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Research Article

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Site and Characteristics of Electrolyte Loss and Effect of Intraluminal Glucose in Experimental Canine Cholera

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ABSTRACT The site and characteristics of gastrointestinal electrolyte loss were investigated in eight dogs with experimental cholera induced by orogastric administration of 6-hr broth cultures of *Vibrio cholerae,* strain Ogawa 395. In these animals, all electrolyte losses originated in the small bowel, predominantly from the jejunum and ileum. The bicarbonate concentration of the small bowel fluid showed a progressive increase from duodenum, where it was less than that of plasma, to the terminal ileum, where it was significantly greater than that of simultaneously obtained plasma.

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INTRODUCTION

The characteristics of the massive gastrointestinal electrolyte losses in cholera have been described during the past decade (1, 2). The site of electrolyte loss has, however, not been defined, nor has the mechanism been delineated. The difficulties inherent in carrying out definitive studies in the cholera patient and the absence of a suitable large animal model have been major obstacles to answering these questions. During the past 2 yr, a canine model has been developed in which the clinical signs and biochemical changes of cholera can predictably be produced by challenge with broth cultures of *Vibrio cholerae* (3). The characteristic gastrointestinal electrolyte losses can also consistently be produced in the dog by intraduodenal challenge with bacteria-free filtrates of broth cultures of *V. cholerae.*[1] The canine model has been employed in three experiments that have been designed to determine the site of electrolyte loss in experimental cholera and to investigate the effect

[1] Craig, J. P., C. C. J. Carpenter, and R. W. Steenberg. Response of the canine gut to intraluminal challenge with a bacteria-free filtrate of broth culture of *V. cholerae.* Manuscript in preparation.

of cholera exotoxin on glucose-associated sodium transport in the small intestine.

METHODS

All studies were performed on adult mongrel dogs, weighing 14–24 kg. Animals were fasted for 18 hr before each study. Sodium and potassium determinations were performed on a Patwin flame photometer with lithium internal standards (National Instrument Laboratories, Inc., Rockville, Md.). Chlorides were determined on a Cotlove chloridometer (Buchler Instruments, Inc., Fort Lee, N. J.). Carbon dioxide content in experiment III was determined by a Natelson microgasometer (Scientific Industries, Inc., Springfield, Mass.). pH measurements were made on a Beckman expanded scale pH meter, Beckman Instruments, Inc., Palo Alto, Calif. Reducing substances were determined by the technique of Hoffman (4) with the Technicon AutoAnalyzer, Technicon Corporation, Ardsley, N. Y. Total proteins were determined by the Folin-Ciocalteu method (5). PSP was determined photometrically by the technique of Schedl and Clifton (6).

The crude cholera exotoxin employed in experiments II and III consisted of a 0.22 μ Millipore filtrate (Millipore Filter Corp., Bedford, Mass.) of a 24 hr shaken culture of *V. cholerae,* strain Inaba 569B, grown in modified Syncase broth; the broth was prepared by a formula of Finkelstein, Atthasampunna, Chulasamaya, and Charunmethee (7) but with sucrose content reduced from 0.5 to 0.1%. In each case, the challenge dose was 40–50 ml of filtrate containing 3–4 million blueing doses of cholera exotoxin as determined by the rabbit skin assay of Craig (8). (Although present evidence suggests that the "permeability factor" measured by the rabbit skin assay is not the toxic moiety responsible for gastrointestinal electrolyte loss in the mammalian gut, the titer of "permeability factor" has correlated well with the electrolyte loss produced in the canine gut after challenge with the crude filtrate employed in these studies. Preliminary studies indicated that crude filtrate containing 1 million rabbit skin blueing doses consistently caused maximal electrolyte loss by the canine small bowel loops.)

Experiment I was designed to determine the origin and solute composition of the fluid produced by the canine gut in experimental cholera. Eight dogs with clinically severe cholera were studied. The challenge technique was as follows. An orogastric tube was placed in the normal, postabsorptive, unanesthetized animal, and the following series of solutions were administered into the stomach over a 5 min period: 50 ml of 6% sodium bicarbonate, 100 ml of a 6 hr Syncase broth (7) culture of *V. cholerae* Ogawa 395, and 100 ml of fresh uninoculated Syncase broth. The viable vibrio count in the 6 hr broth culture ranged from 1×10^9 to 5×10^9 organisms per ml. With this challenge technique, approximately 40% of the animals developed clinical cholera, which was lethal unless treated with intravenous fluids, within 12 hr after challenge.

After challenge, each dog was placed in a metabolic cage, and fluid output was observed over the following 16 hr period. After the initial 16 hr period, eight dogs with clinically severe cholera, manifest by profuse rice-watery diarrhea, poor skin turgor, weak femoral pulse, and cyanosis of the tongue, were studied in detail. Each animal was anesthetized with Nembutal, laparotomy was performed, and the bowel was divided into four segments by tight double ligatures at the pylorus, the ligament of Treitz, and the ileocecal junction. Foley catheters were inserted into the stomach, the terminal duodenum, the terminal ileum, and the rectum and were allowed to drain externally into graduated cylinders during the remainder of the study. Each animal was then rapidly given intravenous fluids (2 parts isotonic sodium chloride to 1 part 1/6 M sodium lactate) in quantities sufficient to restore the hematocrit to the base line level, and the abdominal incision was closed by a series of towel clamps. During the following 14 hr period, each animal was kept under light Nembutal anesthesia and was given the above intravenous fluids in quantities sufficient to maintain a stable hematocrit, as gaged by microhematocrit determinations performed in duplicate at hourly intervals. During this time, the hourly output from each of the segments of bowel was measured, and sodium, chloride, potassium, bicarbonate, and total protein concentrations of the gastrointestinal fluid were determined. In six of the eight dogs, 5 ml samples of jejunal fluid, obtained from a segment approximately 50 cm distal to the ligament of Treitz, were also collected at hourly intervals for electrolyte analysis. The antemesenteric wall of this segment of jejunum was loosely sutured to the abdominal wall adjacent to the laparotomy incision, and the hourly samples were obtained by releasing one of the towel clamps and directly puncturing the jejunal segment with an 18 gauge needle.

In three of the eight animals, the thoracic duct was directly cannulated with polyethylene tubing (I.D. = 0.047 inches) immediately after the abdominal incision was closed; cannulation was performed immediately caudal to the junction of the thoracic duct and the external jugular vein. Hourly thoracic duct lymph flow rates, as well as lymph electrolyte and protein concentrations, were determined throughout the remainder of the study. These observations were made to ascertain whether there was increased leakage of protein from the capillaries to the interstitial fluid of the lamina propria during experimental cholera.

Each animal was sacrificed with Nembutal 30 hr after the initial challenge, and sections of small bowel mucosa were fixed in Hartman's solution and in glutaraldehyde for light and electron microscopic study.

Experiment II was designed to study the response of upper and lower small bowel segments to challenge with crude cholera exotoxin. Studies were performed in 10 dogs with chronic Thiry-Vella small bowel loops. In five dogs, the loops included the 60–70 cm of small bowel immediately distal to the ligament of Treitz, and in five the loops consisted of the 60–70 cm immediately proximal to the ileocecal valve. The loops represented from 20 to 30% of the entire jejunoileal length in these animals.

The isolated small bowel loop was initially challenged with crude cholera exotoxin 2 wk after preparation of the loop. Four of the animals, two with jejunal and two with ileal loops, were then repeatedly challenged by exotoxin at weekly intervals over the following 3 wk and then at monthly intervals over the following 6 months.

The challenge technique was as follows. On the morning of the study, each animal was lightly anesthetized with Nembutal, and Foley catheters (American Cystoscope Makers, Inc., Pelham Manor, N. Y.) were placed, with balloons inflated, in the proximal and distal orifices of the Thiry-Vella loops. The crude exotoxin was then placed in the loop via the proximal Foley catheter. Foley catheters were then clamped and the exotoxin was allowed to remain in the loop for the following 60 min period. After 60 min, both proximal and distal catheters were opened and allowed to drain into graduated cylinders. Volume of fluid output was recorded at 2-hourly intervals over the following 12 hr, and electrolytes were determined on the fluid collected. Hematocrits and plasma electrolyte concentrations were determined at 2-hourly intervals, and intravenous fluids (2 parts isotonic sodium chloride to 1 part 1/6 M sodium lactate) were given at a rate sufficient to maintain a stable hematocrit.

Experiment III was designed to determine the effect of intraluminal glucose on electrolyte movement in isolated small bowel loops before and after administration of cholera exotoxin. Studies were performed in six animals with chronic 60–70 cm jejunal loops and in six animals with chronic 60–70 cm ileal loops. Each experiment consisted of two studies, each consisting of three 4 hr periods. During the initial 4 hr period of the first study, the bowel loop was perfused at a rate of 15 ml/kg per hr with an isotonic electrolyte solution containing the following solutes: sodium, 140 mEq/liter; potassium, 10 mEq/liter; chloride, 100 mEq/liter; and bicarbonate, 50 mEq/liter. At the end of this period the loop was flushed with 200 ml of air, and the rate of absorption of the perfusion fluid was determined. Crude cholera exotoxin, 40–50 ml of bacteria-free filtrate as described above, was then placed in the loop via Foley catheter, and both proximal and distal catheters were clamped for the next 2 hr period. The catheters were then allowed to drain freely over the following 2 hr period, after which the loop was again flushed with 200 ml of air, and fluid output was recorded. The loop was then perfused with the same isotonic electrolyte solution for an additional 4 hr period, at the end of which time the loop was again flushed with 200 ml of air. 1 wk later the second study was done in the same dog, this time with a perfusion fluid containing: glucose, 60 mOsm/liter; Na, 110 mEq/liter; K, 10 mEq/liter; Cl, 80 mEq/liter; and bicarbonate, 40 mEq/liter. In six animals (three with jejunal and three with ileal loops), the perfusion fluid containing no glucose was administered during the first study, and the glucose-containing perfusion fluid during the second study; in the remaining six animals, the reverse sequence was employed.

In each study net electrolyte absorption or production by the isolated gut loop, before and after toxin administration, was determined. In four of the studies, phenolsulfonphthalein (PSP) was added to the perfusion fluid in a concentration of 15 mg/100 ml, and recovery of PSP from the effluent was used to estimate the amount of residual fluid retained in the loops at the end of each 4 hr study period.

RESULTS

Experiment I. In the eight animals with clinically severe cholera secondary to *V. cholerae* infection, the mean gastrointestinal fluid loss during the 30 hr after vibrio challenge was 34.9% of body weight (Table I). The maximum observed hourly rate of gastrointestinal fluid loss was 50 ml/kg per hr. In general, the fluid output was maintained at a relatively constant level from the 20th to the 30th hour of observation, i.e., from the time the catheters had been placed and the animals adequately rehydrated until termination of the study. In each animal, the greatest proportion of electrolyte loss occurred from the jejunoileal segment of the small bowel, with a mean of 91% of fluid loss occurring from this segment (Table I). From 3 to 16% of the fluid loss occurred from the duodenum, and virtually no fluid (less than 1%) was produced proximal to the pylorus. In no animal was any fluid produced by the colon after segmentation of the bowel.

Table II presents the electrolyte composition of fluid produced by the three segments of small bowel during experimental cholera. The duodenal and ileal values represent mean electrolyte concentrations of hourly samples obtained from the 20th to 30th hour after infection in all animals

TABLE I

Gastrointestinal Fluid Losses in Eight Dogs with Experimental Cholera Secondary to Orogastric Administration of V. Cholerae

Dog No.	Gastrointestinal fluid output	Maximum hourly fluid output	Per cent output proximal to ligament of Treitz	Per cent output by jejunoileal segment
	ml/kg	*ml/kg*		
1	358	28	15	85
2	510	45	6	94
3	331	50	3	97
4	240	31	9	91
5	223	30	5	95
6	345	31	12	88
7	269	22	16	84
8	512	28	6	94
Mean values ± SD	349 ± 111	33 ± 9	9 ± 5	91 ± 5

TABLE II

*Electrolyte Composition of Fluid Produced by the Three Portions of Canine Small Intestine during the 20th–30th Hr after Infection with V. Cholerae**

	Sodium	Chloride	Potassium	Bicarbonate
Duodenum, eight dogs	143±13	119±7	7.1±2.1	14± 8
Jejunum, six dogs	153± 4	112±6	6.4±2.0	30± 8
Ileum, eight dogs	143± 5	87±8	9.6±4.1	66±11

* Mean values ± SD.

studied; the jejunal values represent mean concentrations of all hourly samples in the six dogs from which jejunal specimens were obtained. The fluid produced by each of the segments of the small bowel was approximately isotonic with plasma, and in each animal the electrolyte concentrations of fluid produced by a given segment of bowel remained relatively constant throughout the study. Mean bicarbonate concentration of fluid produced by the ileum was significantly greater than that of fluid produced by the jejunum ($t = 6.78$, $P < 0.001$) and duodenum ($t = 10.1$, $P < 0.001$), respectively. (t is used hereafter to denote values derived from Student's t test.) Conversely, mean chloride concentration of fluid produced by the ileum was significantly less than that produced by the jejunum ($t = 6.62$, $P < 0.001$), and duodenum ($t = 7.69$, $P < 0.001$), respectively (Table II). There were no significant differences in the mean potassium concentrations observed at different levels of the bowel, although a wider range of K concentrations were observed in the ileum than in more proximal segments of small bowel. Mean sodium concentration of jejunal fluid was significantly greater than that of ileal fluid ($t = 3.90$, $P < 0.01$).

The protein content of fluid obtained at all levels of the small bowel was consistently less than 500 mg/100 ml, with a mean total protein value of 251 ± 67 (SD) mg/100 ml for jejunoileal fluid obtained from all animals studied.

Total protein concentrations of thoracic duct lymph varied inversely with the lymph flow rate but never exceeded the normal range of values of canine thoracic duct lymph (9). At the time of initial cannulation of the thoracic duct, before saline repletion had been carried out, the mean thoracic duct lymph flow was 3.5 ml/kg per hr, with a mean total protein concentration of 4.0 g/100 ml. The thoracic duct lymph flow rate invariably increased with saline repletion to a mean value of 10.8 ml/kg per hr 4 hr after initiation of intravenous fluid therapy; at this time, the mean total protein concentration was 1.6 g/100 ml. Electrolyte and reducing substance concentrations in thoracic duct lymph were essentially the same as those in simultaneous plasma samples. Table III presents mean values for electrolytes, total protein, and reducing substances in lymph, plasma, jejunum, and ileum, respectively during the period of maximal gastrointestinal fluid output in the three dogs in which thoracic duct lymph studies were performed.

Histologic sections of jejunal and ileal mucosa showed moderate edema of the lamina propria but demonstrated no morphologic changes in epithelial or capillary endothelial cells by either light or electronmicroscopy. These data are described in a separate report (10).

Experiment II. Fluid was produced by all jejunal and ileal loops challenged by the exotoxin preparation. In all instances, a lag period occurred before fluid loss was observed. The filtrate containing exotoxin was completely absorbed by all ileal loops, and largely (75–100%) absorbed by all jejunal loops, within 60 min after challenge. Output of an approximately isotonic fluid was always observed by the end of the 2nd hr after challenge in jejunal loops and during the 3rd hr after challenge in ileal loops. Rate of fluid production usually reached maximum values by the 4th or 5th hr after challenge in both jejunal and ileal loops and then continued at maximum levels for an additional 4–6 hr (Table IV). The rate of fluid

TABLE III

*Correlation of Electrolyte, Protein and Reducing Substance Values in Thoracic Duct Lymph with Simultaneous Plasma and Gastrointestinal Fluid Values during Experimental Cholera**

	Sodium	Chloride	Potassium	Bicarbonate	Total Protein	Reducing Substances
	mEq/liter	*mEq/liter*	*mEq/liter*	*mEq/liter*	*g/100 ml*	*mg/100 ml*
Lymph‡	147	114	2.4	19	1.59	89
Plasma‡	154	119	2.2	19	5.20	87
Jejunum	155	119	4.9	26	0.22	7
Ileum	150	90	3.4	56	0.17	3

* Mean values of determinations in three animals at 22, 24, 26, and 28 hr after vibrio challenge.

‡ All values are corrected to give electrolyte concentrations per milliliter of plasma *water* (11).

TABLE IV

Mean Hourly Rates of Fluid Output by Jejunal and Ileal Loops during 12 Hr Period after Intraluminal Challenge with Cholera Exotoxin

Time after exotoxin challenge	Jejunal loops*	Ileal loops*
hr	*ml/hr*	*ml/hr*
0–2	34±31	0
3–4	94±17	42±12‡
5–6	98±11	48±19‡
7–8	91±12	46±15‡
9–10	74±20	36±12§
11–12	61±20	33±16

* Mean values ± SD, five dogs.
‡ Differences between mean rates of fluid ouput by jejunal and ileal loops significant at 1% level by *t* test.
§ Differences between mean rates of fluid output by jejunal and ileal loops significant at 2% level by *t* test.

output then decreased gradually over the following 6–8 hr period. Fluid output generally ceased entirely by 14 hr after challenge in ileal loops and by 18 hr after challenge in jejunal loops. Mean rates of fluid production by jejunal and ileal loops during the 12 hr after exotoxin challenge are presented in Table IV. The mean total fluid output by the jejunal loops during the 12 hr after exotoxin challenge was 901 ± 134 ml and that by the ileal loops was 421 ± 133 ml (Table V); the differences between mean 12 hr outputs by jejunal and ileal loops are significant ($t = 5.14$, $P < 0.001$).

In the four animals (two with jejunal loops, two with ileal loops) in which exotoxin challenges were administered at weekly intervals during the first 3 wk of the study, the volume responses

TABLE V

Total Fluid Output by Chronic Jejunal and Ileal Loops during the 12 Hr Period after Intraluminal Challenge with Cholera Exotoxin

Jejunal loops		Ileal loops	
Dog No.	Output	Dog No.	Output
	ml		*ml*
1	1016	2	472
3	860	4	339
5	835	6	572
7	735	8	235
9	1060	10	485
Mean±SD	901±134		421±133

TABLE VI

*Volume Responses of Chronic Jejunal and Ileal Loops to Repeated Exotoxin Challenge at Weekly Intervals**

Dog No.	Week	Jejunal loops	Dog No.	Week	Ileal loops
1	1	1016	2	1	472
	2	1055		2	547
	3	990		3	412
5	1	835	6	1	572
	2	760		2	630
	3	883		3	473

* Fluid output during 12 hr after challenge, in milliliters.

showed little variation on the successive challenges (Table VI). Repeated challenges of these animals at 6 months after initial challenge showed decreased fluid production in all animals. Both jejunal and ileal loops showed decreases in response to 30–70% of initial levels. All four dogs, however, showed gross atrophy of the small bowel loops, with decrease in both length and diameter, at the time of the 6 month challenge.

In all animals challenged, the fluid output from the loops was essentially isotonic, but electrolyte composition of fluid produced by jejunal loops varied significantly from that produced by ileal loops in regard to bicarbonate ($t = 15.8$, $P < 0.001$) and chloride ($t = 7.26$, $P < 0.001$) concentrations (Table VII). The mean sodium concentration of jejunal fluid was greater than that of ileal fluid, but the difference was of borderline significance ($t = 2.31$, $P = 0.05$). The mean potassium concentration of fluid produced by jejunal loops was not significantly different from that of fluid produced by ileal loops ($t = 1.12$, $P > 0.5$).

Mean plasma electrolyte concentrations during the studies, corrected for plasma proteins to give concentrations per liter of plasma water (11), are presented in Table VII. There were no significant differences between mean electrolyte concentrations in plasma and in jejunal effluent during the exotoxin challenge studies. There were, however, highly significant differences between mean electrolyte concentrations in ileal effluent and in plasma in regard to bicarbonate ($t = 20.8$, $P < 0.001$) and chloride ($t = 5.12$, $P < 0.001$). Mean potassium concentration in ileal effluent was also greater than that of plasma ($t = 2.32$, $P < 0.05$).

The electrolyte concentrations of intestinal fluid

TABLE VII

Electrolyte Concentrations of Fluid Produced by Jejunal and Ileal Loops in Response to Challenge with Cholera Exotoxin

	Jejunal*		Ileal*	
	Plasma‡	Jejunal effluent	Plasma‡	Ileal effluent
Sodium, *mEq/liter*	153 ±8	159 ±5	153 ±4	145 ±11
Chloride, *mEq/liter*	116 ±5	122 ±6	113 ±4	68 ±17
Potassium, *mEq/liter*	5.1±0.9	6.9±3.2	5.0±0.6	9.6±3.5
Bicarbonate, *mEq/liter*	24 ±2	26 ±4	21 ±2	76 ± 5

* Mean values ± SD, five dogs.

‡ Plasma electrolytes are corrected to give concentrations in milliequivalents per liter of plasma *water* (11).

obtained at 2-hourly intervals after challenge remained essentially constant throughout each study and bore no relationship to the rate of fluid production by the challenged loop. Likewise the electrolyte concentrations of jejunal and ileal fluid remained unchanged in response to repeated weekly challenges over a 3 wk period. The mean total protein concentration of the loop fluid was 242 ± 116 (SD) mg/100 ml for jejunal, and 181 ± 101 (SD) mg/100 ml for ileal loops. Protein concentration of loop fluid never exceeded 500 mg/100 ml.

None of the animals developed diarrhea or showed any clinical evidence of malfunction of the unchallenged intact portion of the gastrointestinal tract during the studies.

Experiment III. All 12 animals absorbed isotonic perfusion fluid containing no glucose during the control period. Mean absorption by jejunal loops was 43 ± 22 ml/hr, and mean absorption by ileal loops was 71 ± 20 ml/hr (Table VIII). The difference between mean control absorption rates by jejunal and ileal loops is significant ($t = 2.23$, $P < 0.05$). Within 2 hr after toxin administration, output of isotonic fluid was observed in all jejunal loops, and within 3 hr isotonic fluid output occurred in all ileal loops. During the perfusion period after toxin administration, all animals exhibited continued fluid and electrolyte loss. The mean rate of production of isotonic fluid by jejunal loops was 68 ± 46 ml/hr and that by ileal loops was 78 ± 19 ml/hr; the differences between jejunal and ileal loops are not significant.

When the perfusion fluid contained glucose, 60 mOsm/liter, an increase in isotonic fluid absorption during the control period was observed in every animal. The mean rate of absorption of the perfusion fluid by the jejunal loops was 100 ± 61 ml/hr and that by ileal loops was 148 ± 63 ml/hr (Table VIII). During the posttoxin glucose perfusion period, the mean rate of gut fluid production was significantly less than that observed when the perfusion fluid contained no glucose in both jejunal ($t = 2.90$, $P < 0.02$) and ileal ($t = 3.35$, $P < 0.01$) loops. The mean rate of fluid production by jejunal loops was 12 ± 17 ml/hr and that by ileal loops was 31 ± 25 ml/hr. Net absorption of isotonic fluid was observed during this period in two of the jejunal and one of the ileal loops.

The net effect of toxin on isotonic fluid movement was not significantly different during perfusion studies with glucose from that observed during perfusion with isotonic fluid containing no glucose. In jejunal loops the mean net effect of toxin was to decrease absorption (or increase production) by 111 ml/hr during perfusion studies without glucose and by 112 ml/hr during studies with glucose. In ileal loops, the mean net effect of toxin was to decrease absorption (or increase pro-

TABLE VIII

Net Absorption or Production of Isotonic Fluid during Perfusion of Small Bowel Loops before and after Intraluminal Administration of Cholera Exotoxin

	Jejunal loops*	Ileal loops*
	ml/hr	*ml/hr*
Perfusion fluid with no glucose		
Control	+43±22	+71±20
After toxin	−68±46	−78±19
Perfusion fluid with glucose, *60 mOsm/liter*		
Control	+100±61	+148±63
After toxin	−12±17	−31±25

\+ indicates net absorption, − indicates production.

* Mean values, ± SD, six dogs.

duction) by 149 ml/hr during studies without glucose and by 179 ml/hr during studies with glucose.

Conversely, the effect of glucose in increasing absorption (or decreasing fluid production) after administration of exotoxin was not significantly different from that observed during control periods in either jejunal or ileal loops. In jejunal loops, the mean net effect of glucose was to increase absorption (or decrease fluid production) by 57 ml/hr in control loops and by 56 ml/hr in toxin-treated loops. In ileal loops, the mean net effect of glucose was to increase absorption (or decrease production) by 77 ml/hr in control loops and by 47 ml/hr in toxin-treated loops.

In the four animals in which PSP, 15 mg/100 ml, was added to the perfusate as a nonabsorbable marker, from 88 to 97% of administered PSP was recovered from the loop effluent at the end of each perfusion period.

Glucose was absorbed from perfusate by both jejunal and ileal loops (Table IX). Administration of cholera exotoxin caused no significant change in rate of glucose absorption by either jejunal or ileal loops.

During perfusion studies of jejunal loops without glucose, the isotonic perfusion fluid showed no significant alteration in electrolyte concentrations. During perfusion with glucose-containing solution, glucose concentration of the perfusate consistently decreased and sodium concentration increased during passage through the loop. Plasma electrolytes were not significantly altered during these studies.

During perfusion studies of ileal loops, the perfusion fluid containing no glucose showed significant increases in bicarbonate concentration during passage through the loop both before ($t = 2.80$, $P < 0.05$) and after ($t = 3.17$, $P < 0.02$) toxin administration. During perfusion with glucose-containing solution a significant increase in perfusate bicarbonate concentration occurred only after toxin administration ($t = 6.00$, $P < 0.001$). A significant decrease in perfusate glucose concentration and increase in perfusate sodium concentration occurred during passage through the ileal loop both before and after toxin administration. Plasma bicarbonate concentration consistently decreased after toxin administration during the ileal studies (Table X), whereas other electrolytes showed no significant changes. The mean plasma bicarbonate concentration at the end of the ileal perfusion studies was significantly less than that at the beginning of the studies ($t = 5.53$, $P < 0.001$ for nonglucose perfusion; $t = 3.10$, $P < 0.02$ for perfusion with glucose-containing solution).

TABLE IX

Glucose Absorption before and after Administration of Cholera Exotoxin by Jejunal and Ileal Loops Perfused by an Isotonic Solution Containing Glucose, 60 mOsm/liter

	Absorption of glucose by jejunal loops*	Absorption of glucose by ileal loops*
	g/hr	*g/hr*
Control	1.70±0.39	1.53±0.58
After toxin	1.44±0.30	1.35±0.44

* Mean values ± SD, six dogs.

TABLE X

Plasma Bicarbonate Concentration (mEq/liter) before and after Cholera Exotoxin Administration in Dogs with Jejunal and Ileal Loops Perfused by Isotonic Electrolyte Solutions

	Jejunal*		Ileal*	
	Perfusion by solution with no glucose	Perfusion by solution with glucose	Perfusion by solution with no glucose	Perfusion by solution with glucose
Control	22±3	23±2	22±3	22±4
After 4 hr perfusion with isotonic solution	23±2	25±4	22±2	21±2
4 hr after toxin administration	22±3	24±5	18±3	19±3
After 4 hr perfusion with isotonic solution	24±3	22±3	15±2	17±3

* Mean values ± SD, six dogs.

DISCUSSION

The studies of animals with clinical cholera after challenge with viable *V. cholerae* clearly indicate that virtually all the electrolyte loss originates in the small bowel. Although all segments of the small bowel contribute to the electrolyte loss, the duodenum, because of its relatively short length, accounts for only a small proportion of the total electrolyte output.

The electrolyte concentrations of the fluid produced by the jejunal and ileal segments in dogs with *V. cholerae* infection were virtually identical

to those in spontaneous secretions from these segments of canine small bowel (12). The observed electrolyte concentrations also closely approximated those which occur in jejunal and ileal segments of small bowel during clinical cholera.[2] Thus in experimental canine as well as in human cholera, the electrolyte composition of the fluid produced by a given segment of small bowel approximates that which is characteristic of secretions normally produced by that segment of bowel.

The protein concentration of the thoracic duct lymph in the current studies was within the normal range observed by other investigators in thoracic duct lymph of dogs anesthetized with Nembutal (13, 14). The absence of an abnormally high thoracic duct lymph protein concentration, together with the consistently low protein concentrations of gastrointestinal fluid produced in response to live vibrio or exotoxin challenge, correlate well with the absence of any observed histologic damage to either gut epithelial cells or capillary endothelial cells in the lamina propria. These data are consistent with the earlier observations of Cohnheim (15) and Goodpasture (16) that histologically demonstrable gut epithelial cell damage is not essential to the pathogenesis of cholera. They are also in agreement with the more recent demonstrations by Gangarosa, Beisel, Benyajati, Sprinz, and Piyaratn (17) that no loss of mucosal integrity occurs in the small bowel of the cholera patient, and by Gordon (18) that intravenously injected polyvinylpyrrolidone does not leak into the gut during clinical cholera. The observations are also consonant with the demonstration by Elliott, Carpenter, Sack, and Yardley (10) that no electromicroscopically detectable lesions occur in the capillaries of the lamina propria of the small bowel during experimental canine cholera, and with the recent demonstration by Norris and Majno (19) that intravenously injected saccharated iron oxide fails to leak through capillaries of the lamina propria of exotoxin-challenged rabbit ileum.

In the current studies, the thoracic duct lumph flow rates were abnormally high at the time of initial thoracic duct cannulation and increased, after intravenous saline repletion, to levels of two to four times the basal rate observed in barbiturate-anesthetized dogs in which thoracic duct cannulation had been performed at the same site (9). It appears likely that the increased thoracic duct lymph flow is related to isotonic fluid absorption during experimental cholera. Previous studies have clearly demonstrated that thoracic duct lymph flow is increased during the absorption of isotonic saline by the mammalian gut (20); and avaliable data suggest that gut sodium absorption is not significantly impaired during clinical[2] or experimental[3] (21) cholera. Absorption of isotonic intraluminal fluid would therefore appear to provide an adequate explanation for the increased thoracic duct lymph flow observed in experimental cholera.

The studies of the response of the isolated small bowel loops to exotoxin challenge demonstrate consistent differences between jejunal and ileal responses in regard to volume and electrolyte content of the fluid produced. The mean rate of fluid production by the jejunal loops is significantly greater than that of ileal loops. As in the dogs with experimental vibrio infection, the bicarbonate concentration is significantly higher, and the chloride concentration significantly lower, in fluid produced by the ileum than in that produced by the jejunum. The electrolyte concentrations of the fluid produced by the canine ileal loops in response to crude exotoxin are essentially the same as those of fluid produced by ligated rabbit ileal loops challenged by sonic lysates of *V. cholerae* (22).

The time sequence of the response to exotoxin was also different in the proximal and distal small bowel loops, as fluid production by jejunal loops began by the end of the 2nd hr after toxin challenge, whereas that by ileal loops did not occur until the 3rd hr after challenge. In both cases, the rate of fluid production continued at maximal levels through the 8th hr after challenge, but fluid output ceased approximately 4 hr earlier in ileal than in jejunal loops. The "latent period" between exotoxin administration and onset of fluid production was less than the 4 hr as observed by Leitch, Iwert, and Burrows in ligated rabbit ileal

[2] Banwell, J. A., N. F. Pierce, R. C. Mitra, G. J. Caranasos, R. I. Keimowitz, A. Mondal, and P. M. Manji. 1968. Preliminary results of a study of small intestinal water and solute movement in acute and convalescent human cholera. *Indian J. Med. Res.* **56:** In press.

[3] Iber, F. L., T. J. McGonagle, H. A. Serebro, E. H. Luebbers, T. M. Baylass, and T. R. Hendrix. 1968. Mechanism of fluid production in experimental cholera toxin-induced diarrhea. *J. Clin Invest.* **47.** (Abstr.) In press.

loops challenged with sonic lysates of *V. cholerae* (22); this finding may represent a species difference in response to cholera exotoxin.

The similar quantitative and qualitative responses to repeated exotoxin challenges over a 3 wk period indicate that neither effective immunity against the responsible exotoxin nor tachyphylaxis develops during this period of time. Subsequent studies have indicated that responses of small bowel loops are essentially unchanged after weekly exotoxin challenges over a 9 wk period. These observations were believed to justify the use of each animal as its own control in repeated studies over a 3 wk period during the subsequent glucose perfusion studies.

The small bowel perfusion studies were performed to determine the effect of cholera exotoxin on glucose-associated sodium transport in the small intestine. Several studies have clearly demonstrated that intraluminal glucose enhances small bowel sodium absorption in both the experimental animal (23) and in man (24). On the basis of these data, Phillips and Wallace (25) performed a series of studies in 1962 that demonstrated that oral administration of hypertonic, glucose-containing electrolyte solutions greatly decreased the negative electrolyte balance in cholera patients. More recently, Pierce, Banwell, Mitra, Keimowitz, Caranasos, Mondal, and Manji [4] and Hirschhorn, Kinzie, Sachar Taylor, Northrup, and Phillips [5] have independently shown that continuous orogastric infusion of isotonic, glucose-containing electrolyte solutions can prevent negative electrolyte balance in the majority of acute cholera patients. These clinical studies did not indicate whether the mechanism of action of intraluminal glucose in cholera patients was to prevent the exotoxin-induced electrolyte secretion or to enhance isotonic fluid absorption to such an extent as to counterbalance the toxin-induced secretion.

The current studies suggest that intraluminal glucose does not alter the effect of cholera exotoxin in the canine gut. The net effect of exotoxin in increasing isotonic fluid production (or decreasing isotonic fluid absorption) was equally great in the presence or absence of intraluminal glucose. Conversely, the effect of glucose in enhancing isotonic fluid absorption (or decreasing isotonic fluid production) was equally great in control and toxin-treated loops. These data indicate that one of the characteristic gut epithelial cell functions, enhanced sodium absorption in the presence of intraluminal glucose, is well maintained in the presence of cholera exotoxin. The data also suggest that the mechanism by which cholera exotoxin produces electrolyte loss in cholera is independent of the mechanism of glucose-enhanced sodium absorption.

The mean rate of isotonic fluid production by toxin-treated ileal loops did not differ significantly from that by toxin-treated jejunal loops during the perfusion studies. These findings appear to conflict with those of experiment II, in which exotoxin was administered to jejunal and ileal loops in the absence of intraluminal perfusion fluid; under those circumstances, fluid production by jejunal loops was significantly greater than that by ileal loops. The following working hypothesis, which is consistent with current data, provides a possible explanation for this apparent paradox. The mean rate of absorption of the isotonic perfusing fluid by the ileal loops during the control period is significantly greater than that by jejunal loops. Several recent studies [2, 3] (21), as well as the current data, suggest that absorptive activity of the small bowel is not affected by the cholera exotoxin. When, therefore, the cholera exotoxin causes increased secretion of isotonic fluid by a given segment of gut, net fluid production occurs only when the rate of secretion exceeds the absorptive capacity for isotonic fluid by that segment of gut (Fig. 1). If we assume that the rates of exotoxin-induced secretion were approximately equal in jejunal and ileal loops, fluid production by nonperfused jejunal loops would be consistently greater than that by nonperfused ileal loops, since the absorptive capacity of jejunal loops is significantly less than that of the ileal loops. During the studies of perfused loops, on the other hand, the absorptive capacities of both jejunal and ileal segments are exceeded by the rate of perfusion of isotonic fluid. All exotoxin-induced secretion, there-

[4] Pierce, N. F., J. G. Banwell, R. C. Mitra, R. I. Keimowitz, G. J. Caranasos, A. Mondal, and P. M. Manji. 1968. Effect of intragastric glucose-electrolyte solution on water and electrolyte enterosorption in Asiatic cholera. *Gastroenterology.* **55.** In press.

[5] Hirschhorn, N., J. L. Kinzie, D. B. Sachar, J. O. Taylor, R. S. Northrup, and R. A. Phillips. Reduction of stool output in cholera by glucose and electrolyte lavage. Manuscript submitted for publication.

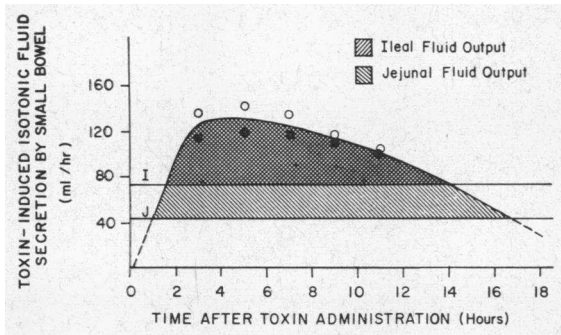

FIGURE 1 Postulated effect of cholera exotoxin on isotonic fluid movement in the canine small bowel. The ordinate represents isotonic fluid secretion induced by cholera exotoxin. The line *J* represents the rate of secretion (43 ml/hr) at which the absorptive capacity of the jejunal loop is equalled (experiment III). Whenever toxin-induced secretion exceeds 43 ml/hr, fluid is produced by the jejunal loop. The line *I* represents the rate of secretion (71 ml/hr) which equals the absorptive capacity of the ileal lop (experiment III). Whenever toxin-induced secretion exceeds 71 ml/hr, fluid is produced by the ileal loop. The points indicated by open circles represent the levels of fluid production by jejunal loops observed in experiment II, with line *J* as base line. The points indicated by closed circles represent the levels of fluid production by ileal loops observed in experiment II, with line *I* as base line. The line representing exotoxin-induced secretion is hand-fitted to the experimental points, on the assumption that the rates of exotoxin-induced secretion are not significantly different in jejunal and ileal loops.

fore, appears as net fluid production because the small bowel mucosa does not absorb any of the additional toxin-induced intraluminal fluid. Thus, during the perfusion studies with nonglucose solutions, the net exotoxin-induced isotonic fluid loss was approximately equal in ileal and jejunal loops.

This working hypothesis would also account for the greater time lag between administration of toxin and onset of fluid production by ileal than by jejunal loops, and also for the shorter duration of toxin-induced fluid loss in ileal than in jejunal loops. If we assume that the time of onset and the rate of increase of toxin-induced secretion were approximately the same in jejunal and ileal loops, the absorptive capacity of jejunal loops would be exceeded sooner and fluid output would be observed earlier, as delineated in Fig. 1. Likewise, as the rate of toxin-induced secretion began to decrease, fluid output would cease sooner in ileal than in jejunal loops. This hypothesis would also account for the recent observation by Banwell, Pierce, and Mitra that, during recovery from clinical cholera, absorption of isotonic fluid from the ileum is observed at a time when isotonic fluid loss is still occurring from the jejunum.[2] It should be emphasized that current data, although consistent with this hypothesis, do not prove that this hypothesis is correct. Critical testing of the hypothesis should be provided by studies of bidirectional fluxes of isotopically labeled sodium during experimental cholera. Such studies are currently underway.[8]

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