Modification of Enterosorption Induced by a Heat Labile *Escherichia coli* Enterotoxin in Rabbit Intestinal Loops (36466)

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Evidence is accumulating that the pathogenesis of diarrhea that is caused by *Vibrio cholerae* in man and that caused by enteropathogenic *Escherichia coli* (EEC) in swine is similar (9, 13). Both organisms produce an enterotoxin which causes net transfer of large volumes of water and electrocytes from plasma to gut lumen (enterosorption) through an intact intestinal mucosa (3, 9).

Enterosorption induced in rabbit ileal loops by *V. cholerae* enterotoxin is reduced in the presence of intraluminal glucose (6), since actively transported sugars tend to enhance intestinal absorption of sodium and water. Leitch *et al.* (5) observed that enterosorption induced by cholera enterotoxin could be reduced by treatment with Diamox (sodium acetoazolamide), an inhibitor of carbonic anhydrase. Subsequently, Norris *et al.* (10) confirmed the above observations and noted that the effects of glucose and Diamox on enterosorption in rabbit ileal loops were additive.

If the mechanisms by which *V. cholerae* and EEC induce diarrhea are similar, enterosorption induced by EEC enterotoxin should be responsive to treatment with either Diamox or intraluminal glucose or both. This report concerns effects of glucose and Diamox on enterosorption induced in rabbit intestinal loops by an enterotoxin derived from an EEC isolated from a pig.

Methods. The crude, heat-labile enterotoxin used in these experiments was derived from an EEC (serotype 08:K87,K88a,b:H19) isolated from a newborn pig with diarrhea. The characteristics of this organism and the methods used to produce and quantitate this enterotoxin have been described (7).

New Zealand White rabbits, 9–12 weeks old, were deprived of feed for 48 hr but allowed access to water until they were used. They were anesthetized[1] and a laparotomy was performed. A silk ligature was placed around the intestine 90 cm anterior to the tip of the appendix. The luminal contents of the terminal 90 cm of small intestine was rinsed posteriorly with 10 cc of saline. A catheter[2] was inserted anteriorly into the intestinal lumen through an incision 10 cm posterior to the initial ligature. The catheter was fixed in place with a silk ligature encircling the intestinal wall, creating a 10-cm intestinal loop which contained 8 cm of catheter. The portion of the catheter in the intestinal lumen was perforated at 0.5 cm intervals. The extraintestinal portion of this catheter was brought through the body wall *via* a stab wound in the right paralumbar fossa. A second loop was prepared in the same manner posterior to the first. The two experimental loops were separated by a 10-cm interloop. The incisions were closed and the rabbits were allowed to regain consciousness. Experimental loops were exposed to either 10 units of enterotoxin in 2 cc of saline or to 2 cc of saline (exposure) and the catheters were sealed. Five hours later, the contents of each loop was aspirated and the volume was recorded. The lumen of each loop was rinsed twice with 5 ml of warm saline (37°). A test solution (8–9 ml) was put into each loop. A 1.5-ml sample was obtained from each loop after a 5-min equilibration period and a sec-

[1] Fluothane, Ayerst Laboratories, 685 Third Avenue, New York, NY.

[2] Sialastic catheter (.040 in. I.D. × .085 in. O.D.), Dow Corning Corporation, Midland, MI.

TABLE I. Effect of Glucose and Diamox on Enterosorption Induced by *E. coli* Enterotoxin in Rabbit Intestinal Loops.

Treatment group	Enterosorption	Electrolyte concentrations (mEq/liter) ± SE			
	Mean volume change[a]	Sodium	Chloride	Bicarbonate	Potassium
Control	0.65 ± 0.15 (17)[b]	145 ± 7 (10)	111 ± 6 (10)	30.9 ± 5.1 (10)	2.46 ± 0.39 (10)
Glucose	0.46 ± 0.13 (17)	142 ± 6 (10)	107 ± 6 (9)	31.7 ± 8.5 (9)	2.77 ± 0.58 (9)
Enterotoxin	2.34 ± 0.15 (16)	144 ± 9 (9)	111 ± 6 (9)	32.2 ± 6.3 (9)	2.39 ± 0.28 (9)
Enterotoxin–glucose	1.99 ± 0.13 (16)	146 ± 5 (13)	108 ± 5 (13)	35.3 ± 5.9 (13)	2.52 ± 0.39 (13)
Enterotoxin–Diamox	1.82 ± 0.14 (19)	145 ± 7 (14)	118 ± 9 (14)	23.9 ± 6.7 (14)	2.21 ± 0.39 (14)
Enterotoxin–Diamox–glucose	1.48 ± 0.13 (19)	142 ± 6 (13)	112 ± 9 (13)	25.0 ± 8.7 (13)	2.42 ± 0.45 (13)

[a] Measured in ml/loop/30 min, ± SE.

[b] Number of observations in parentheses.

ond sample was taken 30 min later. The rabbits were necropsied immediately after collection of the last sample. The status of the interloop was recorded. The data from all loops from any rabbit with a fluid-filled interloop were discarded.

The rabbits were divided into three treatment groups: control rabbits, enterotoxin rabbits, and enterotoxin rabbits treated with Diamox (25–50 mg/kg iv) 30 min before injecting the test solutions. In all treatment groups, the test solution put into one loop of each rabbit contained 150 m*M* NaCl whereas the test solution put into the other loop contained 150 m*M* NaCl plus 20 m*M* glucose. Diamox (500 μg/ml) was added to the test solutions for use in rabbits treated with Diamox. Polyethylene-1,2-^{14}C glycol (PEG-^{14}C mol wt: approx 4000) was added to each test solution (12 μCi/liter) as a volume marker. A portion (100 μl) of each sample was dissolved in 10 ml of Bray's solution (1) containing 20% Triton X-100 and counted on a liquid scintillation spectrometer. Volume changes were calculated from changes in concentration of PEG-^{14}C. The remainder of each sample was frozen for subsequent analysis. Bicarbonate and chloride were determined using automated equipment for clinical chemistry following the manufacturer's recommended procedures.[3] Sodium and potassium were determined by flame photometry.

Results. Mean net secretions during the first 5 hr after exposure were 0.32 ml (control loops), 3.53 ml (enterotoxin loops), and 3.32 ml (enterotoxin loops in rabbits which had been treated with Diamox).

The mean changes in volume of test solution during the 30-min observation period are shown in Table I. In order to determine the significance of volume changes that were due to treatment groups, a separate analysis of variance was done for those loops infused with saline and also for those infused with saline plus glucose.

A mean net transfer of fluid from plasma to intestinal lumen was observed in loops which had not been exposed to enterotoxin. However, loops exposed to enterotoxin secreted significantly more fluid ($p < .05$) than did the control loops. Enterosorption in loops exposed to enterotoxin and treated with Diamox was significantly less ($p < .05$) than in loops exposed to enterotoxin but not receiving Diamox.

The effects of adding glucose to the test solution on treatment groups was evaluated statistically by a paired difference analysis. The change in fluid volume observed in control loops receiving saline tended to be greater than that observed in those receiving saline plus glucose, but this difference was not significant ($.05 < p < .10$). However, the enterosorption observed in loops which received

[3] Auto Analyzer Technical Methods, Technicon Instrument Corporation, Ardsley, NJ.

saline plus glucose was less than in those receiving saline ($p < .05$) for loops exposed to enterotoxin as well as in enterotoxin-treated loops which had been treated with Diamox. Thus, enterotoxin consistently induced enterosorption which was decreased by treatment with either glucose or Diamox. Furthermore, the effects of the combination of glucose and Diamox were significantly greater than either treatment alone ($p < .05$).

The electrolyte profile of the solutions removed 30 min after infusion of test solutions were similar (Table I), regardless of treatment group. However, bicarbonate concentrations in loop fluid of rabbits, treated with Diamox, tended to be lower than those in the other two treatment groups.

Discussion. As previously observed (7), enterotoxin induced large increases in net fluid secretion in rabbit intestinal loops. This was reduced by the presence of intraluminal glucose or by treatment with Diamox and the effects of these two treatments were additive. Thus, the effects of glucose and Diamox on the enterosorption induced by *E. coli* enterotoxin in these experiments are similar to those observed in rabbit ileal loops treated with *V. cholerae* enterotoxin (10).

Although the secretory response of the ileum of the rabbit to either *E. coli* enterotoxin or *V. cholerae* enterotoxin tended to be greater than that of the jejunum (8), the terminal portion of the ileum was avoided in these experiments because it tended to give a higher incidence of "false positive" enterosorptive responses (8, 13). Nevertheless, fluid was present in over half of the control loops at the end of the 5-hr incubation period and a mean net transfer of fluid from blood to intestinal lumen was observed in control loops during the experimental period. We have discussed possible explanations for "false positive" loop responses (8).

The closed loop system has the disadvantage that the highest glucose concentrations and presumably maximal glucose absorption occur during the early part of the experimental period. Since the effects of glucose absorption occurring during the 5-min equilibration period are not included in the observation period, the maximal effects of glucose on enterosorption was probably not observed. Furthermore, the osmotic effect incurred by adding 20 m*M* glucose to the 150 m*M* NaCl would also tend to reduce net absorption of fluid during the early part of the test period.

These results have been interpreted with the assumption that the effects of glucose on enterosorption are mediated through glucose-stimulated sodium absorption (6). Therefore, it is inferred that glucose absorptive mechanisms were functioning and that glucose-stimulated sodium transport occurred in the intestine following exposure to enterotoxin. Bywater (2) reported that glucose absorption was unaffected in Thiry-Vella loops of calf intestine exposed to a heat-stable *E. coli* enterotoxin. The observation that glucose-stimulated sodium absorption was unimpaired by exposure of the intestine to cholera toxin (6) was subsequently exploited successfully in the treatment of clinical cholera in man (4, 11). Thus, the present observations may have therapeutic implications in colibacillosis. They also add additional support to the concept that the mechanism by which EEC enterotoxin exerts its effect on the intestinal mucosa is similar to that of *V. cholerae* enterotoxin.

Summary. Enterosorption was induced in rabbit jejunal loops with a heat-labile enterotoxin produced by an enteropathogenic *E. coli*. Enterosorption was decreased by treatment with Diamox or intraluminal glucose. Furthermore, the effects of these treatments were additive.

The authors wish to thank Mr. Norman Lyon and Mrs. Karen Coburn for their fine technical assistance; Mr. Charles Graham for the statistical analyses; and Dr. Albert Baetz for electrolyte determinations.

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Received Jan. 10, 1972. P.S.E.B.M., 1972, Vol. 140.