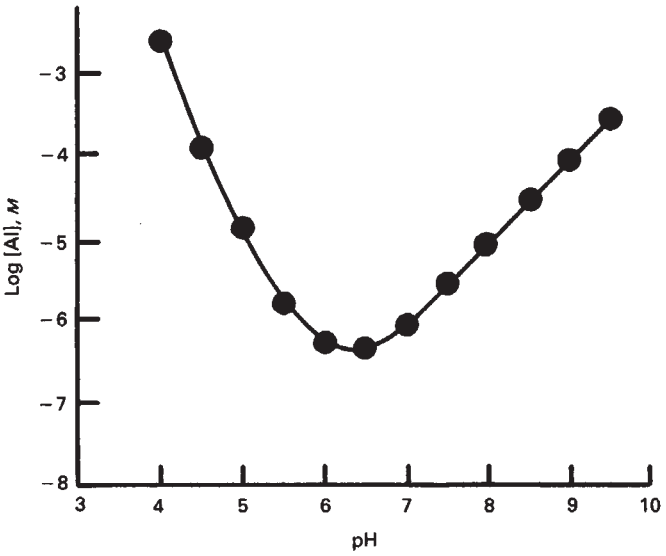

Fig. 1. *Effect of pH on the equilibrium solubility of microcrystalline aluminum hydroxide at 25°C.* From reference 26.

The effect of perfusion with freshly precipitated amorphous aluminum hydroxide on the intestinal absorption of aluminum was investigated by circulating a 2.5×10^{-3} M aluminum chloride-containing perfusate through the in situ intestinal segment for 50 minutes. The pH was maintained at 6.0 by the pH stat titrator. Plasma aluminum levels measured over the course of these perfusion experiments are shown in Figure 3, Curve A. The data represent the mean and standard deviation of perfusion in three rats. These experiments indicate no statistically significant ($P < 0.05$) increase in plasma aluminum levels compared to the values measured before perfusion was begun. Thus, precipitated aluminum hydroxide present in the intestine is not absorbed. This conclusion was confirmed by perfusing a

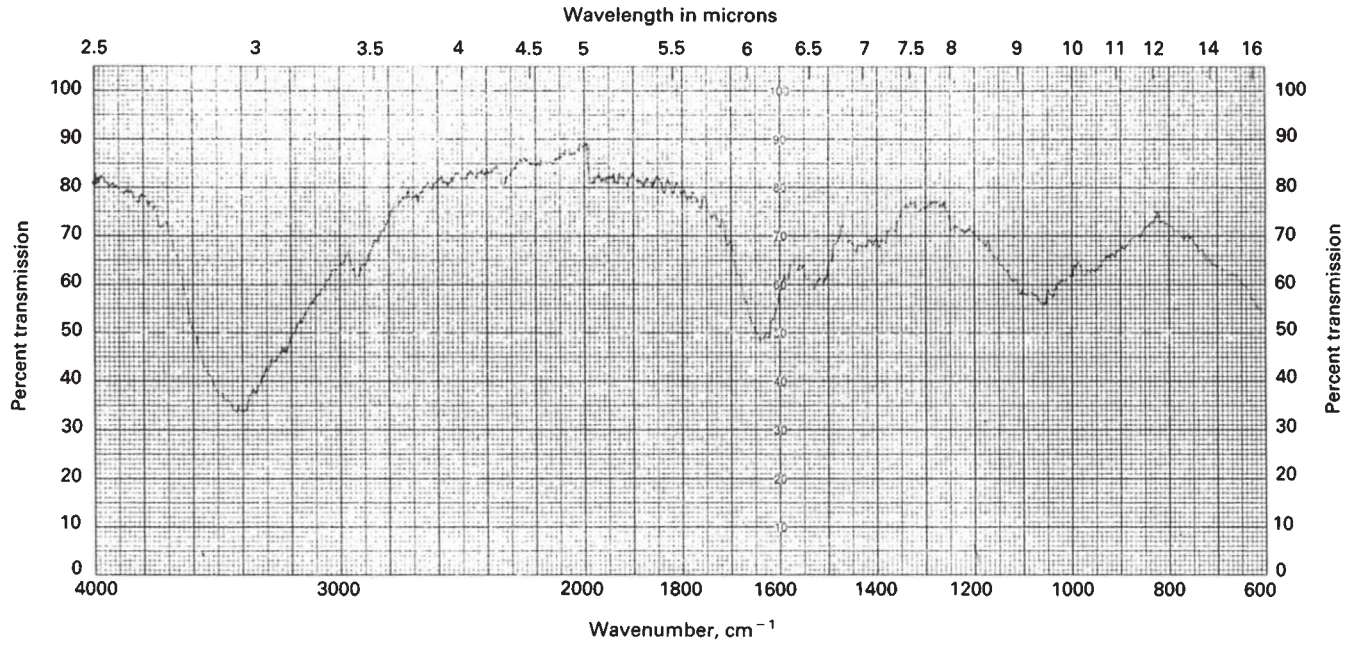

Fig. 2. *Infrared spectrum of the precipitate formed in the in situ intestinal segment during perfusion of a perfusate containing 2.5 × 10^{-2} M aluminum chloride.* The broad band centered at 3400 cm^{-1} is attributed to amorphous aluminum hydroxide; the band at 1635 cm^{-1} is adsorbed water due to the high surface area; bands at 1420 and 1525 cm^{-1} are assigned to carbonate; the bands at 965 and 1060 cm^{-1} are assigned to phosphate.

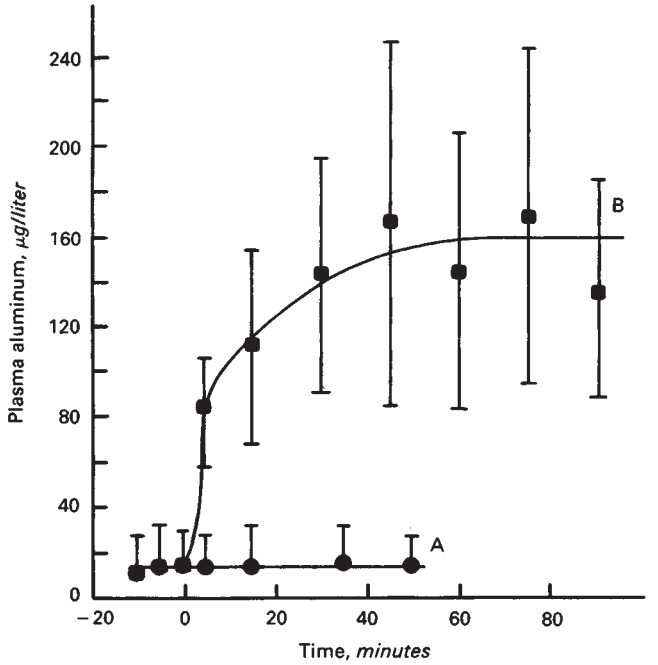

Fig. 3. *Average plasma aluminum levels (N = 3, ± SD) with time for perfusions with:* **A,** *2.5 × 10^{-3} M aluminum chloride-containing perfusate at pH 6;* **B,** *2.5 × 10^{-3} M aluminum chloride and 2.5 × 10^{-3} M sodium citrate-containing perfusate at pH 6.*

suspension of a commercial amorphous aluminum hydroxide containing 2.5 × 10^{-3} M aluminum at pH 6 through the in situ intestinal segment. The results were identical to Figure 3, Curve A, indicating no aluminum absorption.

Absorption of citrate-chelated aluminum, a complex which is soluble at the pH of the intestine, was investigated. Solutions equimolar in aluminum chloride and sodium citrate were prepared, adjusted to pH 6.0 and perfused through the in situ rat gut as before. Plasma aluminum levels in this experiment are shown in Figure 3, Curve B, and illustrate that perfusion with citrate-chelated aluminum species resulted in a statistically significant ($P < 0.05$) sixfold elevation in plasma aluminum levels above baseline values. From this data, it is apparent that the form of aluminum ultimately present in the intestinal lumen has a significant effect on the absorption of aluminum. While insoluble amorphous aluminum hydroxide present in the perfusate did not result in significant increases in plasma aluminum levels, soluble citrate-chelated aluminum species were absorbed.

Aluminum hydroxide normally precipitates at pH 4.5 when an aluminum chloride solution is titrated with sodium hydroxide. However, a number of chemicals present in the diet were found to inhibit precipitation. Table 1 lists the dietary chemicals which were found to prevent precipitation at the pH conditions expected in the intestine.

Discussion

These experiments illustrate the importance of the form of aluminum that ultimately reaches the absorptive surfaces of the intestinal mucosa on the resultant plasma aluminum levels. When aluminum cations, $Al(OH_2)_6^{3+}$, produced by the dissolution of aluminum hydroxide in gastric fluid, encounter the buffered secretions of the intestine, amorphous aluminum hydroxyphosphate carbonate is precipitated. As insoluble species present in the gastrointestinal lumen are not absorbed, this

Table 1. Compounds present in the diet which elevate the pH of precipitation above 8 during the titration of 0.1 M aluminum chloride with 0.1 N sodium hydroxide

Compound	Dietary source
Ascorbic acid	Citrus fruits, food additive: antioxidant
Citric acid	Citrus fruits, food additive: acidulent
Gluconic acid	Food additive: sequestering agent
Lactic acid	Sour foods, molasses, food additive: acidulent
Malic acid	Apples and other fruits, food additive: acidulent
Oxalic acid	Rhubarb, beets
Tartaric acid	Grapes and other fruits, food additive: acidulent

reprecipitation of aluminum hydroxide normally prevents aluminum adsorption. Thus, although environmental and/or therapeutic exposure to aluminum-containing compounds may be high, the low solubility of aluminum at the pH of the intestine renders most orally ingested aluminum-containing compounds unabsorbable.

Forms of aluminum which are soluble at the pH of the intestine were considered as possible sources of the elevated plasma aluminum levels which have been reported following oral ingestion of aluminum-containing compounds. Organic compounds which occur in food were screened to determine if they alter the pH of precipitation of aluminum hydroxide. The compounds listed in Table 1 were all found to elevate the pH of precipitation from the usual value of 4.5 to above 8. The compounds listed share a common structural feature; aluminum-binding groups on two adjacent atoms in the carbon chain backbone.

Citric acid appears to be of most clinical importance as it is available in the diet in high concentrations. For example, edible plant tissues contain as much as 3% citric acid on a fresh weight basis [28]. Citric acid represents 10% of the soluble solids in most citrus fruits [28]. Citric acid is also frequently utilized as a food additive to function as either an antioxidant, acidifier, sequestrant or flavoring agent in amounts ranging up to 5 to 40 g/kg of the food [29].

The presence of aluminum complexing compounds in the gastrointestinal tract in conjunction with gastric acid solubilized aluminum cations may thus result in the equilibrium formation of a soluble complex of aluminum which, by preventing reprecipitation, may result in aluminum absorption and elevated plasma aluminum levels. These experiments suggest that consideration must be given to diet and its relationship to regimens of aluminum-containing phosphate binding agents. For example, chronic renal failure patients should be advised to avoid the concurrent ingestion of fruits or beverages which contain citric acid and the aluminum-containing phosphate binding agent.

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