

# Effects of an Alanyl-Glutamine–Based Oral Rehydration and Nutrition Therapy Solution on Electrolyte and Water Absorption in a Rat Model of Secretory Diarrhea Induced by Cholera Toxin

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**OBJECTIVES:** Recurring diarrhea and persistent diarrhea are commonly associated with malnutrition and long-term functional deficits. A beneficial approach would be to develop an alanyl-glutamine (AlaGln)–based oral rehydration and nutrition therapy (ORNT). We investigated the effect of an AlaGln-ORNT solution on electrolyte and water absorption in a rat model of secretory diarrhea induced by cholera toxin.

**METHODS:** Phenolsulfonphthalein (50  $\mu\text{g/mL}$ ) was used as a non-absorbable marker for calculation of net water and electrolyte transport. Solutions tested were Ringer's solution, a glutamine-based ORNT (Gln-ORNT) solution, and an AlaGln-ORNT solution. Cholera toxin (1  $\mu\text{g/mL}$ ) was injected into lumen of rat small intestinal segments and incubated for 18 h before the initiation of the perfusion.

**RESULTS:** Cholera toxin induced significant secretion of electrolyte and water in the control Ringer's solution. AlaGln-ORNT and Gln-ORNT solutions reduced the sodium secretory effect of cholera toxin by 128% and 36%, respectively. The net water secretion also was reduced by 95% and 60%, respectively, with the AlaGln-ORNT and Gln-ORTN solutions.

**CONCLUSIONS:** These results showed that AlaGln-ORNT solution can enhance water and electrolyte intestinal absorption even better than glutamine or glucose and thus provide a potential novel approach for ORNT to break the vicious cycle of diarrhea and malnutrition. Clinical trials are now needed in children and adults with diarrhea and malnutrition. *Nutrition* 2002;18:458–462. ©Elsevier Science Inc. 2002

**KEY WORDS:** alanyl-glutamine, diarrhea and malnutrition, oral rehydration and nutrition therapy, secretory diarrhea

## INTRODUCTION

Diarrheal diseases remain one of the major public health problems in developing areas throughout the world.<sup>1</sup> Persistent ( $\geq 14$  d) and recurring diarrhea are commonly associated with malnutrition and long-term functional deficits.<sup>2</sup> Three major pathogens have been associated with persistent diarrhea—*Cryptosporidium* spp., entero-

aggregative *Escherichia coli* (EAggEC), and torovirus<sup>3–5</sup>—pathogens for which specific effective drugs are not readily available. A beneficial approach would be to develop an oral rehydration and nutrition therapy (ORNT) to treat dehydration and enhance repair of the intestinal mucosal injury seen with these infections and, hence, prevent malnutrition and to interrupt the vicious cycle of persistent diarrhea and malnutrition.

Glutamine has been identified as a potential candidate to supplement or replace glucose in ORNT.<sup>6</sup> Glutamine is the principal source of energy for enterocytes,<sup>7</sup> and it plays a key role in nucleotide and protein synthesis.<sup>8,9</sup> This non-essential neutral amino acid (molecular weight of 146.1 Da) is the predominant amino acid in the blood, and it can be synthesized and accumulated in some tissues, e.g., skeletal muscles. However, in catabolic conditions such as infections, persistent diarrhea, malnutrition, or stress, it is often depleted and becomes a “provisionally essential” amino acid.<sup>10–15</sup>

The small intestine consumes 40% of the total glutamine supplied by arterial perfusion in experimental rats.<sup>7</sup> Jensen et al. noted that glutamine is critical to the maintenance and repair of intestinal mucosal integrity.<sup>16</sup> This repair is important in intestinal infections, persistent diarrhea, and malnutrition, where the small intestine was damaged and showed striking disruption in intestinal

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permeability.<sup>17-20</sup> Glutamine in enteral and parenteral nutrition increased villous height and mucosal nitrogen and stimulated intestinal mucosal growth in individuals after long periods of fasting.<sup>10,12,13</sup>

Replacement of glucose with glutamine in an oral rehydration solution significantly increased electrolyte and water absorption in a rabbit model of secretory diarrhea induced by cholera toxin.<sup>6</sup> Glutamine also stimulated electrolyte and water absorption in a rabbit model infected with enteropathogenic *E. coli* and in patients with cholera.<sup>21,22</sup> Glutamine derivatives, such as alanyl-glutamine and alanyl-glutamyl-glutamine, are more stable than glutamine in low pH, and they are more stable under the high temperatures normally used to sterilize those enteral and parenteral solutions.<sup>15,23,24</sup> In addition, these di- and tripeptides derivatives would provide a greater number of glutamine molecules at a physiologic osmolality needed in oral rehydration solutions.

We postulated that alanyl-glutamine in a new ORNT solution would improve intestinal electrolyte and water absorption. Therefore, we investigated the effect of an alanyl-glutamine-based oral rehydration solution on electrolyte and water absorption in a rat model of secretory diarrhea induced by cholera toxin.

## MATERIALS AND METHODS

### Experimental Animals

All animals were supplied by the Clinical Research Unit, Federal University of Ceará and the protocol complied with the *Occupational Health and Safety in the Care and Use of Research Animals* (National Research Council, 1997). Wistar rats of both sexes, weighing 180 to 200 g., were fasted for 24 h with water ad libitum before each experiment. After administration of ketamine anesthesia (60 mg/kg intramuscular) and xylazine (10 mg/kg intramuscularly), a median, 8- to 10-cm laparotomy was made for visualization of the small bowel. An approximately 30-cm segment of terminal ileum was washed with 10 mL of phosphate-buffered saline (pH 7.4), and the proximal and distal ends of the segment were ligated for injection of 3 mL of cholera toxin (1  $\mu$ g/mL). The animals were fasted for 18 h and then anesthetized, and the same segments were ligated around a proximal multiperforated polyvinyl cannula (internal diameter 0.0559 inches, outer diameter 0.1239 inches, and wall 0.034 inches; Cole-Parmer Instrument Company, Niles, IL, USA) and a distal polyvinyl cannula (internal diameter 0.085 inches and outer diameter 0.128 inches; Becton Dickinson, Sparks, MD, USA) for perfusion studies. The test solutions were warmed to 37°C, maintained at pH 7.4, and introduced through the proximal cannula with a pump (Holter Roller Pump 900, Extracorporeal Medical Specialities, King of Prussia, PA, USA). Perfusion was maintained at a slow rate of 0.16 mL/min throughout the experiment. At the end of the perfusion, the animals were killed by intracardiac injections of a highly concentrated solution of potassium chloride. The dry weight (after desiccation at 90°C for 48 h) of the intestinal segment was used for flux calculations.

### Drugs and Reagents

Phenolsulfonphthalein (50  $\mu$ g/mL) was used as a non-absorbable marker for calculation of sodium, potassium, chloride, and water net fluxes. Phenolsulfonphthalein was measured spectrophotometrically (Spectrophotometer Model B382; Micronal S.A. Aparelhos de Precisão, São Paulo, SP, Brazil) according to the method developed by Schedl and Clifton.<sup>25</sup> Sodium and potassium concentrations in the perfusate were measured by flame photometry (Flame Photometer Model 443, Instrumentation Laboratory, Lexington, MA, USA). The colorimetric method for chloride (Labtest Bio, Diagnósticos, Belo Horizonte, Brazil) was used according to manufacturer's instructions. The osmolality of the perfusion sam-

TABLE I.

CHEMICAL COMPOSITIONS OF RS, GLN-ORNT, AND ALAGLN-ORNT\*

Components	RS (g/L)	Gln-ORNT (g/L)	AlaGln-ORNT (g/L)
NaCl	6.5	3.5	3.5
KCl	0.14	1.5	1.5
Sodium citrate	—	2.9	2.9
CaCl <sub>2</sub>	0.12	—	—
NaHCO <sub>3</sub>	0.20	—	—
NaHPO <sub>4</sub>	0.01	—	—
Glutamine	—	16.2	—
AlaGln	—	—	24.1

\* The osmolalities of RS, Gln-ORNT, and AlaGln-ORNT were  $267 \pm 6.712$ ,  $285 \pm 3.136$ , and  $304 \pm 11.83$  (mean  $\pm$  standard error). RS versus Gln-ORNT or AlaGln-ORNT was significant at  $P < 0.05$  (unpaired Student's *t* test) and Gln-ORNT versus AlaGln-ORNT was not significant.

AlaGln-ORNT, alanyl-glutamine-based oral rehydration and nutrition therapy; Gln-ORNT, glutamine-based oral rehydration and nutrition therapy; RS, Ringer's solution.

ples was measured with a vapor pressure osmometer (Model 5100C, Wescor, Logan, UT, USA).

### Animal Protocol

Groups of at least seven animals each were used to test the following oral rehydration solutions at pH 7.4: modified Ringer's solution (RS), glutamine-based ORNT solution (Gln-ORNT), and alanyl-glutamine-based ORNT (AlaGln-ORNT; Table I). All three solutions were tested in control rats and rats treated with cholera toxin, as described above. Control animals were treated identically but with phosphate-buffered solution in place of cholera toxin in the 18-h incubation in intestinal segments. The samples were taken every 20 min throughout the 80-min study period for measurement of electrolyte, osmolality, and phenolsulfonphthalein concentrations.

### Data Analysis

The calculations of net fluxes were done with Microsoft Excel 4.0 (Microsoft Corporation, Cupertino, CA, USA) for MS-DOS, and groups were tested for homogeneous variance then used a Student's *t* test. Data are presented as mean  $\pm$  standard error of the mean.  $P < 0.05$  (two-tailed) was considered statistically significant.

### Chemicals

Cholera toxin was obtained from ICN Biomedicals (Aurora, OH, USA). Phenolsulfonphthalein, glutamine, alanyl-glutamine, and glucose were obtained from Sigma Chemical Co. (St. Louis, MO, USA), as were salts for perfusion solutions (NaCl, KCl, CaCl<sub>2</sub>, NaHCO<sub>3</sub>, and NaH<sub>2</sub>PO<sub>4</sub>) and sodium citrate.

## RESULTS

To validate the rat model of secretory diarrhea, cholera toxin (1  $\mu$ g/mL) was injected as described in MATERIALS AND METHODS, and after 18 h the modified RS was perfused for a total of 80 min. Cholera toxin caused significant sodium secretion (from a control net absorptive flux of  $2.341 \pm 0.3323$   $\mu$ Eq/g per minute to a net secretion of  $-1.118 \pm 0.2627$   $\mu$ Eq/g per minute;  $P < 0.05$ ; Fig.

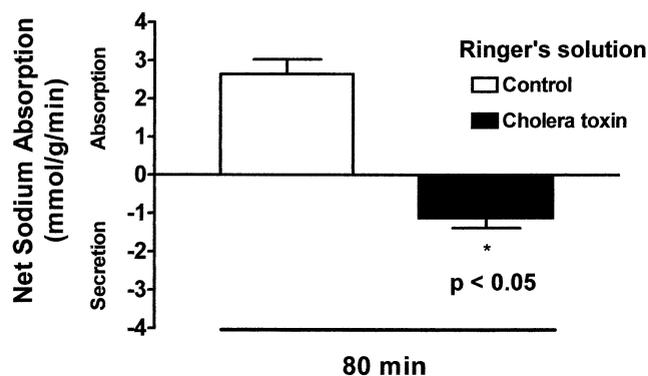


FIG. 1. Net sodium secretion induced by cholera toxin (1  $\mu\text{g/mL}$ ) in the perfused rat small intestine. Data represent mean  $\pm$  standard error for at least seven experimental animals. There was a significant difference between the control and cholera toxin groups,  $P < 0.05$ , unpaired Student's  $t$  test.

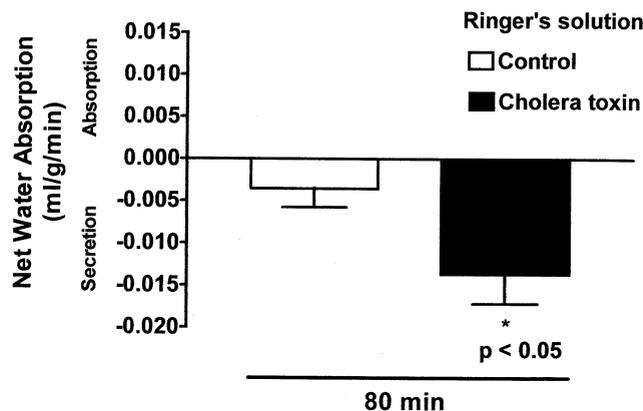


FIG. 2. Net water secretion induced by cholera toxin (1  $\mu\text{g/mL}$ ) in the perfused rat small intestine. Data represent mean  $\pm$  standard error for at least seven experimental animals. There was a significant difference between the control and cholera toxin groups,  $P < 0.05$ , unpaired Student's  $t$  test.

1 and Table II). Change was also seen in net water transport with cholera toxin ( $-0.003563 \pm 0.002206$  versus  $-0.01368 \pm 0.003464$  mL/g per minute;  $P < 0.05$ ) compared with that of control animals with RS without cholera toxin (Fig. 2 and Table II). In addition, cholera toxin caused significant potassium secretion (from a control net absorptive flux of  $0.3206 \pm 0.0187$   $\mu\text{Eq/g}$  per minute to a net secretion of  $-0.7318 \pm 0.06501$   $\mu\text{Eq/g}$  per minute;  $P < 0.05$ ; Table II). The net chloride secretion did not increase with cholera toxin (from control net secretion flux of  $-0.7222 \pm 1.260$   $\mu\text{Eq/g}$  per minute versus  $-0.6861 \pm 0.4583$   $\mu\text{Eq/g}$  per minute;  $P > 0.05$ ; Table II).

Table II summarizes the data on the effects of RS, Gln-ORNT, and AlaGln-ORNT on electrolyte and water absorption in a rat model of secretory diarrhea induced by cholera toxin. The AlaGln-ORNT solution significantly decreased sodium secretion (from an RS control net secretion of  $-1.118 \pm 0.2627$   $\mu\text{Eq/g}$  per minute to a net absorptive flux of  $0.3104 \pm 0.07726$   $\mu\text{Eq/g}$  per minute;  $P <$

$0.05$ ; Fig. 3). This solution significantly changed the net water transport with cholera toxin ( $-0.01368 \pm 0.003464$  versus  $-0.00064 \pm 0.00179$  mL/g per minute;  $P < 0.05$ ; Fig. 4) compared with that of RS with cholera toxin. In addition, AlaGln-ORNT solution significantly changed secretion of chloride (from an RS control net secretion of  $-0.6861 \pm 0.4583$   $\mu\text{Eq/g}$  per minute to a net absorption of  $2.913 \pm 0.4752$   $\mu\text{Eq/g}$  per minute;  $P < 0.05$ ) and potassium (from an RS control net secretion of  $-0.7318 \pm 0.06501$   $\mu\text{Eq/g}$  per minute to a net absorption of  $0.7154 \pm 0.1051$   $\mu\text{Eq/g}$  per minute;  $P < 0.05$ ).

Net sodium secretion was consistently altered by the Gln-ORNT ( $-1.118 \pm 0.2627$  versus  $-0.7125 \pm 0.8330$   $\mu\text{Eq/g}$  per minute;  $P > 0.05$ ) as was net water transport ( $-0.01368 \pm 0.003464$  versus  $-0.005438 \pm 0.004487$  mL/g per minute;  $P > 0.05$ ) compared with that of control animals with RS solution with cholera toxin (Table II and Figs. 3 and 4). This Gln-ORNT

TABLE II.

EFFECTS OF THE ORNT SOLUTIONS ON THE NET SODIUM, NET CHLORIDE, NET POTASSIUM, OSMOLALITY, AND WATER WITH AND WITHOUT CHOLERA TOXIN (1  $\mu\text{G/ML}$ )\*

	Net sodium balance ( $\mu\text{Eq} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ )	Net chloride balance ( $\mu\text{Eq} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ )	Net potassium balance ( $\mu\text{Eq} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ )	Change in osmolality ( $\mu\text{Osm} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ )	Net water balance ( $\text{mL} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ )
<b>RS</b>					
Control	$2.341 \pm 0.3323$	$-0.7222 \pm 1.260$	$0.3206 \pm 0.0187$	$-4.062 \pm 1.622$	$0.003563 \pm 0.002206$
Cholera toxin	$-1.118 \pm 0.2627$ †	$0.6861 \pm 0.458$ ‡	$-0.7318 \pm 0.06501$ †	$-9.915 \pm 1.875$ ‡	$-0.01368 \pm 0.003464$ †
<b>Gln-ORNT</b>					
Control	$0.7881 \pm 0.1049$	$9.225 \pm 1.056$	$1.3210 \pm 0.07828$	$-1.314 \pm 0.4105$	$-0.001438 \pm 0.001588$
Cholera toxin	$-0.7125 \pm 0.833$ ‡	$7.346 \pm 1.745$ ‡§	$0.7603 \pm 0.07804$ ‡§	$-18.56 \pm 1.790$ ‡§	$-0.005438 \pm 0.004487$ ‡
<b>AlaGln-ORNT</b>					
Control	$2.9640 \pm 0.4193$	$9.769 \pm 0.6943$	$1.3650 \pm 0.1119$	$-27.61 \pm 1.057$	$-0.001716 \pm 0.001505$
Cholera toxin	$0.3108 \pm 0.0773$ †	$2.913 \pm 0.4752$ †  ¶	$0.7154 \pm 0.1051$ †	$-38.11 \pm 2.091$ †  ¶	$-0.000643 \pm 0.001793$ †

\* Data represent mean  $\pm$  standard error of at least seven animals studied from the 80 min of perfusion. Negative values are the net secretory balance and positive values are the net absorption balance.

† Control versus cholera toxin for each group was significant at  $P < 0.05$  (unpaired Student's  $t$  test). ‡Not significant at  $P > 0.05$  for the same groups.

§ RS plus cholera toxin versus Gln-ORNT plus cholera toxin, significant at  $P < 0.05$ .

|| RS plus cholera toxin versus AlaGln-ORNT plus cholera toxin, significant at  $P < 0.05$ .

¶ Gln-ORNT plus cholera toxin versus AlaGln-ORNT plus cholera toxin, significant at  $P < 0.05$ .

AlaGln-ORNT, alanyl-glutamine-based oral rehydration and nutrition therapy; Gln-ORNT, glutamine-based oral rehydration and nutrition therapy; RS, Ringer's solution.

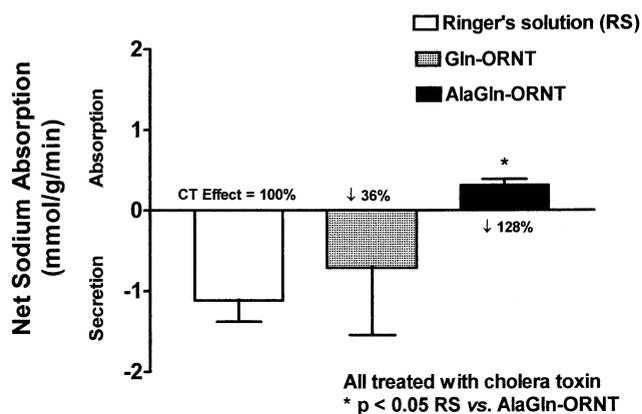


FIG. 3. Reduction of CT-induced sodium secretory effect by AlaGln-ORNT and Gln-ORNT solutions in the perfused rat small intestine. The AlaGln-ORNT and Gln-ORNT solutions reduced, by 128% and 36%, respectively, the sodium secretory effect induced by CT (1  $\mu\text{g/mL}$ ). AlaGln-ORNT, alanyl-glutamine–based oral rehydration and nutrition therapy; CT, cholera toxin; Gln-ORNT, glutamine-based oral rehydration and nutrition therapy; RS, Ringer's solution.

solution also significantly decreased net secretion of chloride (from an RS control net secretion of  $-0.6861 \pm 0.4583 \mu\text{Eq/g}$  per minute to a net absorption of  $7.346 \pm 1.745 \mu\text{Eq/g}$  per minute;  $P < 0.05$ ) and potassium (from an RS control net secretion of  $-0.7318 \pm 0.06501 \mu\text{Eq/g}$  per minute to a net absorption of  $0.7603 \pm 0.07804 \mu\text{Eq/g}$  per minute;  $P < 0.05$ ).

These data showed that the AlaGln-ORNT and Gln-ORNT solutions reduced the sodium secretory effect of cholera toxin by 128% and 36%, respectively. The net water secretion also was reduced by 95% and 60%, respectively, by these ORNT solutions (Fig. 3 and Table II).

RS control did not significantly reduce the net osmolality of the intestinal fluid induced by cholera toxin, an effect that was significantly reduced by AlaGln-ORNT and Gln-ORNT (Table II).

## DISCUSSION

The basis for the ORNT was suggested first in 1831 by O'Shaughnessy. He suggested that patients with cholera should be

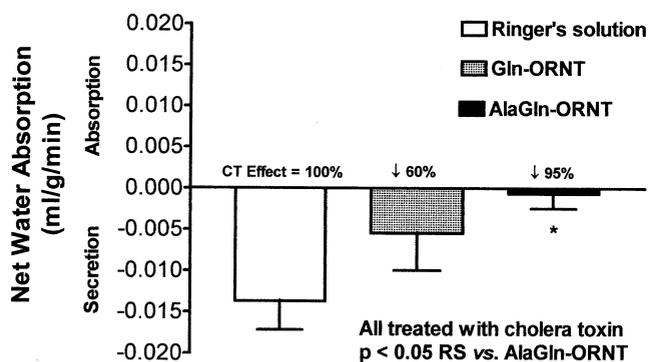


FIG. 4. Reduction of CT-induced water secretory effect by AlaGln-ORNT and Gln-ORNT solutions in the perfused rat small intestine. The AlaGln-ORNT and Gln-ORNT solutions reduced, by 95% and 60%, respectively, the water secretory effect induced by CT (1  $\mu\text{g/mL}$ ). AlaGln-ORNT, alanyl-glutamine–based oral rehydration and nutrition therapy; CT, cholera toxin; Gln-ORNT, glutamine-based oral rehydration and nutrition therapy; RS, Ringer's solution.

treated with a solution with the electrolyte and water levels equivalent to those in their liquid stools.<sup>26</sup> In the late 1940s, sodium absorption was found to be increased by the addition of glucose to the luminal side of the intestinal epithelium.<sup>27</sup> In the 1950s, Darrow, Harrison, and others reported the successful use of an oral rehydration solution to maintain the electrolyte and water balance in children with dehydration.<sup>28–32</sup> An oral rehydration solution with electrolytes and glucose was successful in treating patients with cholera in India.<sup>33</sup>

In the 1990s, a new ORNT with glutamine was investigated in several studies in vitro, in vivo, and clinical trials. Rhoads et al. found that glutamine stimulates the electroneutral transport of NaCl in the small intestine in vitro.<sup>34</sup> In addition, they reported the electrogenic absorption of sodium in the presence of glutamine. Lima et al. showed that the absorption of sodium with glutamine or alanine in the rabbit ileum mounted in Ussing chambers was more effective than that with glucose.<sup>35</sup> These cotransporters of sodium with amino acids and glucose were not impaired in tissues treated with cholera toxin.<sup>36</sup> Ribeiro et al. studied an oral rehydration solution containing L-glutamine and compared the efficacy of adding 90 mM/L of L-glutamine with that of 90 mM/L of L-glucose to the standard World Health Organization (WHO) oral rehydration solution to treat infants 1 mo to 1 y of age with acute non-cholera diarrhea and dehydration.<sup>37</sup> They found that the glutamine-based oral rehydration solution is well tolerated and has effects similar to those of the standard WHO oral rehydration solution in treating dehydration in infants with non-cholera diarrhea.

Two animal studies reported the efficacy of Gln-ORNT solution in a rabbit diarrhea model induced by cholera toxin and enteropathogenic *E. coli*.<sup>6,21</sup> The present results from a perfused rat model corroborated those of a study by Silva et al.<sup>6</sup> and add new data on the AlaGln-ORNT solution. Somewhat to our surprise, these results with the new ORNT solution suggested that the stable glutamine derivative, AlaGln, is actually more effective than glutamine alone on electrolyte and water absorption. The mechanism for this improvement is not known, but preliminary experiments suggested that it is not due to enhanced sodium cotransport because AlaGln did not increase the electrogenic sodium transport in vitro when using ileal mucosa mounted in Ussing chambers (Lima et al., unpublished observations). In fact, cumulative data showed that di- and tripeptide transporters are not cotransported with sodium but with  $\text{H}^+$ .<sup>38</sup> We postulated that AlaGln provides critical support for intestinal epithelial metabolism by increasing such ion transporters as neutral NaCl transport,<sup>34</sup> thereby improving electrolyte and water absorption compared with RS or Gln oral rehydration solution. Because experiment took only 80 min, we do not feel that this effect is due to an effect on villous growth, which takes considerably longer (Brito et al., unpublished observations). AlaGln is a stable derivative of glutamine, resistant to low pH and high temperature for sterilization, and has been given parenterally to humans without toxicity.<sup>23</sup>

These data documented in vivo that an AlaGln-ORNT solution can enhance water and electrolyte absorption and thus provides a potential novel approach for rehydration and nutrition to break the cycle of diarrhea and malnutrition. Clinical trials are needed in children and adults with diarrhea and malnutrition. In addition to its effect on rehydration, AlaGln provides the potential of a new ORNT solution to supply the key nutrients for repair of intestinal barrier function and treatment of persistent diarrhea and malnutrition.

In summary, these results showed that the new AlaGln-ORNT solution is even more effective than Gln-ORNT solution, which is more effective than glucose-based oral rehydration solution or RS solution on electrolyte and water absorption in secretory diarrhea induced by cholera toxin.

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