Mechanisms of aluminum absorption in rats\textsuperscript{1-3}

Mark W Whitehead, Gillian Farrar, Gillian L Christie, John A Blair, Richard PH Thompson, and Jonathan J Powell

ABSTRACT Aluminum has become a dietary toxin in modern times but its mechanism of absorption is poorly understood. After ingestion, the systemic transfer of aluminum is small but it is greatly affected by the coingestion of certain dietary agents, such as citrate, that complex with the metal in the intestinal lumen or transiently alter the permeability of the mucosa. Here, mechanisms of aluminum absorption were studied by using freshly prepared aluminum hydroxide and aluminum citrate. Everted sacs of rat gut were used to investigate the site of absorption, effect of chemical charge on absorption of aluminum citrate, and presence of active or passive absorption with use of the metabolic inhibitor ouabain. Absorption was biphasic with a large tissue uptake that was consistent with adhesion to mucus-mucosal surface but little tissue transport, which was consistent with passive paracellular permeation. Citrate reduced the uptake-transport ratio both by competing with the mucosal uptake and by increasing mucus-mucosal permeation but not by affecting the charge of the luminal aluminum species. Despite the potential for hydroxypolymerization of aluminum at intestinal pH, the small bowel and colon absorbed aluminum passively and paracellularly but the stomach did not. The predominantly proximal absorption of aluminum observed in vivo is a reflection of the proximal absorption, and therefore removal, of dietary constituents (eg, citrate) that enhance mucosal permeation of aluminum. The colon should be investigated further as a site of significant paracellular permeability. Am J Clin Nutr 1997;65:1446–52.

KEY WORDS Gut sac, aluminum, intestinal absorption, transport, uptake, poorly absorbed solute, rats

INTRODUCTION There has been renewed interest in the pathophysiology of aluminum. The element is an established toxin in cell and animal models and in patients with reduced or immature renal function. In addition, aluminum is found in elevated concentrations in the neurofibrillary tangles of Alzheimer disease (1), and at low concentrations in vitro it induces specific \( \beta \)-sheet conformations of modeled Alzheimer tangles and \( \beta \)-amyloid plaques (2). Although aluminum is ubiquitous, it has only recently been noted as a toxin because it is strongly associated with silicates in the environment and is therefore biologically unavailable. New man-made forms are more available (3), although only \( \approx 0.1\% \) of an ingested dose is absorbed (3). Thus, not only are studies of aluminum absorption important, but the underlying mechanisms will also be relevant to a wide range of other poorly absorbed solutes.

The exact proportion of aluminum that is absorbed depends on the gastric solubility of the form ingested and the presence of various dietary cofactors (3–5). Most forms of aluminum become more soluble in the acid environment of the stomach, and the poor solubility of aluminum at the neutral pH of the intestinal tract has led to speculation that absorption occurs in the stomach (6, 7), but this has not been assessed quantitatively. In contrast, when rats were given oral doses of aluminum citrate, the time of peak aluminum absorption into blood (40 min) closely matched absorption of the glucose that was used as a marker for proximal small bowel absorption (8). Other work has also suggested that aluminum absorption occurs from this area (9).

There is also evidence that the colon may be able to absorb metals. Some studies with bismuth show a similar early peak in blood that corresponds to proximal small bowel absorption, but, in addition, there can be a late peak consistent with colonic transport (10). Indeed, the colon is important in the absorption of water even against a high osmotic gradient although a recent study shows that rat colon has a higher permeability to inulin than does the small bowel (11).

Neither the site of absorption nor the mechanism of aluminum absorption are understood. Association of aluminum with the mucosa is usually termed uptake and is far greater than transport through the mucosa (3, 9). This may be due to interaction of the aluminum ion or a larger polymeric hydroxy-aluminum species formed in the lumen (3) with the mucus-mucosa. Although most work suggests that aluminum is passively transported (3), both energy-dependent (12, 13) and carrier-mediated, energy-dependent mechanisms (9) have been proposed. Some dietary ligands such as citrate greatly increase aluminum absorption (8, 14, 15), probably both by forming low-molecular-weight luminal complexes that are then better absorbed (3) and also by binding intercellular calcium, thus reducing the integrity of the tight junctions and increasing

\textsuperscript{1} From the Gastrointestinal Laboratory, The Rayne Institute, St Thomas’ Hospital, London; the Biology Division, Aston University, Aston Triangle, Birmingham; and the School of Applied Chemistry, Kingston University, Penrhyln Road, Kingston on Thames, United Kingdom.

\textsuperscript{2} Supported by grants from the Wellcome Trust, The University of London Central Research Fund, and the Special Trustees for St Thomas’ Hospital.

\textsuperscript{3} Address reprint requests to JJ Powell, Gastrointestinal Laboratory, The Rayne Institute, St Thomas’ Hospital, London SE1 7EH, United Kingdom. E-mail: t.burden@umds.ac.uk.

Received July 16, 1996.
Accepted for publication December 2, 1996.

absorption through the paracellular pathway (8). Some authors (15), however, have suggested that the overall charge of the aluminum citrate species is important for absorption, particularly under the more acidic conditions in the stomach in which an uncharged species should predominate (15).

We therefore investigated the site and mechanisms of absorption of both citrate-bound and fresh hydroxypolymerized aluminum using everted sacs of rat stomach and intestine as a model for gastrointestinal absorption (16).

**MATERIALS AND METHODS**

Twenty-five male 3-mo-old Wistar rats weighing 250–300 g were maintained on a standard laboratory diet containing 1.1 μmol Al/g and then fasted for 12 h with free access to deionized water. Immediately after cervical dislocation, the whole gastrointestinal tract from the lower esophagus to the colon was removed. The stomach, and one 5-cm segment from each of the proximal, mid, and distal small bowel and colon were removed. One end of the stomach or bowel segments was ligated with a silk suture, everted with a glass rod, and filled with a solution of 16 mmol HEPES buffer/L, 125 mmol NaCl/L, 3.5 mmol KC1/L, and 20 mmol glucose/L. The other end of the sac was then tied and the sacs were incubated as described previously (9, 16, 17) at 37 °C for 20 min in a thermoplastic incubation chamber filled with 10 mL experimental solution bubbled with 95% O2 and 5% CO2. The gut sacs were weighed both empty and full before and after incubation and the aluminum content of the bathing solution and gut sac contents were also analyzed both before and after incubation. Five different experimental solutions were used with five rats in each group. Two different sets of aluminum concentrations were used—low and high. All animal procedures in this study were carried out by licensed investigators and in accordance with regulated procedures under the Animals (Scientific Procedures) Act (1986).

**Experiments with a low aluminum concentration**

Aluminum chloride (0.2 mmol/L) at a pH of 3.5 was used for stomach incubations and at a pH of 7.4 for intestinal incubations.

Aluminum chloride (0.2 mmol/L) plus ouabain (1 mmol/L) (AlCl3 + ouabain) at a pH of 3.5 was used for stomach incubations and at a pH of 7.4 for intestinal incubations.

Aluminum (0.2 mmol/L), citrate (50 mmol/L), plus ouabain (1 mmol/L) (aluminum citrate + ouabain) at a pH of 3.5 was used for stomach incubations and at a pH of 7.4 for intestinal incubations.

**Experiments with a high aluminum concentration**

Aluminum (2 mmol/L) plus citrate (50 mmol/L) (aluminum citrate) at a pH of 3.5 was used for stomach incubations and at a pH of 7.4 for intestinal incubations.

Aluminum (2 mmol/L) plus citrate (50 mmol/L) (aluminum citrate) at a pH of 2.0 was used for stomach incubations and at a pH of 8.5 for intestinal incubations.

The Na+-K+ pump inhibitor ouabain was used to see whether aluminum absorption is mediated by active transport. In the high-aluminum experiment, conditions were chosen to give marked differences between the two groups in the chemical charges on the aluminum-citrate species formed (Table 1).

The experimental design therefore allowed three aspects of aluminum absorption to be studied: site of absorption (all experiments), type of absorption (active or passive; low-aluminum experiments), and the mechanism of the citrate effect on absorption of aluminum (high-aluminum experiments).

**Analysis**

The aluminum contents of the samples were analyzed by using inductively coupled plasma–optical emission spectrometry (ICP-OES; Philips PV 8050 at Royal Holloway and Bedford New College, Egham, United Kingdom) as described previously (19), except that for aluminum concentrations below the detection limit of this polychromatic ICP-OES (2.2 μmol/L in the sample), furnace atomic absorption spectrometry (FAAS) was used (Water Research Centre, Medmenham, United Kingdom) with a sample detection limit of 0.1 μmol Al/L, described elsewhere (20).

**Ultrafiltration**

AlCl3 (0.2 mmol/L) at pH 3.5 and pH 7.4, as used in the low-aluminum experiments, was incubated for 15 min at 37 °C and ultrafiltered through cleaned Centricon C-30 filters (30 000 molecular weight cutoff; Amicon Ltd, Stonehouse, United Kingdom) as described previously (19).

**Speciation**

All the aluminum citrate solutions were prepared from aluminum chloride and citric acid and were fully soluble under the experimental conditions. The chemical speciation computer modeling program ESTA (21) was used to predict the citrate complexes that would occur under the conditions of the high-aluminum experiments by using appropriate stability constant data (18).

**Histology**

Sections of gut, before and after incubation, were fixed in 10% formalin, embedded in wax, and 10-μm sections were stained with hematoxylin and eosin before examination and photography under a light microscope.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Aluminum speciation*</th>
<th>Gastric tissue</th>
<th>Intestinal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 2.0</td>
<td>pH 3.5</td>
<td>pH 7.4</td>
</tr>
<tr>
<td>Species</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Al(citrate)H+</td>
<td>66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al(citrate)2+</td>
<td>24</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Al(H2O)2Al2−</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al(citrate)2−</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al(citrate)(OH)−</td>
<td>2</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>Al(citrate)(OH)3−</td>
<td>40</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Al(citrate)(OH)5−</td>
<td>57</td>
<td>94</td>
<td></td>
</tr>
</tbody>
</table>

* Speciation of aluminum citrate determined by ESTA computer-modeling program (species present at < 1% are not shown) at the experimental pH values used in the high-aluminum experiments (see Materials and Methods). Data are from Vobe and Williams (18).
**Calculations**

Aluminum uptake and transport (both μmol Al/g wet wt of tissue), were calculated as follows:

\[
\text{Aluminum transport} = \frac{[\text{Al}]_{\text{SF}} V_{\text{SF}} - [\text{Al}]_{\text{SI}} V_{\text{SI}}}{W_{\text{SI}}} \tag{1}
\]

Aluminum uptake

\[
\text{Aluminum uptake} = \frac{[\text{Al}]_{\text{IU}} V_{\text{IU}} - [\text{Al}]_{\text{BF}} V_{\text{BF}}}{W_{\text{SI}}} - \text{aluminum transport} \tag{2}
\]

The following calculation indicates the concentration of aluminum inside the sac at the end of the incubation period as a percentage of the ultrafiltrable aluminum concentration outside the sac at the start of the incubation period. The large differences in volume between the inside and the outside of the sacs means that any concentration gradient should be observed, such as with facilitated and active transport:

Percentage “transported” ultrafiltrable aluminum concentration

\[
\text{Percentage} = \frac{[\text{Al}]_{\text{UFV}}}{[\text{Al}]_{\text{IU}}} \times 100 \tag{3}
\]

Water uptake (mL water/g wet wt of tissue), and water transport (g water/g wet wt of tissue) were calculated as follows:

\[
\text{Water transport} = \frac{V_{\text{SF}} - V_{\text{SI}}}{W_{\text{SI}}} \tag{4}
\]

\[
\text{Water uptake} = \frac{W_{\text{SF}} - W_{\text{SI}}}{W_{\text{SI}}} \tag{5}
\]

where [Al]_{SF} is the final aluminum concentration inside the gut sac (mmol/L), [Al]_{SI} is the initial aluminum concentration inside the gut sac (mmol/L), V_{SF} is the final volume inside the gut sac (L), V_{SI} is the initial volume inside the gut sac (L), W_{SI} is the initial wet weight of the empty gut sac (g), [Al]_{IU} is the initial aluminum concentration in the incubation chamber (mmol/L), [Al]_{BF} is the final aluminum concentration in the incubation chamber (mmol/L), V_{IU} is the initial volume of fluid in the incubation chamber (L), V_{BF} is the final volume of fluid in the incubation chamber (L), [Al]_{BF} is the initial ultrafiltrable aluminum concentration in the incubation chamber (mmol/L), [Al]_{UFV} is the final aluminum concentration inside the gut sac [corrected for any small change in gut sac fluid volume during incubation (mmol/L)], and W_{SF} is the final wet weight of emptied gut sac (g).

**Statistics**

Because the data were not normally distributed, log aluminum uptake and transport values that normalized the data were used for statistical analysis. The data were analyzed by analysis of variance (ANOVA; one- and two-way), and if a significant difference was found Tukey’s method for multiple comparisons was used. The computer program (MINITAB; Minitab, State College, PA) used for statistical analysis gave significance values as \(P < 0.05\) for Tukey’s test and \(P < 0.001\) for ANOVA. Correlations to investigate the association between tissue transport of water and aluminum were also calculated and significance ascertained by Student’s t test. Mann-Whitney U tests and Wilcoxon signed-rank tests were used for inter- and intraexperimental analysis of the percentage of ultrafilterable aluminum transported. Data are given as means ± SDs.

**RESULTS**

**Experiments with low aluminum concentrations**

At pH 3.5, 98.0 ± 2.1% \((n = 4)\) of aluminum was recovered from 0.2 mmol AlCl\(_3\)/L whereas at pH 7.4 recovery was only 10 ± 0.4% \((n = 4)\). For all three low aluminum groups tissue uptake of aluminum by stomach sacs was markedly less than that by the corresponding sacs of small bowel and colon, but within groups, there was no difference in the uptake between any of the three small bowel sites or the colon (Figure 1). The uptake of aluminum in all sacs with aluminum citrate and ouabain was markedly less than with AlCl\(_3\) + ouabain or AlCl\(_3\) alone. In contrast, uptakes for AlCl\(_3\) + ouabain and AlCl\(_3\) alone were not different; ie, ouabain had no significant effect.

In the AlCl\(_3\) alone and AlCl\(_3\) + ouabain groups (Figure 2A and B) the transport of aluminum was similar, so ouabain had no significant effect, and for both groups was much lower than their uptakes, ranging from 0.25 to 1.4 \(\times\) \(10^{-8}\) mol Al/g tissue wet wt. Furthermore, the concentration of ultrafiltrable aluminum inside the gut sacs after incubation was not significantly different between AlCl\(_3\) alone and AlCl\(_3\) + ouabain (Figure 2B); in neither group was aluminum concentrated within the sacs compared with the surrounding fluid. Both groups of colon sacs, however, achieved a higher concentration of aluminum than did the small bowel segments \((P < 0.05)\), but this difference (Figure 2B) was not due to differences in the amount of aluminum transported nor to the volume of water transported; it was largely due to the lower fluid volumes into which the aluminum was transported, because less fluid was required to fill the colon sacs than the small bowel sacs.

In the aluminum citrate + ouabain group the transport of aluminum was much greater than in the AlCl\(_3\) + ouabain or

![Figure 1](https://academic.oup.com/ajcn/article-abstract/65/5/1446/4655479/1448)

**Figure 1.** Aluminum uptake (see Methods, calculations) by isolated rat gut sacs with low aluminum concentrations. AlCl\(_3\) (0.2 mmol/L), AlCl\(_3\) (0.2 mmol/L) + ouabain (1 mmol/L), aluminum citrate (0.2 mmol Al/L and 50 mmol citrate/L) + ouabain (1 mmol/L). [Cl], stomach; [□], proximal small bowel; [■], mid small bowel; [■], distal small bowel; and [●], colon. *Significantly different from all other sites in the same experimental group, \(P < 0.05\) (Tukey test); **aluminum citrate + ouabain significantly different from AlCl\(_3\) + ouabain or AlCl\(_3\) alone for all small bowel and colon sacs, \(P < 0.05\) (Tukey test). \(\bar{x} ± SD\); \(n = 5\) for each site of each group except proximal small bowel of AlCl\(_3\) and colon of AlCl\(_3\) for which \(n = 4\) each.
Aluminum absorption in rats

Experiments with high aluminum concentration

The uncharged aluminum citrate species accounted for 24% of total aluminum at pH 2.0 but only 7% at pH 3.5, and it was not significantly present at pH 7.4 and 8.5. Full speciations are indicated in Table 1.

Again, as for the low-aluminum experiments, tissue uptake of aluminum by the stomach sacs in the high-aluminum experiments was markedly less than in the small bowel and colon. However, there were no differences in tissue uptake of aluminum between any of the three sites of the small bowel or the colon, nor between any equivalent site in the two groups (aluminum citrate at pH 3.5 and 7.4 compared with aluminum citrate at pH 2.0 and 8.5; Figure 3). In both of the high-aluminum groups aluminum transport was much smaller than uptake but was greater by all the small bowel sacs than by the colon, which showed greater transport than the stomach. However, there was no difference in aluminum transport between the two groups (aluminum citrate at pH 3.5 and 7.4 compared with aluminum citrate at pH 2.0 and 8.5; Figure 4) for any equivalent site.

Water transport

There was no significant correlation in any experiment between tissue water transport and tissue aluminum transport ($r = -0.2$ for AlCl$_3$, $r = -0.45$ for AlCl$_3$ + ouabain, $r = 0.2$ for aluminum citrate + ouabain, $r = -0.06$ for aluminum citrate at pH 3.5 and 7.4, and $r = -0.01$ for aluminum citrate at pH 2.0 and 8.5; $P > 0.05$ for all correlations).

Histologic examination

In experiments without ouabain (AlCl$_3$, aluminum citrate at pH 3.5 and 7.4, and aluminum citrate at pH 2.0 and 8.5) the integrity of the mucosa remained intact after incubation, but the process of eversion did cause occasional tissue damage to the tips of the villi, otherwise the tissues were histologically normal. However, in both experimental groups with ouabain (AlCl$_3$ + ouabain and aluminum citrate + ouabain) there were marked changes after incubation showing disruption of cells and villi and loss of integrity of the intestinal mucosa. This effect was less marked for stomach than for intestinal sacs.

**FIGURE 2.** A: Aluminum transport (see Methods, calculations) by isolated rat gut sacs. AlCl$_3$ (0.2 mmol/L), AlCl$_3$ (0.2 mmol/L) + ouabain (1 mmol/L), aluminum citrate (0.2 mmol A/L and 50 mmol citrate/L) + ouabain (1 mmol/L). [ ], stomach; [ ], proximal small bowel; [ ], mid small bowel; [ ], distal small bowel; and [ ], colon. *Significantly different from all other sites for aluminum citrate + ouabain, $P < 0.05$ (Tukey test); **aluminum citrate + ouabain for all small bowel sacs significantly different from AlCl$_3$ + ouabain or AlCl$_3$ for all small bowel and colon sacs, $P < 0.05$ (Tukey test). $\bar{x} \pm SD$; $n = 5$ for each site of each group except proximal small bowel of AlCl$_3$ and colon of AlCl$_3$ for which $n = 4$ each. B: Equilibrated ultrafiltrable aluminum concentration (see Methods, calculations) within the gut sac after incubation, as a percentage of the ultrafiltrable concentration of aluminum outside the gut sac, for AlCl$_3$, and AlCl$_3$ + ouabain. AlCl$_3$ : small bowel, median = 31.9 ($n = 14$, one data point lost); colon, median = 64.4 ($n = 5$) ($P < 0.05$). AlCl$_3$ + ouabain: small bowel, median = 33.8 ($n = 15$); colon, median = 57.8 ($n = 5$) ($P < 0.05$). There was no difference between small bowel with AlCl$_3$ compared with AlCl$_3$ + ouabain or between colon sacs with AlCl$_3$ compared with AlCl$_3$ + ouabain. The differences between sites (colon compared with small bowel sites) not reflected in A are due to the significantly smaller fluid volume required to fill the colon sacs.

**FIGURE 3.** Aluminum uptake (see Methods, calculations) by isolated rat gut sacs from aluminum citrate (2 mmol A/L and 50 mmol citrate/L). Aluminum citrate at 3.5/7.4: stomach pH 3.5, all other sites pH 7.4; Aluminum citrate at 2.0/8.5: stomach pH 2.0, all other sites pH 8.5. [ ], stomach; [ ], proximal small bowel; [ ], mid small bowel; [ ], distal small bowel; and [ ], colon. *Significantly different from other sites for both pH values, $P < 0.05$ (Tukey test). $\bar{x} \pm SD$; $n = 5$ for each site of each group except proximal small bowel of aluminum citrate at pH 2.0 and 8.5 for which $n = 4$. 
DISCUSSION

These experiments were designed, first, to investigate the site of aluminum absorption from the gastrointestinal tract, second, to study the mechanism of the well-known citrate effect on aluminum absorption and, third, to test the hypothesis that aluminum is absorbed by an energy-dependent system by using the metabolic inhibitor ouabain. The two groups of experiments were chosen to give the maximum information from a reasonable number of experiments, whereas the concentration of aluminum (2 mmol/L) in the high-concentration experiments was chosen to facilitate analysis and minimize the well-known problems of adhesion or contamination (19, 22, 23). Addition of 50 mmol citrate/L to 2 mmol Al/L ensured that even around neutral pH, soluble aluminum-citrate complexes were formed that at the varying pHs used were of predictable and significantly differing chemical charge and speciation (Table 1).

In contrast, around neutral pH and in the absence of effective chelators such as citrate, it is more difficult to predict the in vitro aqueous speciation of aluminum because complex hydroxypolymerization of the metal occurs, leading to the precipitation of aluminum hydroxides. Nevertheless, this process partly mimics the in vivo speciation of aluminum in the intestinal lumen, although in vivo, the hydroxypolymers of aluminum interact with endogenous molecules and are maintained as smaller soluble species that are more easily dissociable than are those in vitro (3, 20). This in vitro precipitation of aluminum salts such as AlCl₃, which occurs as the pH is neutralized [in simplified form: AlCl₃ + 3H₂O + 3OH⁻ → Al(OH)₃ + 3Cl⁻ + 3H₂O, precluded the use of the higher concentrations of aluminum (2 mmol/L) in the experimental group without citrate (low-aluminum experiments). Indeed, in the low-aluminum (0.2 mmol/L) ultrafiltration experiments a precipitate still occurred, but it was a relatively stable suspension that avoided the rapid sedimentation seen with 2.0 mmol Al/L (data not shown).

ICPOES was used as the main analytic technique. Our recent work with monochromatic ICPOES has achieved detection limits approaching those for FAAS (24), but for the polychromatic instrument used in these experiments detection limits were higher, and so FAAS was used to analyze those samples with quantities of aluminum not detectable by ICPOES. FAAS was not used routinely because it is less reliable for higher concentrations and is not suited to such large batches of samples.

The everted gut sac model is a simple system that excludes many physiologic variables such as peristalsis, blood flow, gastric emptying, and gastrointestinal transit times, allowing mechanisms of absorption to be studied in isolation. Nevertheless, there are limitations with isolated organ techniques, including the lack of systemic control and the short incubation times that are required to maintain viable tissue. Furthermore, in this model, tiny leakage of aluminum from the surrounding fluid and into the sacs through the sutured ends of bowel may be anticipated. Accurate measurements of changes in solute and fluid volume were made but would not detect such leakage. Indeed, it is difficult to control for this background because aluminum is itself a poorly absorbed solute, and a marker without any penetration through tissue but with leakage through the sutured ends identical to aluminum is not available. However, because the stomach sacs always showed the lowest transport rates in their groups, the upper limit of this background was less than or similar to the transport shown for stomach sacs.

Mechanism of absorption

This study confirms previous work showing that the tissue uptake of aluminum is much greater (up to 1000-fold) than its transport (3, 9). This suggests mainly that there is retention of the metal at the mucus either intracellularly (25) or by binding to the mucus-mucosal surface (19) because metals bind to glycoproteins in the mucus-glycocalyx layer (3, 5, 26). Absorption of aluminum is therefore biphasic, with both uptake and transport.

Uptake

The presence of citrate reduced the ratio of aluminum uptake to transport and although mucosal permeability was increased in the presence of citrate (ie, an increase in transport; see below), competition for metal binding between citrate and the mucus-mucosa (ie, a large reduction in uptake); Figure 1 was another reason. Indeed, in all experiments aluminum uptake by the stomach (at pH 2–3.5) was markedly less than by other sites (at pH 7.4–8.5), supporting the concept of avid metal uptake by the mucus-mucosa, which would be reduced by competing protons at the lower pH. Although other factors may also be important in explaining this observation, such as the potentially greater affinity of freshly polymerized aluminum (pH 7.4–8.5) for mucus-mucosa than unpolymerized aluminum (pH 2–3.5), and the smaller surface area of stomach than of intestine, these observations are consistent with a mechanism of largely extracellular (5) rather than intracellular mucosal uptake.

Transport

Ouabain clearly disrupted mucosal integrity and function, and yet had no effect on the transport of freshly prepared...
hydroxypolymerized aluminum (Figure 2), suggesting that there was no significant active transport of the metal. Other results confirmed the absence of an active process. First, there was no increase in percentage transport of aluminum above simple chemical equilibration (Figure 2B). Second, the large enhancement of transport by citrate argues against active transport. Finally, in the colon, which is more permeable to the passive diffusion of insulin than the small bowel (11), the percentage transport of aluminum was greater than that in the small bowel (Figure 2B), again supporting paracellular permeability, and this was independent of water transport.

**Effect of citrate on transport**

As expected from many previous reports (3, 5, 8, 14, 15), aluminum transport, ie, absorption, was increased in the presence of citrate. However, in the intestine, transport of aluminum from aluminum citrate (Figure 4) was independent of the magnitude of the negative charge on the overall complex (Table 1). Furthermore, in the stomach, generation of an uncharged aluminum-citrate species (Table 1) still did not allow permeation of the metal (Figure 4). Thus, the increase in aluminum transport by citrate may be because the small aluminum-citrate complex is better able to pass through the mucus-mucosa than the polyhydroxy species, or because citrate, like other calcium chelators, alters the permeability of the mucus (27) and mucosa (3, 8) or a combination of these. Whichever the reason, it is not due to the charge of the aluminum-citrate complex, as some have suggested (15).

**Site of absorption**

Transport of aluminum is a measure of systemic absorption and stomach sacs showed the lowest transport in all experiments, although not significantly so in the absence of citrate (AlCl₃ and AlCl₃ + ouabain groups), but such low values for transport are close to the error of the technique (see above). Differences in vivo between absorption from the stomach and the rest of the bowel will be greater than those shown in the AlCl₃ and AlCl₃ + ouabain groups, because first, the surface area of the stomach is much smaller than that of the whole of the small bowel, second, the transit time in the stomach is short compared with that of the small bowel and colon, and third and most importantly, the aluminum species used in the intestinal sacs here (fresh hydroxypolymerized aluminum) is not representative of the more labile and easily dissociable hydroxymetal-ligand polymer that probably forms in vivo (5). Hence, we support the view that, despite better solubility of aluminum under conditions in the stomach, its major absorption is from the bowel (3, 8).

In our study, the small bowel and colon absorbed aluminum almost equally, despite their surface areas being so different, and indeed, because of the differences in luminal fluid volumes, the colon achieved a higher concentration of aluminum in the period of transport studied. As before, there are other differences to consider in vivo. First, the colon absorbs water rapidly, producing a semisolid phase from which the diffusion of solutes is inhibited (28) and second, bacteria in the colon will greatly affect the intraluminal chemical environment. These effects cannot be easily modeled. Nevertheless, the colon clearly allows the passive transport of some poorly absorbed solutes. We were unable to confirm that aluminum absorption is greatest in the proximal small bowel (9), although in vivo this is the site likely to show enhanced absorption because any dietary ligands that are ingested with aluminum and promote its absorption (3, 5, 8), such as citrate, ascorbate, and maltol will be absorbed in the proximal bowel and therefore lost from the lumen more distally.

Thus, the whole bowel, and to a much lesser extent the stomach, has the capacity to absorb aluminum, but the mucosa of the distal bowel is largely not exposed to aluminum complexes capable of being well absorbed because these (and/or their constituent-promoting ligands) are absorbed and therefore removed more proximally. However, more poorly absorbed forms of aluminum are probably typical of most dietary situations and could be absorbed in small amounts throughout the length of the intestinal tract, including the colon. Such low, persistent exposure may be important for the accumulation of the metal and would have pharmacokinetics different from those for aluminum absorbed in bolus form such as from aluminum citrate.

**Conclusions**

This gut sac model has allowed further investigation of the absorption of the poorly absorbed solute aluminum. The results support recent work and suggest that the colon should be investigated as a site of significant paracellular transport of poorly absorbed solutes (11). In addition, the effects of citrate on mucosal permeability should be followed up with different markers of paracellular permeation. Similarly, it would be of interest to determine the effects of other substances on aluminum absorption, such as glucose, which, at least in the rat, may influence the integrity of the paracellular pathway (29) and could therefore increase systemic bioavailability of this toxin.

The results of this study suggest that although the stomach has little capacity in vivo to absorb aluminum, the metal may be absorbed passively in any other part of the intestinal tract, although the enhancing effects of ligands on absorption will chiefly occur proximally in the small bowel.

We thank Julie Simpson for statistical advice; N Walsh for use of the ICPOES facility at the Department of Geological Sciences, Royal Holloway and Bedford New College, Egham; and the Water Research Centre, Medmenham, for FAAS analyses.

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