

# Evaluation of Oral Rehydration Solution by Whole-Gut Perfusion in Rats: Effect of Osmolarity, Sodium Concentration and Resistant Starch

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## ABSTRACT

**Background:** Reduced osmolarity oral rehydration solution (ORS) improved small bowel absorption of fluid and electrolytes in segmental perfusion in experimental animals; this was borne out in clinical practice. Adding amylase-resistant starch (RS) to ORS is expected to increase colonic fluid absorption. This study used combined small and large bowel perfusion to evaluate combinations of reduced osmolarity and starch in ORS.

**Methods:** Single-pass steady-state perfusions of the whole gut at 30 mL/h, using the nonabsorbable marker  $^{14}\text{C}$ -polyethylene glycol 4000, were performed in Wistar rats after exposure to cholera toxin or *Escherichia coli* heat-stable enterotoxin (STa).

**Results:** Steady state was established within 90 minutes after commencing perfusion. Net secretion of water, sodium and chloride induced by cholera toxin was partially reversed by standard glucose-ORS (G-ORS). Substituting glucose in G-ORS with RS (RS-ORS) substantially increased net water

absorption ( $P < 0.001$ ) as did reduced osmolarity ORS (RO-ORS) ( $P < 0.001$ ); addition of RS to RO-ORS further increased water absorption ( $P < 0.001$ ). In STa-treated intestine, RO-ORS and RS-ORS significantly improved water absorption compared to G-ORS ( $P < 0.005$ ). RO- and RS-RO-ORS did not significantly augment net electrolyte absorption compared with G-ORS. RS-ORS was associated with highest net absorption of sodium and chloride compared with all other groups.

**Conclusions:** RS increased net water (and sodium) absorption from isosmolar and reduced osmolar ORS consistent with increased absorption by the colon. RS in reduced osmolar ORS may have advantages to reduce severity of diarrhea and prevent hyponatremia in severe diarrhea and may be applicable to diarrhea of different etiologies. *JPGN* 43:568–575, 2006.

**Key Words:** Oral rehydration therapy—Diarrhea—Cholera—Dehydration—Starch. © 2006 Lippincott Williams & Wilkins

Oral rehydration solution (ORS) is a well-established treatment for acute diarrhea by virtue of its stimulation of fluid and electrolyte absorption in the small intestine. The physiological basis for ORS is that glucose-stimulated sodium absorption is not altered by cyclic adenosine monophosphate (cAMP), while cAMP stimulates active chloride secretion and inhibits electroneutral Na-Cl absorption (1). Despite the substantial benefit provided by ORS to correct dehydration and metabolic acidosis in children with acute diarrhea, ORS does not substantially reduce stool output (2). As a consequence, there have been many attempts to modify ORS to reduce stool output as

well as to increase small intestinal fluid and electrolyte absorption (3). Until recently, all of these approaches focused on ORS modifications that would enhance small intestinal fluid absorption. For example, different amino acids have been used without substantial benefit (3). The use of food polymers, which result in glucose production in the proximal small intestine following pancreatic hydrolysis, has been effective. Thus, rice-based ORS was more effective in the treatment of acute diarrhea than WHO (World Health Organization)-ORS, although this benefit appears to be confined to cholera (4). The identification of an optimal ORS formulation has been facilitated by parallel studies in experimental animals, usually rats, in which segments of isolated portions or all of the small intestine have been perfused with  $\geq 1$  solutions (5–7).

A different approach to improve ORS was recently demonstrated that involved enhancement of colonic fluid and electrolyte absorption (8) by the addition of starch

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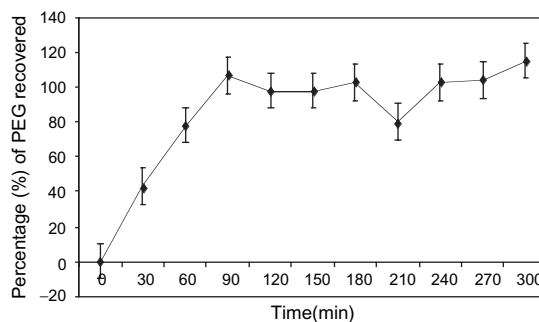
that is relatively resistant to amylase digestion (resistant starch, or RS). The use of RS-ORS was based on the demonstration that stimulation of active Na-Cl absorption by short-chain fatty acids (SCFA) in the rat distal colon was not inhibited by cAMP (9,10). It was proposed that RS-ORS would result in an increase in the delivery of nonabsorbed carbohydrate to the colon, where they would be metabolized by colonic bacteria to SCFA (11). Indeed, a clinical trial demonstrated that RS-ORS was better than both WHO-ORS and rice flour (RF) ORS in the treatment of acute cholera in adults (12).

Other studies had demonstrated that hypoosmolar ORS was better than WHO-ORS (13–16). As a result, hypoosmolar or reduced osmolar ORS was established as the preferred ORS by the World Health Organization despite concerns of the potential development of hyponatremia in some patients with acute cholera (17–19). A critical issue is to determine whether RS would also enhance the treatment of acute diarrhea in patients treated with reduced osmolar ORS because it had been shown to do with WHO-ORS. However, the formulation of an optimal RS-reduced osmolar ORS would require prior perfusion studies in experimental animals. Because the presumed site of absorption for RS is the colon and that for reduced osmolar ORS is the small intestine, it would be necessary first to establish a model in which both the small and large intestines could be perfused together with different modifications of ORS.

The present study was designed to develop a whole-gut perfusion model in rats to identify the optimal formulation of an RS-containing reduced osmolar ORS. In these studies, different formulations of ORS that varied the composition of RS and reduced osmolarity (RO) were studied in rats exposed to either cholera toxin (CT) or the heat-stable enterotoxin of *Escherichia coli* (STa).

## METHODS

Adult Wistar albino rats (180–220 g body weight) used in all experiments were fasted for 16 h with free access to water. These experiments were approved by the Animal Ethics Committee of the Christian Medical College, Vellore. The rats were anesthetized with intraperitoneal injection of sodium pentobarbitone (30 mg/kg body weight). Anesthesia was maintained throughout the experiment by interval intraperitoneal injection (10–15 mg/kg), as required. The abdomen was opened through a midline incision, and the first part of the duodenum was cannulated. The cecum was excluded by a ligature, taking care to avoid ischemia. The colon was cleared of luminal feces and a catheter placed in the rectum and secured by a ligature, taking care not to compromise the blood supply. The intestine was returned to the abdominal cavity and the abdomen closed. Single-pass continuous perfusion of the whole gut was performed at a rate of 30 mL/h using a syringe infusion pump (Vickers). The tubing containing the infused solutions passed through a water bath to maintain the temperature at 37°C. The



**FIG. 1.** Mean (SEM) recovery of PEG-4000 from rectal effluent, expressed as percentage of PEG infused into the gut during that same time, in each 30-min time period. PEG recovery reached 100% of the amount infused in the 30-min period from 60–90 min after onset of perfusion, and remained at 95%–110% thereafter, indicating steady-state conditions.

body temperature of the animal was maintained at  $37^{\circ} \pm 0.5^{\circ}\text{C}$  using an overhead lamp.

In these experiments, net fluid movement was determined by changes in the concentration of polyethylene glycol of average molecular weight 4000 (PEG-4000), a nonabsorbable marker, which was added to all perfusion solutions together with  $^{14}\text{C}$ -PEG. PEG concentration was determined by measuring  $^{14}\text{C}$ -PEG in a liquid scintillation counter. Preliminary experiments were designed to determine the time required to establish steady-state conditions in both control animals and those in whom fluid secretion had been induced by CT. In these experiments, a Ringer solution (Na 140, K 5.2, Cl 119.8,  $\text{HCO}_3$  25,  $\text{H}_2\text{PO}_4$  0.4,  $\text{HPO}_4$  2.4, Mg 1.2, Ca 1.2, glucose 10 mmol/L) was used as the perfusate. Rectal effluent was collected from the beginning of perfusion at 15-minute intervals for a total of 5 hours and  $^{14}\text{C}$ -PEG activity was measured (Fig. 1). Steady-state conditions were established between 60 and 90 minutes of perfusion, as assessed by total  $^{14}\text{C}$ -PEG recovery and/or by constant concentrations of  $^{14}\text{C}$ -PEG in the rectal effluent. The mean  $\pm$  SEM recovery of PEG for 30-minute periods over 300 minutes as the percentage of recovery of the amount infused into the intestine during the corresponding period is shown in Fig. 1. Similar results were observed in the animals that were not exposed to CT (results not shown). As a result of these observations, an equilibration period of 90 minutes was used followed by 15-minute collection periods of effluent fluid in subsequent experiments. Net water and electrolyte movement was calculated from the 2 final collection periods. At the end of the experiment, rats were killed by an overdose of anesthesia, the abdomen was opened, and the perfused segment of bowel was removed, opened out and stretched out on paper. The total length of perfused bowel was measured. Each rat was perfused with 1 solution. Results were expressed as microliters per minute per centimeter or micromoles per minute per centimeter of perfused intestine.

## Studies with CT

In studies with CT (C-3015, Sigma Chemical, St Louis, MO) 100  $\mu\text{g}$  of CT added to 8 mL 0.9% NaCl was instilled into the intestine, evenly dispersed by gentle squeezing of the intestine

and then left in situ for 2 hours. Control studies were done similarly by exposing the intestine to 8 mL 0.9% NaCl for 2 hours, after which perfusion studies were commenced.

### Studies with STa

In experiments with STa (E-5763, Sigma Chemical), the intestine was perfused with the test ORS for 90 minutes to establish steady state, after which the same test ORS containing 300  $\mu\text{g/L}$  STa was perfused for another 90 minutes. The concentration of STa required for these studies was established in initial studies using various concentrations; the final dose that was used was one that abolished net fluid absorption from the intestine.

### Analyses

$\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$ , and  $\text{HCO}_3^-$  in perfusate and effluent samples were measured using ion-sensitive electrodes (Beckman). [ $^{14}\text{C}$ ]-Polyethylene glycol was measured by liquid scintillation spectrometry using an LKB 1210 Rackbeta counter.

### Solutions

Table 1 lists the composition of the several ORS that were studied. Polyethylene glycol-4000, consisting of 3 g/L unlabeled PEG-4000 (catalog no. 81240, Fluka) and 1.2  $\mu\text{Ci/L}$  [ $^{14}\text{C}$ ]-PEG-4000 (CFA 508, Amersham Biosciences), was added to all solutions as a nonabsorbable marker.

Solutions with different osmolalities were used in these experiments. As detailed in Table 1, osmolality of the basic ORS was either 311 mOsm/kg  $\text{H}_2\text{O}$  (standard WHO glucose-ORS) or 245 mOsm/kg (reduced osmolality WHO-ORS). High amylose maize starch (Hi-Maize) used in these studies and referred to as RS because it provided amylase-resistant starch. RS, when added to ORS, did not increase measured osmolality; in contrast, because RS replaced glucose in ORS, there was a drop in osmolality. To provide appropriate controls for the further reduction of osmolality induced by adding RS to ORS in place of glucose, digestible starch (DS) or RF was included in additional test solutions to provide solutions of

similar osmolality as RS-containing ORS. In RS, 30% of the starch was amylase resistant, the other 70% being digestible. The digestible component of the starch was expected to provide glucose in the small intestine by luminal hydrolysis, and glucose was therefore excluded from these solutions. In their composition, they mimic currently used cereal-based ORS. RO refers to reduced osmolality ORS that is currently recommended by the WHO. RF refers to rice flour suspended in reduced osmolar ORS instead of glucose. RS-RO-ORS was similar to RO-ORS in electrolyte composition but contained RS in place of glucose and was therefore of lower osmolality.

### Calculations

Net absorption or secretion of water across the gut epithelium was calculated from changes in the concentration of PEG.

Net water transport ( $\mu\text{L}/(\text{min}/\text{cm})$ )

$$= V_i - [V_i(\text{PEG}_i)/(\text{PEG}_o)]/\text{length of gut (cm)} \times 1000$$

Net electrolyte transport (nmol/(min/cm))

$$= [E_i V_i] - [E_o V_i(\text{PEG}_i)/(\text{PEG}_o)]/\text{length of gut (cm)} \times 1000$$

where  $V_i$ ,  $\text{PEG}_i$  and  $E_i$  are volume (in milliliters), PEG concentration (in milligrams/milliliters) and ion concentration (in millimoles per liter) of infusate, and  $\text{PEG}_o$  and  $E_o$  are PEG and ion concentrations of effluent fluid.

The mean PEG and electrolyte concentrations of 2 consecutive 15-minute effluent samples after attaining steady state were used to calculate transport using standard formulas. Steady-state conditions were ensured in all of the experiments by ensuring that PEG recovery during the test period equaled the PEG input during that time period.

### Statistics

All of the values were expressed as mean (SEM). Differences between study groups in water and ion movement were examined statistically using Kruskal-Wallis analysis of variance

**TABLE 1.** Composition of the experimental oral rehydration solutions tested in this perfusion system

	G-ORS	RS-ORS	DS-ORS	RO-ORS	RS-RO ORS	RF-RO-ORS
$\text{Na}^+$	90	90	90	75	75	75
$\text{Cl}^-$	80	80	80	65	65	65
$\text{K}^+$	20	20	20	20	20	20
Citrate	10	10	10	10	10	10
Glucose	111	—	—	75	—	—
Resistant starch	—	50 g/L	—	—	50 g/L	—
Digestible starch	—	—	50 g/L	—	—	—
Rice flour	—	—	—	—	—	50 g/L
Osmolarity	311	200	200	245	170	170

Concentrations are expressed as mmol/L except where shown as g/L. G-ORS indicates the standard glucose-ORS containing 111 mmol/L glucose and 90 mmol/L sodium that was until recently recommended as the universal ORS by the WHO; RS, high-amylose maize starch, 30% of which was amylase resistant, the other 70% being digestible; DS, digestible starch, expected to undergo complete digestion in small intestine; RO, reduced osmolality ORS that is currently recommended by the WHO; RS-RO-ORS, similar to RO-ORS in electrolyte composition but contained RS in place of glucose; RF-RO-ORS, rice flour suspended in reduced osmolar ORS instead of glucose.

and differences between pairs of individual means were tested by the Wilcoxon rank sum test.

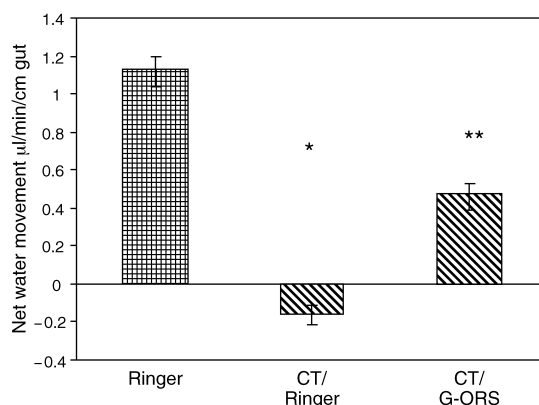
## RESULTS

### Validation of the Whole-Gut Perfusion Model with CT

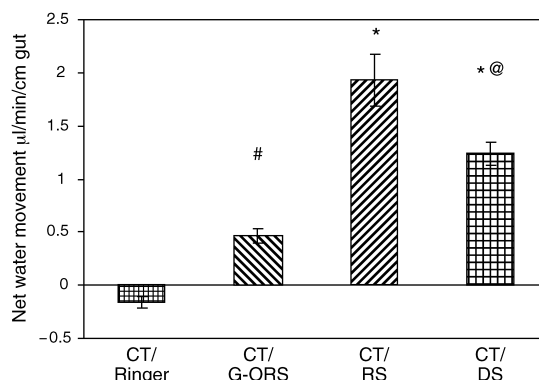
The initial experiments were designed to compare the results of whole-gut perfusion with Ringer and glucose-ORS (G-ORS) solution in control animals and those pretreated with CT to determine whether the pattern of results was similar to that observed in similar studies that were performed with either the entire small intestine or segments of small intestine. Net water absorption [ $1.12 \pm 0.08 \mu\text{L}/(\text{min}/\text{cm})$ ] was observed in control animals perfused with Ringer solution (Table 2). As expected, pretreatment with CT induced net water secretion [ $-0.16 \pm 0.05 \mu\text{L}/(\text{min}/\text{cm})$ ;  $P < 0.001$ ], which was reversed to net water absorption [ $0.46 \pm 0.07 \mu\text{L}/(\text{min}/\text{cm})$ ] by perfusion with G-ORS (Fig. 2). Net absorption of sodium and chloride was noted in control animals perfused with Ringer solution. As anticipated, pretreatment with CT induced net secretion of sodium and chloride as well as of potassium and bicarbonate (Table 2). Perfusion with G-ORS in CT-treated animals resulted in significantly higher net absorption of sodium, chloride and potassium than in CT-treated animals perfused with Ringer solution (Table 2).

### Effect of Amylase-Resistant Starch on Water and Electrolyte Absorption in CT-Treated Gut

Substitution of glucose in G-ORS by RS-ORS resulted in substantially higher net absorption of water [ $1.93 \pm 0.24 \mu\text{L}/(\text{min}/\text{cm})$ ,  $P < 0.001$ ] compared with G-ORS (Fig. 3). Net water absorption from DS-ORS [ $1.24 \pm 0.11 \mu\text{L}/(\text{min}/\text{cm})$ ] was significantly higher than from G-ORS ( $P < 0.001$ ), but was also significantly lower than that from RS-ORS ( $P < 0.02$ ), indicating that reduced osmolarity and amylase-resistant starch both played roles in the effect of the addition of starch to ORS. Effects of the different ORS on electrolyte transport paralleled their effects on water transport (Table 3).



**FIG. 2.** Effect of CT and G-ORS on net water movement (mean  $\pm$  SEM) across the whole gut. Values above the baseline denote net absorption, and values below denote net secretion into the lumen of the gut. Compared with control perfusion with Ringer solution, CT induced net water secretion (\* $P < 0.001$  compared with Control-Ringer). This was reversed to net water absorption in CT-treated animals perfused with G-ORS (\*\* $P < 0.001$  compared with CT/Ringer).



**FIG. 3.** Effect of complex carbohydrate on net water absorption from whole gut treated with CT. G-ORS, RS-ORS and DS-ORS were used. # $P < 0.001$  compared with CT/Ringer; \* $P < 0.001$  compared with G-ORS and @ $P < 0.02$  compared with RS-ORS.

### Effect of Reduced Osmolarity on Water and Electrolyte Absorption in CT-Treated Gut

Osmolarity is a critical determinant of fluid absorption with increased fluid absorption observed during perfusion with hypoosmolar solutions and net fluid secretion

**TABLE 2.** Effect of CT and G-ORS on whole gut net absorption of electrolytes

	Na nmol/(min/cm)	Cl nmol/(min/cm)	K nmol/(min/cm)	Bicarbonate nmol/(min/cm)
Ringer	172.7 ± 17.6	31.5 ± 12.8	-0.66 ± 1.2	46.2 ± 6.9
CT/Ringer	-35.4 ± 5.9 <sup>#</sup>	-11.9 ± 5.5 <sup>#</sup>	-5.07 ± 2.2	-13.4 ± 1.6 <sup>##</sup>
CT/G-ORS	2.1 ± 12.4*	29.5 ± 9.6*	19.1 ± 3.6 <sup>@</sup>	-60.6 ± 6.0**

All values shown are the mean  $\pm$  SEM of 8 perfusion studies.

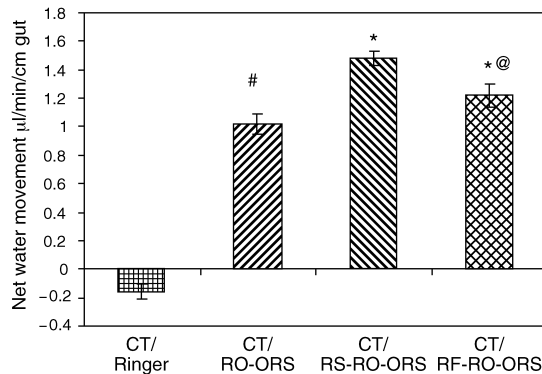
<sup>#</sup> $P < 0.001$  and <sup>##</sup> $P < 0.005$  compared with control Ringer. \* $P < 0.01$ , @ $P < 0.05$  and \*\* $P < 0.001$  compared with CT-Ringer.

**TABLE 3.** Effect of substituting RS and DS for glucose in G-ORS in CT-treated whole gut perfusions

	Na nmol/(min/cm)	Cl nmol/(min/cm)	K nmol/(min/cm)	Bicarbonate nmol/(min/cm)
G-ORS	2.1 ± 12.4	29.5 ± 9.7	19.1 ± 3.6	-60.6 ± 6.0
RS-ORS	106.3 ± 29.4*	109.6 ± 16.8*	22.6 ± 3.4*	-40.1 ± 7.9**
DS-ORS	23.0 ± 9.4#	68.3 ± 9.8	21.3 ± 5.4*	-43.3 ± 4.8

Values shown are mean ± SEM of 8 experiments.

\*P < 0.001 and \*\*P < 0.05 compared with G-ORS. #P < 0.02 and @P < 0.02 compared with RS-ORS.



**FIG. 4.** Effect of reduced osmolarity and complex carbohydrate on net water absorption from whole gut treated with CT. RO-ORS, RS-RO-ORS and RF-RO-ORS were used. #P < 0.001 compared with CT/Ringer. \*P < 0.001 compared with RO-ORS. @P < 0.05 compared with RS-RO-ORS.

with hyperosmolar solutions. In the present experiments, reducing the osmolarity to 245 mOsm/kg significantly increased net water absorption compared to G-ORS (P < 0.001) (Fig. 4); these results are identical to prior experiments that only studied the effect of hypoosmolarity on water absorption in the small intestine. The substitution of glucose in reduced osmolarity ORS by RS (RS-RO-ORS) significantly increased net water absorption from CT-treated intestine compared with RO-ORS (P < 0.001) (Fig. 4). Because this substitution reduced osmolarity of the ORS further, an additional control was studied (ie, substitution of glucose by RF). The latter solution (RF-RO-ORS) was of the same osmolarity as RS-RO-ORS, but did not contain amylase-resistant starch. Net

water absorption from the RF-RO-ORS was significantly higher than from RO-ORS (P < 0.001), but was significantly lower than that observed with RS-RO-ORS (P < 0.02) (Fig. 4). Net electrolyte absorption during perfusion with different solutions is shown in Table 4. Of note, net sodium secretion was observed during perfusion of RO-ORS, which has a lower sodium concentration than standard G-ORS. Induction of hyponatremia during rehydration therapy is a clinical concern when using reduced osmolarity ORS in severe diarrhea. Substitution of glucose in RO-ORS by RS or RF significantly increased net sodium absorption compared with RO-ORS (P < 0.02 and 0.002, respectively). Net sodium absorption from RF-RO-ORS was significantly higher than from RS-RO-ORS (P < 0.05).

#### ORS Evaluation in STa-Treated Whole Gut

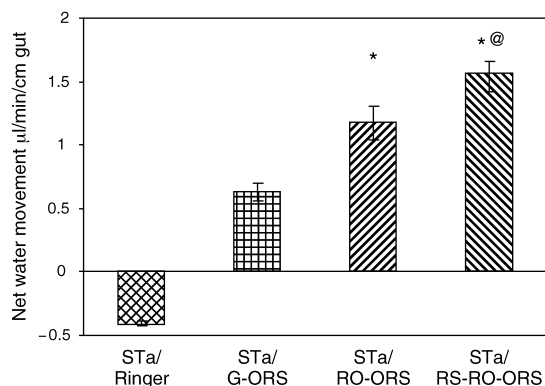
Additional experiments were designed to determine the importance of both RS and RO in fluid movement in a model in which increased mucosal cyclic guanosine monophosphate and not cAMP was responsible for the changes in intestinal function. As a result, parallel studies were performed in which fluid secretion was induced by STa. Perfusion of the intestine with Ringer solution in the presence of STa induced net fluid secretion (Fig. 5). Perfusion with G-ORS significantly increased net fluid absorption in the presence of STa (P < 0.001) (Fig. 5). Experiments were also performed with RS and reduced-osmolarity ORS to determine whether these 2 modifications also enhanced fluid absorption in the presence of STa, as was observed with CT. Net fluid absorption with RO-ORS was increased compared with that observed with G-ORS (P < 0.005) (Fig. 5). When the entire

**TABLE 4.** Effect of substituting RS and RF for glucose in RO-ORS in CT-treated whole gut perfusions

	Na nmol/(min/cm)	Cl nmol/(min/cm)	K nmol/(min/cm)	Bicarbonate nmol/(min/cm)
RO-ORS	-62.4 ± 8.1	32.7 ± 6.0	4.3 ± 3.0	-57.5 ± 5.6
RS-RO-ORS	8.5 ± 6.5*	66.9 ± 3.5*	27.2 ± 5.6*	-49.6 ± 5.1
RF-RO-ORS	34.8 ± 9.1**,@	59.7 ± 8.5 <sup>S</sup>	15.3 ± 6.3	-26.4 ± 5.5**,@

Values shown are mean ± SEM of 8 experiments.

\*P < 0.02, \*\*P < 0.002 and <sup>S</sup>P < 0.05 compared with RO-ORS. @P < 0.05 compared with RS-RO-ORS.



**FIG. 5.** Effect of STa on net water absorption from the whole gut. G-ORS, RO-ORS, and RS-RO-ORS were used. Ringer solution is shown for comparison. \* $P < 0.005$  compared with G-ORS. @ $P < 0.05$  compared with RO-ORS.

intestine was perfused with STa with a solution that both contained RS and was hypoosmolar, net fluid absorption was significantly increased compared with that observed with G-ORS and RO-ORS ( $P < 0.001$ ). Net secretion of sodium, chloride and potassium observed in STa perfusions with Ringer solution were converted to net absorption in G-ORS perfusions (Table 5,  $P < 0.01$ ), whereas net secretion of bicarbonate was increased in the presence of G-ORS. No statistically significant differences in ion absorption were noted between G-ORS, RO-ORS and RS-RO-ORS groups.

## DISCUSSION

This study had 2 objectives: to establish and validate an experimental method to determine fluid and electrolyte movement simultaneously in both small and large intestine, and to use this method to study different formulations of ORS that may be primarily absorbed in both small and large intestine. Traditional ORS formulations have been considered to stimulate fluid absorption exclusively in the small intestine as glucose-stimulated sodium absorption is not present in the colon (1,2). Modifications of ORS including glucose polymers, amino acids and reduced osmolarity also enhance small intestinal and not colonic fluid absorption.

As a result, prior studies with isolated segments of proximal or distal or the entire small intestine were appropriate to evaluate the different ORS formulations because these are absorbed solely in the small intestine.

The recent development of a modified ORS containing starch that is relatively resistant to amylase digestion (RS) is based on the concept that RS is delivered to the colon, where it exerts physiological effects. Administration of RS to adults with cholera and to children with non-cholera diarrhea resulted in reduced fecal output and quicker recovery from diarrhea (12,20). Because the effectiveness of RS-ORS is based on enhanced colonic fluid absorption, an experimental model to study different ORS that included RS would require one that included a colonic segment. We are not aware of prior use of perfusion of small and large intestine in continuity. Figure 1 demonstrates that steady-state conditions were established within 60 minutes of the initiation of perfusion of the entire small and large intestine (exclusive of the cecum) and persisted up to 300 minutes. The present experiments were performed using data collected during a 30-minute period following the initiation of steady-state conditions.

Consistent with many prior observations in small intestinal segments, whole gut perfusion with CT induced electrolyte changes mimicking that seen in cholera. The stimulation of fluid secretion induced by CT was considerably greater than that by STa. The validity of this model of perfusion of the whole intestine was confirmed by the ability of G-ORS to stimulate fluid absorption induced by both CT and STa (Figs. 2 and 5). Recent studies have demonstrated the efficacy of reduced osmolarity RO-ORS in the treatment of several diarrheal disorders in both children and adults (14–16,21). As a result, RO-ORS has been established as the preferred ORS formulation by the WHO. These present experiments demonstrated that RO-ORS enhanced fluid absorption in the presence of both CT and STa compared with G-ORS (Figs. 4 and 5), observations that are consistent with both clinical studies and experiments with isolated segments of small intestine (13).

A critical issue was to determine whether the combination of reduced osmolarity and resistant starch would result in a truly "super-ORS." Before performing clinical trials, it would be important to provide evidence in an

**TABLE 5.** Net electrolyte absorption from whole gut in STa-perfused animals

	Na nmol/(min/cm)	Cl nmol/(min/cm)	K nmol/(min/cm)	Bicarbonate nmol/(min/cm)
Ringer	-24.1 ± 37.9	-34.4 ± 16.2	-9.3 ± 4.3	-14.2 ± 12.3
G-ORS	13.8 ± 12.0*	49.7 ± 11.8*	28.7 ± 4.5*	-47.5 ± 4.4*
RO-ORS	-5.6 ± 12.6 <sup>ns</sup>	50.5 ± 14.2 <sup>ns</sup>	31.4 ± 7.6 <sup>ns</sup>	-46.5 ± 6.2 <sup>ns</sup>
RS-RO-ORS	16.5 ± 13.6 <sup>ns</sup>	72.0 ± 14.8 <sup>ns</sup>	33.7 ± 6.6 <sup>ns</sup>	-38.6 ± 6.5 <sup>ns</sup>

\* $P < 0.01$  compared with Ringer solution. <sup>ns</sup> indicates nonsignificant differences between compared groups.

experimental system about whether the combined use of RO and RS was better than RO or RS alone. The present results provide such evidence that the combination of RS and RO in a single solution designated as RS-RO-ORS resulted in an enhanced level of fluid absorption in the presence of both CT and STa. This observation is consistent with the physiological importance of the colonic reserve capacity to absorb fluid as a critical determinant of the severity of diarrhea and fecal fluid losses (22,23) and the demonstration that colonic absorption is impaired in cholera and other diarrheal disorders (8,24).

The reduced-osmolarity ORS now recommended by the WHO is especially useful in diarrhea in children. A meta-analysis of 15 randomized trials of reduced osmolarity ORS in children revealed a small (21%) but significant decrease in stool output compared with standard G-ORS (16). In cholera, the use of reduced osmolarity solution was associated with hyponatremia in some subjects (19,21), presumably related to its lower sodium concentration. In the current perfusion studies, reduced osmolarity ORS was associated with significantly increased water absorption from the CT-treated gut compared with the standard G-ORS, but net sodium secretion was observed. Substituting RS for glucose in low-sodium reduced-osmolarity ORS (RS-RO-ORS) significantly increased net sodium absorption, suggesting that RS could provide an advantage in patients with cholera, resulting in less hyponatremia compared with reduced-osmolarity ORS. Depleted body potassium and hypokalemia is another problem that occurs in malnourished children with diarrhea; ReSoMal is an oral hydration solution that specifically corrects this (7). The finding in the present study that net potassium absorption was significantly reduced in CT perfusions with RO-ORS but was significantly augmented by RS-RO-ORS suggests that there would be an advantage in using RS-RO-ORS in malnourished children with cholera.

The RS used in these studies was actually a mixture of amylase-resistant starch and digestible starch. The addition of RS to ORS confers 2 advantages. The digestible component is rapidly hydrolyzed in the small intestine to glucose leading to stimulation of sodium-glucose cotransport, without the osmotic penalty of glucose. The amylase-resistant component is expected to enter the colon and provide additional effects on absorption. RS-ORS was of substantially lower osmolarity (200 mOsm/kg) than standard G-ORS (311 mOsm/kg) because the glucose was replaced by starch that was not osmotically active. As an additional control, we studied the effect of adding (completely amylase-sensitive) digestible starch to ORS (DS-ORS, osmolarity 200 mOsm/kg) in the place of RS. This led to a solution of similar osmolarity to RS-ORS, which provided glucose to stimulate sodium absorption in the CT-induced secreting small intestine. The

finding that RS-ORS increased net water and sodium absorption to a significantly greater extent than digestible starch indicates that the effect of the starch was not solely caused by the reduced osmolarity of RS-ORS but that there was a colonic component that provided a superior effect. Rice flour also had an effect on net water and sodium absorption compared with RO-ORS; compared with resistant starch, net water absorption was lower but net sodium absorption higher and net bicarbonate secretion lower with RF (Table 4). It is possible that RF has an effect on Na-HCO<sub>3</sub> secretion at some level of the gut. Rice flour contains both digestible and amylase-resistant starch and may provide some of the effect of amylase-resistant starch.

It seems clear that malabsorbed carbohydrate can be useful in the treatment of acute diarrhea (25); however, not all malabsorbed carbohydrates may be equally useful in management of acute diarrhea (26). There have been variable results with the addition of malabsorbed carbohydrates such as partially hydrolyzed guar gum and a mixture of nondigestible carbohydrates in treating children with non-cholera diarrhea. The former hastened recovery from diarrhea (27), whereas the latter was not effective (28). The differences between these clinical trials may result from the nature of the carbohydrate chosen (ie, whether it increased osmolarity) as well as the nature of the diarrhea. Thus, rice-based ORS is generally not more effective than G-ORS in children with non-cholera diarrhea. We speculate that the amylase-resistant component of RS stimulates SCFA-dependent sodium absorption from the colon, although this was not directly examined in the present study. Administration of RS to rats results in its being fermented in the colon to SCFA by luminal bacteria (29). Short chain fatty acid-stimulated sodium absorption in the colon is not inhibited by cAMP, despite its requirement for Na-H exchange (9,30). Short chain fatty acids increase sodium absorption via the Na-H exchange isoform 2 (NHE2), which is expressed on the apical membrane of colonic epithelial cells and is upregulated by cAMP (31). In addition, SCFAs inhibit active chloride secretion in the colon (30,32), which may explain the significantly increased chloride absorption from the starch-containing ORSs compared with the glucose-containing ORSs (Tables 3 and 5). The perfusion system described here may wash out fecal bacteria to an extent that it prevents colonic fermentation. This is also a situation that may occur in severe acute diarrhea. Alternative effects of malabsorbed carbohydrate on colonic fluid absorption, including reduced nitric oxide levels in colon as has been suggested for gum arabic (33), cannot be excluded. In the present perfusion studies of the whole gut, increased net water absorption from RS-RO-ORS suggests that it may reduce fecal fluid loss in acute diarrhea compared with RO-ORS. This solution should be tested in clinical trials in children and adults with diarrhea with the expectation that it may provide

clinical improvement compared with either RS-ORS or RO-ORS alone.

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