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Proabsorptive effect of glycerol as a glucose substitute in oral rehydration solutions

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We hypothesized that glycerol, a readily diffusable hydrophilic substance, may effectively substitute for glucose and enhance intestinal water and sodium absorption in an oral rehydration solution (ORS). This was evaluated using a low osmolality (230–240 mOsm/kg) ORS containing 75 mmol/L sodium and a combination of glucose:glycerol (in mmol/L) 75:0, 50:25; 37.5:37.5, 25:50, 10:65, or 0:75 during 3-hour long in vivo rat jejunal perfusions. Water, sodium, potassium, glucose and glycerol absorption, and unidirectional fluid movement (J_{in}, J_{eff}) were determined. Sodium and net water absorptions were maximal at glucose:glycerol ratios between 37.5:37.5 and 10:65 mmol/L. In the absence of glucose (0:75), absorption of water and electrolytes was lower than at any other concentration. The greater net rehydration seemed to be due to a higher J_{in} as glycerol was increased up to 65 mmol/L. Potassium absorption followed a similar pattern. With 50 mmol/L glycerol and 25 mmol/L glucose, there was a marked expansion of the lamina propria extracellular space and increased intercellular expansion between enterocytes. These results indicate that glycerol may be an effective partial substitute for glucose in ready-to-use ORS by producing an improved rate of water and electrolyte absorption.
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Keywords: oral rehydration solutions; water absorption; sodium absorption; glycerol; jejunal perfusions; hyperhydration

Introduction

Oral rehydration solutions (ORS) are life-saving treatments for diarrheal disease because of their effectiveness in the replacement of water and electrolytes.[1] In the standard World Health Organization ORS, the energy source is 111 mmol/L (20 g/L) glucose with 90 mmol/L sodium to replace electrolyte losses during diarrheal episodes. Although effective in ameliorating fluid and electrolyte losses in cholera-induced diarrhea, the use of this formulation in noncholera type diarrheas can cause hypernatremia.[2] Consequently, many clinicians are inclined to use a lower sodium ORS in the treatment of diarrheal disease caused by rotavirus or bacteria other than *V. cholerae*.[3] Although concentrations of sodium less than 60 mmol/L are generally found in commercially bottled or hospital-made ORS in the United States and Europe,[4,5] these products typically have an excess of sugar and relatively high osmolality, resulting in a decreased sodium:glucose ratio, which is detrimental for absorption.[6,7] Therefore, a preparation with an intermediate sodium and glucose concentration (75 mmol/L) has been formulated[8] and adopted in this study.

In addition to modifications of the sodium:glucose ratio of ORS, research has focused on ORS additives that stimulate greater absorption of water and electrolytes while providing a source of energy to the patient in an easily absorbable form.[2] Efforts aimed at improving ORS efficacy have included the addition of amino acids, glucose polymers, or partially hydrolyzed starch additives in attempts to provide a supplementary absorbable nutrient source to maintain a positive energy balance during diarrheal episodes.[3,9] An alternative approach, not yet examined for the treatment of diarrhea, is the use of a hydrophilic agent such as glycerol. Glycerol is a low molecular weight, readily absorbable, and metabolizable solute, with a slightly higher energy content than glucose (4.3 vs. 3.8 kCal/g; 18.0 vs. 15.9 kJ/g),[10] used to induce hyperhydration under exercise-related fluid replacement conditions.[11,12] In view of these

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Table 1 Composition of the modified oral rehydration solutions used in the experiments

Glycerol:Glucose*	0:75	25:50	37.5:37.5	50:25	65:10	75:0
NaCl*	45	45	45	45	45	45
Na_3 Citrate*	10	10	10	10	10	10
KCl*	20	20	20	20	20	20
Glycerol*	0	25	37.5	50	65	75
Glucose*	75	50	37.5	25	10	0
Osmolality†	243 ± 0.8	237 ± 0.3	232 ± 0	228 ± 0.6	225 ± 0.7	228 ± 1.5

*Expressed as mmoles/L.
†Actual values, in mOsm/kg, are means ± SEM; $N = 4$.

advantages we designed the present study (1) to test the hypothesis that partial substitution of glycerol for glucose in ORS would enhance water, electrolyte, and glucose absorption and (2) to evaluate the possible mechanism(s) involved.

Methods and materials

Animals and perfusion procedure

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN USA) weighing 90 to 120 g were acclimatized to the animal facility environment for a minimum of 48 hours and then fasted overnight prior to surgery. Rats were anesthetized with ketamine-xylazine (87–13 mg/mL, 1.0 mL/kg, administered intramuscularly), a 20- to 30-cm segment of rat jejunum was isolated in situ, and proximal and distal ports were cannulated with polyethylene tubing. At the end of the experiment, the jejunal segment perfused was excised and measured under a 3 g tension. The perfused solutions were prewarmed so that they entered the abdomen at 37°C and were introduced into the intestine via a peristaltic pump (model 1203, Harvard Instruments, Boston, MA USA) at a rate of 10 to 12 mL/h. Each experiment consisted of perfusing 12 rats simultaneously; however, each animal was perfused with only a single solution. Following a 1-hour equilibration period, eight 15-minute aliquots of perfusate were collected and analyzed as indicated below. The rates of absorption were calculated with algorithms applied in previous studies.[13–16] The number of animals perfused with each solution is noted in the tables and figure legends. The protocol for this study was approved by the Institutional Animal Care and Utilization Committee of North Shore University Hospital.

Solutions and chemicals

The composition of the solutions used in these experiments is presented in *Table 1*. The design consisted of modifying the molar proportions of glycerol and glucose while minimizing osmolality changes. All chemicals were purchased from Sigma (St. Louis, MO USA). Tracer amounts of tritiated water (^3H_2O, 2 μCi/L = 74 MBq/L; NEN-Dupont, Boston, MA USA) were added to the luminal perfusion solutions to evaluate lumen-to-serosa water flux. Net water absorption was determined by the difference between the weight of the fluid entering and leaving the intestinal segment, making it unnecessary to use nonabsorbable markers.[16] Disappearance of tritiated water was used to calculate lumen-to-serosa influx (J_{in}), considering that recirculation of the label during the experiment was negligible.[16] Serosa-to-lumen efflux (J_{eff}) of water was estimated by the difference between J_{in} and net water absorption. Sodium in perfusates was determined by atomic absorption spectrophotometry (SpectrAA10, Varian Instruments Inc., Sugar Land, TX USA), and absorption/secretion rates were adjusted for volume shifts, as determined gravimetrically from the net water absorption. Glucose was assayed by an enzymatic method (Sigma 510), and its absorption was computed in the same manner as the electrolytes. Glycerol also was determined enzymatically, with glycerol dehydrogenase (EC 1.1.1.6; Sigma G 3512). Absorbable carbon was calculated from the absorption rates of both glucose and glycerol. Absorption rates of glucose and glycerol were graphically analyzed (Sigmaplot, Sigma) as a function of the proportion of both solutes to determine the equations that best fit the data. Kinetic parameters of glucose absorption were similarly treated using the Hanes-Woolf plot, which allows for the direct calculation of kinetic and diffusion constants with minimal influence by noncarrier mediated transport.[17,18] Osmolality was determined by vapor pressure changes (model 5500, Wescor Inc., Logan, UT USA).

Light and electron microscopy

Segments of jejunum (1–2 cm) were excised after the perfusion and fixed in 20 g/L glutaraldahyde in 50 mmol/L cacodylate buffer, pH 7.3 (300 mOsm/kg), at room temperature and then at 4°C for 2 to 18 hours. Segments were rinsed in cold 100 mmol/L cacodylate buffer with 75 g/L sucrose and dissected to provide properly oriented tissue slices spanning the serosal to mucosal villus surface. The slices were rinsed in buffer, postfixed in 10 g/L buffered osmium tetroxide at 4°C for 1 hour, rinsed in cold 75 g/L sucrose, soaked in uranyl acetate for 30 minutes at room temperature, rinsed again in cold 75 g/L sucrose, treated with 10 g/L tannic acid in cacodylate buffer, dehydrated in increasing concentrations of ethanol, and embedded in effapoxy resin (E. Fullam, Schenectady, NY USA). For light microscopy, 1-μm sections of multiple jejunal samples from each animal were evaluated. Selected areas were thin sectioned, stained with uranyl acetate and lead citrate, and examined on a JEM 100CXII (JEOL, Peabody, MA USA) electron microscope operated at 80 kV.

Analysis of results

Results are presented as means ±SEM and were analyzed by analysis of variance and Tukey's test for multiple contrasts after examining for normal distribution of data (Sigmastat, Sigma).[19] Regression analysis of data was carried out with a computer program (Sigmaplot). The threshold of significance was 0.05.

Results

Electrolyte, water, and solute absorption

The data indicated that substitution of glycerol for glucose led to increased sodium (*Figure 1*) and water (*Figure 2*) absorption up to 65 mmol/L of glycerol. By contrast, 75 mmol/L glycerol abolished sodium absorption and impaired net water absorption compared with the solution without

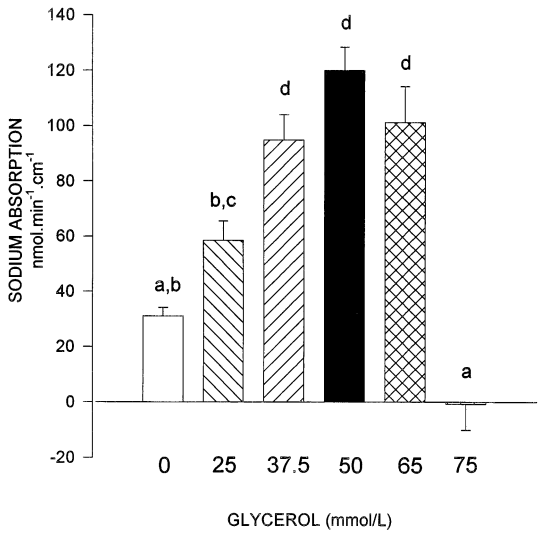

Figure 1 Sodium absorption rates during perfusions with an oral rehydration solution containing 75 mmol/L sodium and variable concentrations of glycerol. The error bar equals the SEM. Bars that do not share a letter are different at the $P < 0.05$ level (Tukey's test).[17] The number of rats per group is indicated in *Table 2*.

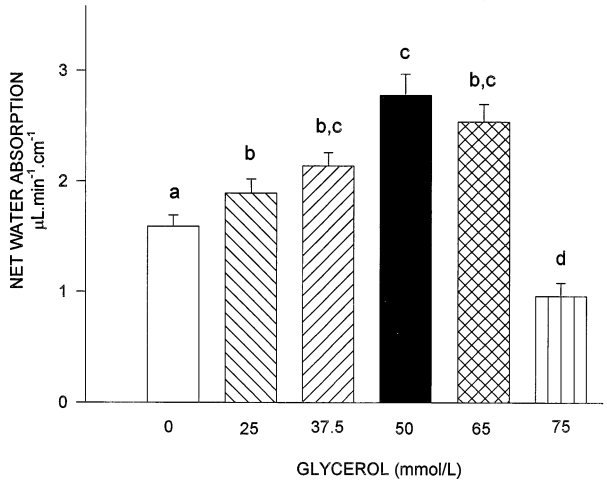

Figure 2 Net water absorption rates under conditions described in the text and in *Figure 1*. Data that do not share a letter are significantly different ($P < 0.05$).

glycerol. Sodium absorption was highest between 37.5 and 65 mmol/L glycerol (*Figure 1*). At 37.5, 50, and 65 mmol/L glycerol sodium absorption rates were also higher than at 25 mmol/L glycerol. Potassium absorption was unaffected by modifications in the glycerol and glucose concentrations up to 65 mmol/L glycerol, and decreased sharply at 75 mmol/L [glycerol (in mmol/L): 0, 1.71 ± 0.19; 25, 1.89 ± 0.13; 37.5, 1.72 ± 0.04; 50, 1.63 ± 0.17; 65, 1.57 ± 0.13; and 75, 0.93 ± 0.07 nmol × min^{-1} × cm^{-1}).

Net water absorption presented a similar pattern to that described for sodium absorption (*Figure 2*). With 75 mmol/L glycerol, net water absorption was not as affected as sodium, but was nevertheless reduced compared with the solution containing no glycerol. As shown in *Table 2*, J_{in} was highest at glycerol concentrations greater than 50 mmol/L. At 25, 37.5, and 75 mmol/L, J_{in} was indistinguishable from the baseline solution containing no glycerol. J_{eff} was increased with 75 mmol/L glycerol compared with all other solutions. The J_{in}:J_{eff} ratio, an index of the relative contribution of unidirectional fluxes, reached a peak at 50 mmol/L glycerol and fell sharply at 75 mmol/L glycerol. Glucose absorption declined as the proportion of glucose in the perfusion solutions decreased, whereas glycerol absorption increased up to 65 mmol/L glycerol. Surprisingly, at 75 mmol/L glycerol, absorption of this solute was indistinguishable from that measured at 25 mmol/L glycerol (*Table 2*). Absorbable carbon reached a peak when both glycerol and glucose concentrations were 37.5 mmol/L. At the highest glycerol concentration, however, absorbable carbon was reduced to a value well below all others.

Plots of glucose and glycerol absorption rates as a function of the glycerol:glucose ratio were consistent with quadratic functions (*Figure 3*). Glycerol absorption was best fit by the equation $y = 88.7 + 118.2\,X - 12.8\,X^2$, $r^2 = 0.952$, and glucose absorption rates could be represented by the equation $y = 337.5 - 34.8\,X + 0.50\,X^2$, $r^2 = 0.997$. Both curves intersected at a glycerol:glucose ratio of 1.96, a value close to that of the solution with 50 mmol/L glycerol and 25 mmol/L glucose shown earlier to yield maximal sodium and water absorption. As expected, there was a significant inverse correlation between glucose and glycerol absorption rates ($r = -0.514$, $P < 0.01$) (*Figure 3, insert*).

Table 2 Water mucosa-to-serosa influx (J_{in}), serosa-to-mucosa efflux (J_{eff}), J_{in}/J_{eff} ratios, glucose, and glycerol absoprtion rates

Glycerol:Glucose*	0:75	25:50	37.5:37.5	50:25	65:10	75:0
J_{in} (µL/min/cm)	5.50 ±0.20^{a,b}	5.30 ±0.13^{a}	5.70 ±0.21^{a,b,c}	6.26 ±0.18^{b,c}	6.37 ±0.29^{c}	5.94 ±0.24^{a,b,c}
J_{eff} (µL/min/cm)	3.89 ±0.14^{a}	3.43 ±0.10^{a}	3.56 ±0.13^{a}	3.48 ±0.12^{a}	3.61 ±0.20^{a}	4.98 ±0.17^{b}
J_{in}/J_{eff}	1.45 ±0.05^{a,b}	1.56 ±0.08^{a}	1.66 ±0.06^{a}	1.83 ±0.11^{c}	1.72 ±0.07^{a,c}	1.20 ±0.02^{b}
Glucose absorption (nmol/min/cm)	339 ±29^{a}	314 ±16^{a}	310 ±28^{a}	268 ±21^{a}	133 ±21^{b}	—
[n]	[11]	[13]	[11]	[11]	[10]	[8]
Glycerol absorption (nmol/min/cm)	—	130 ±19^{a}	218 ±10^{b}	264 ±10^{c}	315 ±9^{c}	104 ±11^{a}
[n]		[9]	[10]	[10]	[10]	[8]
Absorbable carbon (nmol/min/cm)	2,033 ±176^{a,b,c}	2,288 ±117^{a,b,c}	2,759 ±174^{d}	2,487 ±152^{a,b}	1,739 ±47^{c}	311 ±34^{e}

*Expressed as mmoles/L.
Data presented as means ± SEM.
Data in the same row that do not share a superscript letter are significantly different ($P < 0.05$).

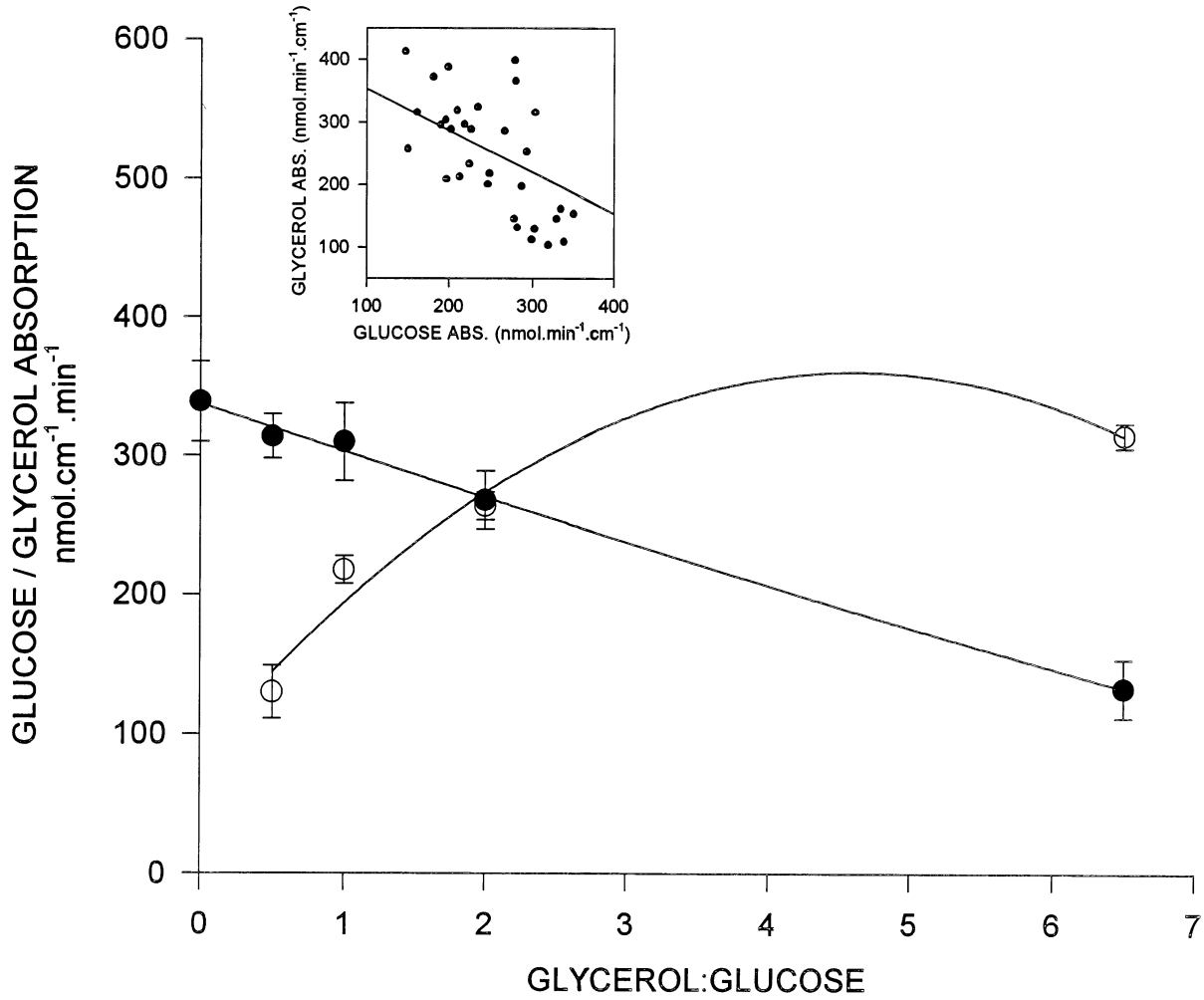

Figure 3 Absorption rates of glycerol [o] and glucose [●] as a function of the glycerol:glucose ratio. The error bars represent the SEM. The regression curves represent quadratic equations and intersect at a value indicated in the text. The insert is the regression of the absorption rates of the two solutes, when simultaneously perfused. Each point corresponds to the values obtained for a single rat. The total number of animals is indicated in *Table 2*.

Glucose absorption kinetics, under our experimental conditions of fixed sodium and variable glucose concentration, revealed a nearly linear regression between glucose concentration [S] and its ratio against [V], the absorption rates[17,18] (Figure 4). Data points fit the equation $y = 0.04166 + 2.33 \times 10^{-3}$ X, $r^2 = 0.981$, and yielded a K_m of 17.9 mmol/L and V_m of 430 nmol/min × cm.

Morphologic observations

Light and electron microscopy of jejunal epithelium from rats perfused with a solution containing 50 mmol/L glycerol and 25 mmol/L glucose showed increased expansion of the lamina propria extracellular space and intercellular space between villus absorptive epithelial cells compared with specimens not exposed to glycerol (*Figure 5* and *Figure 6*). Absorptive cells from perfusions with 50 mM glycerol contained normal organelles, including microvilli, mitochondria, and Golgi apparatus (*Figure 6*), indicating no morphologic alterations of the ultrastructure.

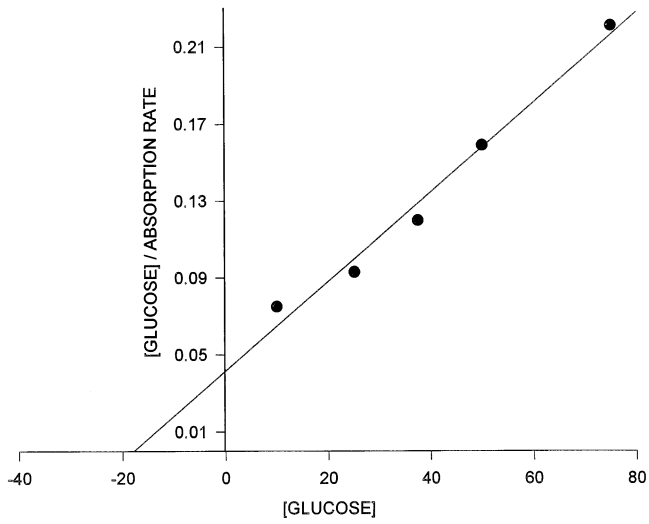

Figure 4 Hanes-Woolf representation of glucose absorption kinetics from the mean data presented in *Table 2*. Figures in abscissa are expressed in mmol/L. This plot yields $-K_m$ at the intersection with abscissa and K_m/V_m at the intercept with ordinates.

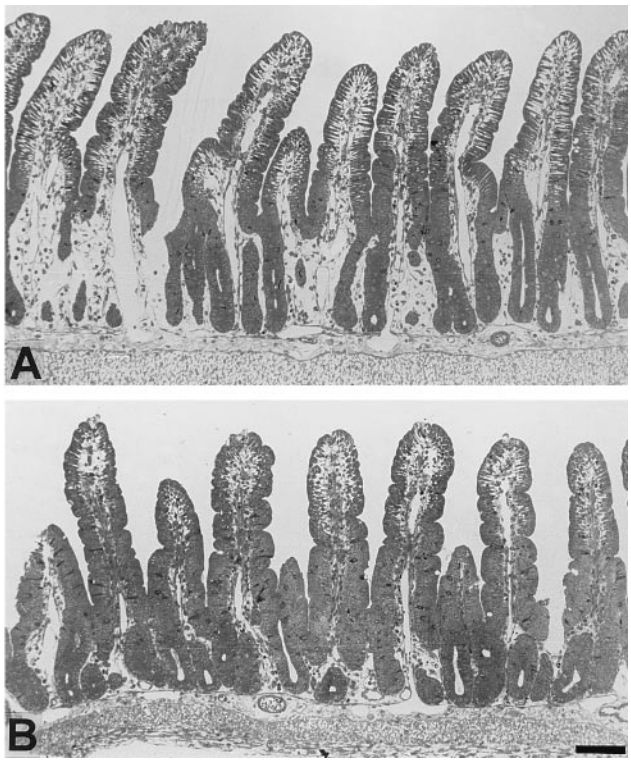

Figure 5 Light micrograph of 1 μm plastic sections stained with toluidine blue, from a typical area of rat jejunum perfused with a 75 mmol/L sodium oral rehydration solution (ORS) containing 50 mmol/L glycerol and 25 mmol/L glucose (*Figure 5A*) or an ORS containing 75 mmol/L glucose without any glycerol (*Figure 5B*). Note the increased expansion of the extracellular spaces between more apical villus enterocytes and the lamina propria from the glycerol containing ORS perfusion (*Figure 5A*) compared with perfusion with the ORS that did not contain glycerol (*Figure 5B*). Magnification: ×100. Bar = 100 μm.

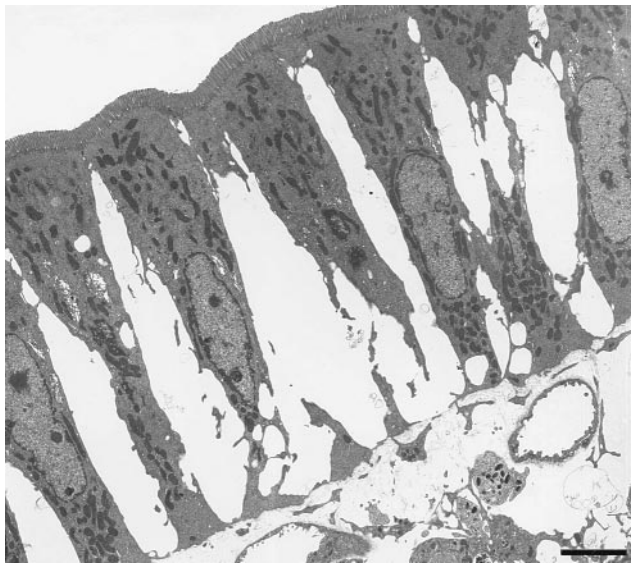

Figure 6 Electron micrograph of jejunal villus epithelium and portion of the lamina propria following perfusion with a 75 mmol/L sodium oral rehydration solution containing 50 mmol/L glycerol and 25 mmol/L glucose. Note the marked expansion of the lateral intercellular spaces between the absorptive epithelial cells and the clear extracellular space of the lamina propria. Organelles, including microvilli, mitochondria, and Golgi apparatus, all appear normal. Magnification: ×3,700. Bar = 5 μm.

Discussion

Our observations indicate that substitution of glycerol for a portion of glucose in ORS has a positive effect on net jejunal sodium and water absorption. These results can be explained by the hydrophilic properties of glycerol operating in conjunction with the active transport of glucose in the range between 37.5 and 65 mM glycerol. Formulations resulting in maximal sodium absorption contained a sodium:glucose ratio between 2:1 to 7.5:1, values that include or excede the sodium:glucose transporter stoichiometry of 2:1.[20,21] This suggests that, in vivo, a relative excess of sodium over glucose in the jejunum favors sodium and water absorption. The sodium:glucose cotransport takes place in conjunction with other sodium exchange mechanisms such as diffusion, the Na^+/H^+ antiport or the Na^+-Cl^- symport.[20,21] The poor performance of the ORS with 75 mmol/L glycerol and no glucose can be attributed to the increase in J_{eff}, suggesting that glycerol can easily recirculate from and to the intestinal lumen, a phenomenon apparently masked by the absorptive drive of glucose.

The increase in net water absorption at 50 and 65 mmol/L glycerol appears to be due to an increase in water J_{in}. The hypotonic nature of the solutions tested and the hydrophilic nature of glycerol may lead to fluid retention in the intercellular space as evidenced by the morphologic studies. An examination of the J_{in}:J_{eff} ratios, a more sensitive indicator of changes in unidirectional fluxes, showed a maximum in the solutions with 50 and 65 mmol/L glycerol. The proabsorptive effects of the glycerol:glucose combination is confirmatory of previous perfusion experiments, carried out at a lower sodium concentration (24 mmol/L), where substitution of 60 or 80 mmol/L glycerol for equimolar glucose, in the presence of corn syrup, reversed net fluid secretion.[15] The effect of high concentration glycerol on J_{eff}, in the absence of glucose, was unexpected, because it was anticipated that substitution of glycerol for glucose would lead to an increase bulk flow and thus a progressive increment J_{in} due to the hydrophilicity of glycerol. This seems to be true up to 65 mmol/L glycerol with 10 mmol/L glucose. However, it appears that glycerol remains in the intercellular space longer than sodium, as suggested by the electron microscopic observations. Sodium is translocated through the basolateral membrane via the Na^+,K^+-ATPase, which maintains the electrogenic gradient,[20] and is free to diffuse into the circulation; however, glycerol transfer to the extracellular space is presumably a slower, diffusive process.

The preconditions set for the experiments presented in this report (i.e., an equimolar substitution of glycerol for glucose at each of the test solutions in order to minimize osmolality differences) and the transport kinetics of glucose discussed earlier may explain why sodium and water absorption peak between 37.5 and 65 mmol/L glycerol, corresponding to glucose concentrations between 37.5 and 10 mmol/L. The glycerol:glucose ratio of 2:1, corresponding to the highest values, is very close to the intersection point of the plots of glycerol and glucose absorption rates (*Figure 3*). This signifies that the conditions optimal for

sodium and water absorption coincide with the maximal absorption rates of organic nonelectrolytes.

Glycerol did not alter glucose kinetic parameters. The values obtained in the present experiments are close to those previously reported for glucose K_m (24 mmol/L) and V_m (414 nmol/min × cm) in comparable in vivo studies.[22] The linearity of the regression in *Figure 4* indicates that, under the conditions of these experiments, glucose is overwhelmingly transported by a carrier-mediated mechanism, with little contribution by a nonsaturable component.[18] This finding is also in agreement with the minor role attributable to the paracellular pathway of glucose absorption, as determined in rats with isotonic solutions.[23]

Glycerol is generally well tolerated as an ingredient of many pharmaceutical and over-the-counter oral liquid preparations. At 50 or 65 mmol/L glycerol, where water and sodium absorption are at the maximum, the solutions are neither noticeably viscous nor unpalatable. Glycerol is metabolized by phosphorylation in the liver and enters the glycolytic pathway.[24,25] Its use has been proposed in sports medicine as a hyperhydrating agent with generally satisfactory results,[11] including a diminished thirst.[26] However, if ingested in hypertonic solutions (10%), it does not appear to provide cardiovascular or thermoregulatory advantages.[26,27] Inclusion of glycerol in isotonic or hypotonic rehydration solutions may be particularly useful in endurance events, when sweat losses are great, under extreme environmental conditions, or when water is in short supply.[28] The concentration of glycerol in the rehydration solution appears to be important, because the osmotic shock of a hypertonic solution is physiologically demanding, even in healthy individuals. For rehydration of patients with diarrhea, ready-to-use fluid preparations are extensively used in industrialized countries, in contrast to the dry packages prepared for distribution in the field prevailing in developing areas of the world. Although the present studies were comparative and were conducted in rats under anesthesia, it is conceivable that glycerol, in beverages containing sodium and glucose, could find more extensive application for therapeutic and recreational purposes in industrialized countries.

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