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**Research Article**

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# Intestinal Fluid and Electrolyte Transport in Human Cholera

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ABSTRACT The site, nature, magnitude, and duration of fluid and electrolyte loss into the small intestine during the acute and recovery phase of human cholera was defined in 27 Indian patients. 11 subjects without cholera served as controls. The marker perfusion technique employed was shown, in preliminary experiments, to measure accurately jejunal and ileal fluid and electrolyte transmucosal transport rates under conditions of cholera diarrhea. Fluid loss into the lumen occurred from jejunal and ileal mucosa. The fluid was isotonic in both regions. Bicarbonate concentration was significantly higher in ileal than jejunal fluid during all phases of the disease. Bicarbonate concentration in both regions was significantly higher in acute cholera than during convalescence. Fluid loss into the intestinal lumen ranged from 0.07 to 10.9 ml/hr per cm. Losses were significantly greater from jejunum than ileum. Net ileal absorption was recorded in five of 10 acute cholera studies. During the acute phase of the disease, net jejunal fluid transport showed a positive correlation with fasting intestinal flow rate and stool output. Stool output was also positively correlated with jejunal fasting intestinal flow rates. Recovery of normal fluid and electrolyte absorptive function was usually complete in both jejunum and ileum by the sixth day after admission.

These findings in human cholera validate the animal models of choleraic diarrhea and suggest that similar measurements of small intestinal secretory function in other nonspecific diarrheal diseases using the marker perfusion technique may be rewarding.

A preliminary report of this study has already been presented (1).

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## INTRODUCTION

The metabolic consequences of diarrhea in human cholera (2–5) are well known and many pathophysiological features of the disease can be reproduced in animal models (6–9). However, the source of this diarrheal fluid within the human intestinal tract is unknown, although Greenough (10) has previously demonstrated that a significant portion of the fluid loss in human cholera was generated above the upper portion of the jejunum.

The marker perfusion technique (11–14), recently utilized for studying absorption from the normal human intestine, has been used in this investigation to define the site, nature, magnitude, and duration of fluid and electrolyte loss in the small intestine during different phases of human cholera. This information is required for a better understanding of the pathogenesis of cholera diarrhea and to validate the animal models of this disease.

## METHODS

The study was performed between March 1967 and July 1968 at the Infectious Disease Hospital, Calcutta, India and included 27 male Indian patients aged 16–66 (mean 35 yr) with a history of the acute onset of severe watery diarrhea within 24 hr of admission. The patients were weighed and placed on metabolic beds which allowed accurate and separate collection of urine and stool. A standard clinical and laboratory evaluation was performed on each patient as previously described by this unit (15).

The severity of the clinical state on admission and the magnitude and duration of the subsequent diarrhea ranged from mild to severe (Table I). Dehydration, hypotension, and metabolic acidosis were present in most patients. Diarrhea continued for up to 6 days with a mean duration of 3.8 days.

TABLE I

*Summary of Admission Clinical and Laboratory Data for 27 Patients with Acute Cholera*

| | Mean | Range |
|---|---|---|
| Age, *yr* | 35 | (16–66) |
| Duration of diarrhea before entry, *hr* | 8 | (1.5–18) |
| Weight, *kg* | 41 | (33.8–46.4) |
| Systolic blood pressure,* *mm Hg* | 54 | (0–100) |
| Pulse,* *min* | 125 | (0–180) |
| Respiration, *min* | 34 | (5–51) |
| Arterial pH | 7.19 | (6.93–7.52) |
| Plasma specific gravity | 1.039 | (1.027–1.049) |
| Serum osmolality, *mOsm* | 276 | (250–288) |
| Serum Na, *mmoles/liter* | 141 | (136.0–156.0) |
| Serum K, *mmoles/liter* | 4.6 | (3.4–6.9) |
| Serum Cl, *mmoles/liter* | 117 | (91–129) |
| Serum $HCO_3$, *mmoles/liter* | 9.0 | (5.4–15.3) |

* Groups contain two patients in whom blood pressure and heart rate were unobtainable and given a value of zero.

Intravenous fluid replacement was started promptly on admission with a saline-lactate solution containing Na—154, Cl—103, lactate—51, mmoles/liter respectively, and KCl—20 mmoles/liter added after the 3rd liter. Complete rehydration, as judged by normal blood pressure, a full radial pulse at a rate of less than 90/min, normal skin turgor, and normal plasma specific gravity (1.024–1.027) was achieved 1–2 hr after admission together with the reappearance later of an adequate urine flow. After this, stool loss was measured and replaced on a volume for volume basis; rehydration was monitored by frequent plasma specific gravity determinations. No chemotherapy was given to 24 patients during the course of their illness. Three others received tetracycline (500 mg every 6 hr for 48 hr) after the first perfusion study, 24–36 hr after admission.

Stool volume was measured throughout the diarrheal period. The mean stool output before (10–14 hr), during (5–6 hr), and 10–14 hr after the perfusion study were calculated and expressed as milliliters per hour. Stool output during perfusion was derived by substracting the volume of perfused solution from the measured stool output during the 5–6 hr period.

Liquid stool was obtained on admission and rectal swabs daily thereafter until discharge for bacteriological culture as previously described (15).

25 patients had infection with *Vibrio cholerae* (Ogawa), biotype *eltor*. Two others had an Inaba serotype (biotype *eltor*). These findings are similar to the pattern of cholera in this locality for the past 4 yr.

11 Indian male subjects (aged 16–60 yr) served as controls. All were asymptomatic and denied gastrointestinal symptoms. None had steatorrhoea although three had low *d*-xylose excretion and four had mild, nonspecific changes on jejunal biopsy. These features are frequently encountered in the normal population from this tropical region (16).

## Plan of study

The aim of the study was to measure (*a*) the electrolyte composition of intestinal fluid during acute cholera, (*b*) the fasting intestinal flow rate (FIFR), and (*c*) net transmucosal movement of fluid and electrolytes in jejunum and ileum at different stages of cholera. The data were compared with values from control subjects.

Approximately 4–12 hr after admission, when nausea and vomiting had subsided and acidosis had been corrected, patients swallowed a multilumen polyvinyl[1] perfusion tube similar to that employed by Whalen, Harris, Geenen, and Soergel (13). It consisted of a proximal (P) and distal (D) aspiration port, 15 and 45 cm distal to the infusion port (I) respectively. The segment of intestine between I and P was the "mixing" segment and between P and D the "study" segment. A balloon containing 2 ml of metallic mercury, which could be inflated with an additional 8 ml of water to aid small intestinal transit, was attached by a fourth tube 20 cm distal to D. Water and mercury were withdrawn by siphonage to collapse the balloon before perfusion studies were performed. The internal volume of each polyvinyl tube between aspiration port and attachment of the sampling syringe was 7.5 ml.

The infusion port (I) was positioned fluorscopically at the ligament of Treitz, 90–100 cm from the incisor teeth for the acute jejunal study.[2] In 10 patients, the balloon was reinflated with mercury and water and the tube was allowed to reach the ileum for a repeat study (230–270 cm from the incisor teeth). All subjects were fasting for at least 8 hr before commencing the studies.

The following series of studies were performed. (*a*) *Acute studies* were performed (day 1–4). The initial acute jejunal or ileal studies were arbitrarily defined as those performed within 48 hr of admission and additional repeat acute studies were obtained later during the phase of diarrhea (day 2–4) either by withdrawing the tube from the ileum manually by applying gentle traction for 20–40 min to relocate the perfusion system in the jejunum or, alternatively, by allowing the tube to advance with balloon inflated from jejunum to ileum .After deflating the balloon, a 60 min equilibration period was allowed in all studies before sampling intestinal fluid. (*b*) *Early convalescent studies* (day 4–14), 15 jejunal and four ileal studies, were performed on 16 patients. In all but four cases who were perfused during day 4–6 diarrhea had ceased at the time the early convalescent study was performed. (*c*) *Late convalescent studies* (> 21 days after admission) were also performed. Full recovery had occurred in all cases at the time of the late convalescent study. The multilumen perfusion tube was reintroduced orally for all early and late convalescent studies.

## Perfusion technique

The marker perfusion technique has been shown to have precision and accuracy with mean errors that are close to zero in normal human studies (12, 13). However, in severe cholera diarrhea errors may be introduced into the method by a variety of factors arising from fluid secretion. A series of studies were carried out on acute cholera patients to define these conditions in more detail and to investigate whether they altered the precision and reliability of the method. These results are recorded in an Appendix.

*Intestinal fluid.* Samples of fasting intestinal fluid were obtained by slow aspiration from D after the "steady-state" equilibration period and before measurement of FIFR or fluid and electrolyte transport rates.

*Fasting intestinal flow rates (FIFR).* FIFR were measured in the jejunum immediately before a perfusion study

[1] Kindly supplied by the Pharmaseal Laboratories, Glendale, Calif.

[2] A variable time (8–53 hr) elapsed before the tube reached the study position due to differences in the rate of passage of the tube.

using a constant slow infusion (0.5–1.0 ml–min) of the isotonic perfusion solution containing $Co^{58}$–Vitamin $B_{12}$ (5 $\mu$Ci/liter) as the poorly absorbable marker. The infusion solution entered the bowel lumen at I; two successive 15 min samples were obtained from D after a 45 min equilibration period by steady siphonage with a syringe (0.5 ml/min). The FIFR was calculated from the infusion rate (ml/min) multiplied by the ratio of $Co^{58}$–Vitamin $B_{12}$ concentration in the infusion solution to the mean concentration in the two aspirated samples. Sulfobromophthalein (BSP) was used as the marker in seven measurements of FIFR in the ileum.

*Measurement of net transmucosal fluid and transport rates.* Isotonic perfusion solutions (jejunum; Na—145 mmoles, K—5 mmoles, Cl—140 mmoles, $HCO_3$—10 mmoles, and ileum; Na—140 mmoles, K—10 mmoles, Cl—120 mmoles, and $HCO_3$—30 mmoles–liter) were delivered at a constant rate of 9.2 ±0.9 ml/min (mean ±SD) for acute studies and 10.1 ±0.3 ml/min for convalescent studies, the pump rate being calibrated immediately before each study. BSP (5–15 mg/100 ml) was used as the poorly absorbable marker. For each study a 60 min infusion equilibration period and a 20 min period for tube clearance by aspiration preceded the study period of 120 min. Intestinal contents were sampled from P and D by syringe at a steady rate of 0.5 ml/min, and four 30-min pooled collections of 15 ml each were made and collected on ice. Samples were stored at 4°C and centrifuged before analysis. Total $CO_2$ content[3] was measured by a volumetric Van Slyke technique with an error of 1% on duplicate analysis. BSP was determined by the method of Seligson and Marino with an error of 1.5 ±2% on duplicate analysis (17). Sodium and potassium were measured in duplicate by flame photometer with internal lithium standard and chloride with a Buchler-Cotlove chloridometer.[4]

## Calculations

Calculations of net transmucosal transport rates for water, sodium, potassium, chloride, and bicarbonate were performed with an IBM computer using the following formulae:

$$Fe = I \times \frac{[BSP]_I}{[BSP]P} - 0.5$$

$$Fl = Fe \times \frac{[BSP]P}{[BSP]D}$$

$$\Delta H_2O = \frac{Fl - Fe}{30} \times 60$$

and

$$\Delta Na = \frac{[Fl\ Na]D - [Fe\ Na]P}{30} \times 60 \times 1000$$

Fe = flow rate entering study segment (ml/min), Fl = flow rate leaving study segment (ml/min), I = infusion rate (ml/min), $BSP_{I,P,D}$ = BSP concentration in the infusion solution and at P and D respectively (mg/100 ml), and $\Delta H_2O$ = net transmucosal fluid transport rate (ml/hr per cm). Positive values indicate net secretion and negative values, net absorption of fluid. "Secretion" means net gain of fluid within the intestinal lumen; its use provides no indication whether this is an active or passive process. $\Delta$Na, K, Cl, and $HCO_3$ = sodium, potassium, chloride, and bicarbonate net transmucosal transport rates ($\mu$moles/hr per cm).

[3] Total $CO_2$ was determined but as the pH of the luminal fluid was 7.0 over 97% of $CO_2$ was in the form of $HCO_3$. The term "bicarbonate" has been used in this text although "total $CO_2$" may be more precise.

[4] Buchler Instruments, Inc., Fort Lee, N. J.

# RESULTS

## A. Intestinal fluid composition

All fluid had an osmolality close to isotonicity with plasma (Table II). Sodium and potassium content of intestinal fluid was similar in all phases of the disease and in control subjects. Bicarbonate concentration in ileal fluid was significantly higher than jejunal fluid in both acute and convalescent stages. Furthermore, jejunal and ileal bicarbonate concentration were greater in the acute period than in recovery ($P < 0.005$) (Table II). Potassium, sodium, and chloride concentrations were similar in both areas of small bowel. Acute cholera stool had increased potassium and decreased sodium concentrations.

## B. Acute studies

*Jejunum.* Acute jejunal perfusions were performed on 26 of the 27 patients (Table III). Net fluid secretion into the lumen occurred in 21 patients (0.07–10.88 ml/hr per cm) with corresponding movement into the lumen of Na (5–1441 $\mu$moles/hr per cm), K (−3–73 $\mu$moles/hr per cm), Cl (25–1335 $\mu$moles/hr per cm), and $HCO_3$ (10–125 $\mu$moles/hr per cm). Mean stool rates varied from 42–886 ml/hr for the 10–14 hr period preceding the perfusion. The mean FIFR was 6.2 ±3.2 ml/min for 19 acute measurements. In four cases diarrhea had ceased and in one other stool output was small when the initial acute perfusion study was performed (case 22, 94 ml/hr). In these five patients net fluid and electrolyte absorption was demonstrated.

*Ileum.* Five of 10 acute subjects had net fluid movement into the lumen which was accompanied by diarrhea (140–606 ml/hr, mean 318 ml/hr), (Table IV). However, five others had net water absorption in the ileum at this early stage of their disease in the presence of diarrhea which continued even after the perfusion study had been completed at a rate similar to that of the patients who were secreting in the ileum. (Rate before perfusion study, 167–383 ml/hr, mean 284 ml/hr. Rate after perfusion study, 38–833 ml/hr, mean 334 ml/hr). The net movement of bicarbonate was into lumen in all instances whereas net movements of other electrolytes were in the direction of net water movement. The mean FIFR measured in four studies was 8.19 ml/min.

*Correlation of ileal and jejunal studies (Fig. 1).* In nine patients both jejunal and ileal studies were obtained during the acute phase. The jejunum was studied first in six of the patients, the ileal study being performed 6–12 hr later. In three other patients studies were performed first in ileum and in the jejunum 6–14 hr later.

Jejunal net fluid secretion in these nine patients (+3.4 ±2.3 ml/hr per cm) significantly exceeded ileal output (+0.8 ±2.1 ml/hr per cm) ($P = < 0.02$). In five studies net ileal fluid absorption was already occurring although during the same period the jejunum was secreting. Patient 2 provides an example of this situation (Fig. 2). There was evidence of persistent diarrhea and jejunal fluid secretion 2 days after net ileal absorption had been demonstrated by two consecutive perfusion studies.

*Correlation between jejunal fluid transport, stool output, and FIFR (Fig. 3).* There was a significant correlation betwen stool output for 10–14 hr before the jejunal perfusion and $H_2O$ flux (Fig. 3 *a*), stool output 10–14 hr before the perfusion study and FIFR (Fig. 3 *b*), and $H_2O$ flux and FIFR (Fig. 3 *c*) in acute cholera patients. By extrapolation, at zero stool output the net $H_2O$ absorption of approximately −2.6 ml/hr per cm was of similar magnitude to values of $H_2O$ measured by the perfusion technique in convalescent patients (−1.53 ±0.88 ml/hr per cm). The measured stool output during the perfusion study and after the study had somewhat less correlation with $H_2O$ ($r = +0.73$ and +0.68 respectively). At zero net fluid transport in the jejunum, FIFR was still increased at 4.3 ml/min above normal control value and stool output was 160 ml/hr which most probably represented the result of fluid secreted into the bowel lumen which occurred in the duodenum above the site of jejunal perfusion.

*Rate of recovery of net $H_2O$ absorption (Fig. 4).* Sequential jejunal studies were performed in nine patients from the acute study through convalescence (Fig. 4 *a*). Return to net fluid absorption from an initial secretory state occurred within 6 days in all cases. In addition, all patients restudied in early and late convalescence demonstrated a return to net fluid absorption.

In four of nine patients the second measurement of fluid transport during the acute phase of cholera showed a greater secretion into the lumen than during the initial study even though all subsequently returned to a net

TABLE II

*Electrolyte Composition of Intestinal Fluid in Acute and Convalescent Cholera Patients and Control Subjects**

| Intestinal fluid | Electrolytes | | | | Osmolality |
|---|---|---|---|---|---|
| | Na | K | Cl | $HCO_3$ | |
| | *mmoles/liter* | *mmoles/liter* | *mmoles/liter* | *mmoles/liter* | *mOsm/liter* |
| Acute: jejunum (n = 13) | 147 ±6 | 5.6 ±0.7 | 138 ±5 | 15 ±4 | 292 ±8 |
| Acute: ileum (n = 9) | 140 ±4 | 5.7 ±0.9 | 122 ±8 | 41 ±5‡ | 290 ±12 |
| Acute:§ stool (n = 10) | 133 ±21 | 20.1 ±7.7 | 100 ±7 | 41 ±9 | 301 ±9 |
| Convalescent:§ stool at 30 hr (n = 10) | 132 ±16 | 8.1 ±4.7 | 79 ±13 | 73 ±15 | 288 ±6 |
| Convalescent: jejunum (n = 6) | 142 ±7 | 5.7 ±1.1 | 129 ±15 | 5 ±6‖ | 294 ±10 |
| Convalescent: ileum (n = 10) | 143 ±7 | 6.2 ±2.0 | 131 ±10 | 26 ±12§‖ | 289 ±11 |
| Control subjects: jejunum (n = 7) | 142 ±7 | 4.8 ±.5 | 135 ±8 | 8.2 ±5 | 285 ±10 |
| Control subjects: ileum (n = 9) | 140 ±6 | 4.9 ±1.5 | 125 ±12 | 30 ±11 | 292 ±12 |

* Mean values ±SD.

‡ Significantly different from corresponding jejunal study ($P = < 0.01$).

§ Data from Pierce et al. (26).

‖ Significantly different from corresponding region during acute study ($P = < 0.005$).

TABLE III

*Fasting Intestinal Flow Rates, Stool Output, and Net Jejunal Fluid and Electrolyte Transport Rates for Acute Cholera Studies*

| Patient | FIFR | Stool output* | Net transmucosal transport rate, jejunum | | | | |
|---|---|---|---|---|---|---|---|
| | | | $H_2O$ | Na | K | Cl | $HCO_3$ |
| | *ml/min* | *ml/hr* | *ml/hr per cm* | *μmoles/hr per cm* | *μmoles/hr per cm* | *μmoles/hr per cm* | *μmoles/hr per cm* |
| 1 | 4.6 | 184 | +0.36 | +155 | +4 | +150 | +23 |
| 2 | 4.6 | 314 | +0.61 | +182 | +5 | +143 | +59 |
| 3 | 10.1 | 273 | +1.59 | +326 | +6 | +268 | +2 |
| 4 | 9.3 | 182 | +1.77 | +293 | +3 | +282 | +35 |
| 5 | 4.6 | 260 | +3.06 | +523 | +17 | +472 | +5 |
| 6 | 6.6 | 316 | +0.48 | +70 | −3 | +78 | +2 |
| 7 | 8.5 | 267 | +2.10 | +444 | +3 | +371 | +46 |
| 8 | 6.7 | 175 | +0.24 | +94 | +10 | +49 | +32 |
| 9 | 10.5 | 438 | +0.38 | +106 | −5 | +118 | +75 |
| 10 | 11.6 | 677 | +9.92 | +1441 | +73 | +1335 | +115 |
| 11 | 3.3 | 0 | −2.05 | −276 | −12 | −134 | −59 |
| 12 | 2.6 | 0 | −0.95 | — | — | — | — |
| 13 | — | 218 | +1.81 | +346 | +4 | +252 | +14 |
| 14 | — | 166 | +1.19 | +263 | +4 | +256 | +12 |
| 15 | — | 886 | +10.88 | +1267 | +44 | +1321 | +57 |
| 16 | — | 246 | +1.91 | +355 | +11 | +336 | +52 |
| 17 | — | 326 | +1.70 | +262 | +8 | +282 | +8 |
| 18 | — | 616 | +4.25 | +630 | +21 | +619 | +125 |
| 19 | — | 413 | +4.03 | +600 | +23 | +579 | +74 |
| 20 | — | 288 | +3.93 | +740 | +14 | +612 | +85 |
| 21 | 3.2 | 0 | −1.12 | — | — | — | — |
| 22 | 2.7 | 94 | −0.82 | −124 | −6 | −145 | −18 |
| 23 | — | 375 | +1.12 | +239 | +6 | +244 | +9 |
| 24 | — | 104 | +0.01 | +5 | −1 | +25 | −10 |
| 25 | — | 0 | −0.34 | −56 | −4 | −33 | 0 |
| 26 | — | 520 | +3.31 | +720 | +21 | +495 | +164 |
| 27 | — | 383 | Ileal studies performed | | | | |
| Mean ±SD | 6.2 ±3.2‡ | 286 ±214 | 1.90 ±3.00 | 344 ±399 | 10 ±18 | 332 ±374 | 39 ±49 |

+ = net secretion into intestinal lumen; − = net absorption from lumen.

* Measured stool output 10–14 hr before initial acute perfusion studies.

‡ Mean of 19 values. Includes five additional measurements obtained before repeat acute jejunal perfusion studies during days 2–4 in acute cholera diarrhea.

absorptive state. Three sequential ileal studies on patients with initial net fluid secretion also showed a return towards net fluid absorption by the early convalescent period (Fig. 4 *b*).

Net fluid absorption was present in the jejunum and ileum in late convalescence and in all control subjects. The mean net transport rates for fluid and Na, K, Cl, and $HCO_3$ in the jejunum and ileum at all stages of the disease are shown in Table V. The calculated mean cation concentration ($Na^+ + K^+$) in transported fluid was 147 ±31 mmoles/ml.

*Correlation of bacteriological status with jejunal fluid transport rates.* In general, stool cultures were positive for *V. cholerae* throughout the period when net fluid movement was into the lumen. In three mild cases (no. 21, 22, and 25) stool cultures were positive on admission although net water absorption was occurring.

## DISCUSSION

In human cholera, fluid secretion into the intestine originates from the jejunum and ileum and most probably from the duodenum as well. These studies confirm the previous observations of Greenough (10) in man and more recent measurements in the dog (18, 19) and rabbit (20, 21) which have demonstrated that fluid production by the small intestine follows exposure to the live vibrio or its metabolic products. Furthermore, linear correlations between jejunal net water flux,

TABLE IV

*Fasting Intestinal Flow Rates, Stool Output, and Net Ileal Fluid and Electrolyte Transport Rates for Acute Cholera Studies*

| Patient | FIFR | Stool output* | Net transmucosal transport rate, ileum | | | | |
|---|---|---|---|---|---|---|---|
| | | | $H_2O$ | Na | K | Cl | $HCO_3$ |
| | *ml/min* | *ml/hr* | *ml/hr per cm* | *μmoles/hr per cm* | *μmoles/hr per cm* | *μmoles/hr per cm* | *μmoles/hr per cm* |
| 1 | 3.8 | 167 (38) | −0.09 | −41 | −3 | −140 | +126 |
| 2 | 13.5 | 269 (252) | −2.65 | −378 | −14 | −506 | +53 |
| 3 | 9.0 | 273 (333) | −1.31 | −306 | −31 | −419 | +93 |
| 4 | 9.4 | 214 (400) | +2.11 | +406 | +17 | +274 | +163 |
| 6 | — | 179 (226) | −0.73 | −92 | −8 | −196 | +114 |
| 8 | — | 383 (200) | −0.50 | −62 | −6 | −84 | +40 |
| 15 | — | 140 (527) | +3.52 | +1069 | +22 | +491 | +458 |
| 16 | — | 246 (544) | +2.58 | +522 | +29 | +497 | +71 |
| 18 | — | 606 (833) | +5.25 | +708 | +41 | +517 | +227 |
| 27 | — | 383 (383) | +4.37 | +821 | +9 | +695 | +148 |
| Mean ±SD | +8.9 ±4.0 | +286 ±140 374 ±211 | +1.26 ±516 | +247 ±516 | +6 ±22 | +113 ±432 | +149 ±122 |

+ = net secretion into intestinal lumen; − = net absorption from lumen.

* Measured stool output 10–14 hr before and after (in parentheses), the initial acute perfusion studies.

FIFR, and stool output in human cholera provide evidence that small intestinal fluid secretion can account for the major portion of the diarrheal fluid and the clinical and biochemical features of this disorder. These findings do not entirely exclude the possibility of gastric, biliary, pancreatic, or colonic contributions to the diarrheal fluid. However, these regions are already known to make only a small contribution to fluid loss in the dog (19) and rabbit (20).

In the acute disease jejunal fluid production was greater and usually of longer duration than that of the ileum. The loss in secretory response of the ileum might be attributed partly to the spontaneous improvement which occurred with time. However, the mean stool output was similar for patients with and without net ileal fluid secretion which suggested that the fluid output was primarily derived from the jejunum. This impression was supported by the observation that two of

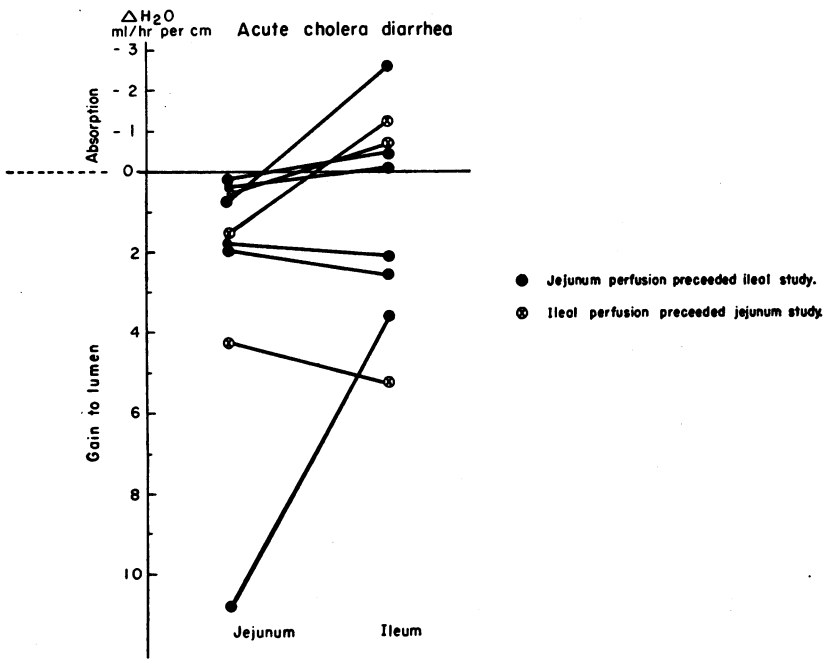


FIGURE 1 Net fluid transport rates for jejunum and ileum in the same nine patients with acute cholera diarrhea. Jejunal net fluid secretion significantly exceeded ileal secretion ($P = < 0.02$).

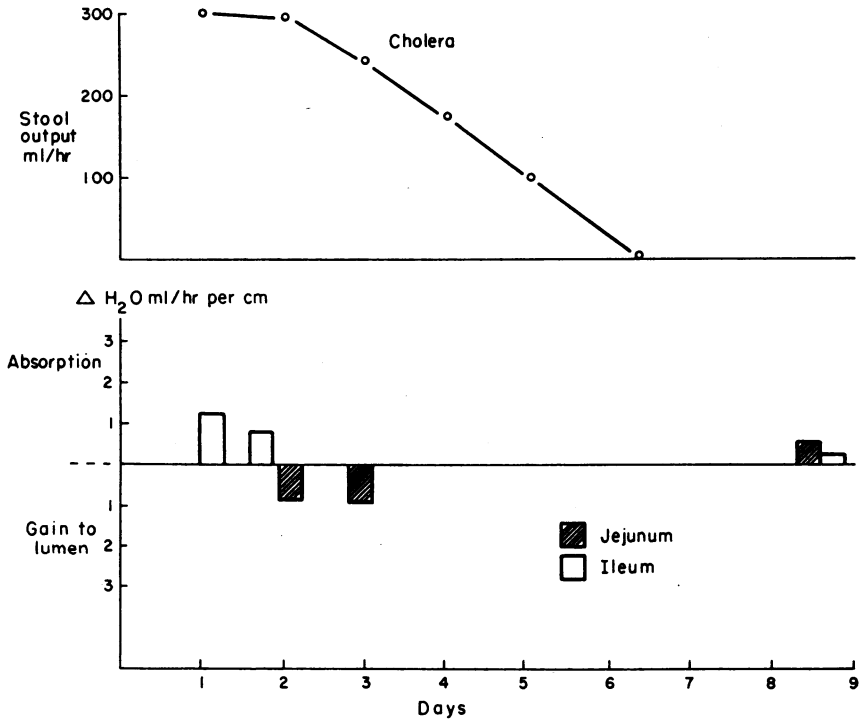


FIGURE 2 Case 2. Net fluid absorption was occurring in the ileum on the first day after admission although stool output and jejunal fluid secretion were recorded on day 2 and 3 while diarrhea continued.

three ileal perfusions performed before the jejunal study showed less secretion than the corresponding jejunal region. Carpenter, Sack, Feeley, and Steenberg (18) have reported similar observations in dogs exposed to cholera toxin. They have suggested that the jejunum and ileum may secrete fluid at equal rates in response to this toxin but the ileum may have the greater net absorptive capacity. While this occurs in the dog, human studies in convalescent patients and normal subjects have shown that fluid absorption in the jejunum usually equaled or exceeded that in the ileum. An explanation for this difference between the human jejunum and ileum exposed to cholera must await further studies.

Intestinal fluid was approximately isotonic with plasma suggesting that the small intestine in acute cholera retains its normal ability to elaborate isotonic fluid. However, the bicarbonate concentration was significantly higher in both jejunal and ileal fluid in the acute disease than in convalescence. These findings cannot be explained by chance variation in the entry of gastric, biliary, or pancreatic fluid into the upper intestine or by variable placement of the tube in the ileum. Similar observations on duodenal fluid have been made previously by Beisel, Watten, Blackwell, Benyajati, and Phillips (22) and Greenough (10). Leitch and Burrows (20) demonstrated a rise in amylase in rabbit duodenal loops exposed to cholera which paralleled the accumulation of bicarbonate. This could be attributed to a secretin-stimulated release of pancreatic fluid but it is unlikely to be the whole explanation for the increased bicarbonate concentration found in man. Elevated bicarbonate might result from an increased anionic transport into the lumen during the acute disease. Field, Fromm, Wallace, and Greenough (23) have shown that cholera exotoxin could stimulate an active anionic pump in isolated rabbit ileum which resulted in chloride secretion. There is also recent evidence that normal jejunal bicarbonate absorption in man depends on active hydrogen ion movement into the lumen (24). A reduction in $H^+$ ion secretion might also explain the high bicarbonate concentration found in acute cholera fluid. However, further clarification of this observation must await a detailed analysis of the intestinal fluid composition in the animal model with acute and convalescent cholera.

All patients in the study had positive stool cultures for *V. cholerae* associated with a history of diarrhea. In three patients, however, diarrhea ceased after admission and subsequent measurement of jejunal fluid transport indicated that net absorption was already occurring in the jejunum. This emphasizes that the relationship of the vibrio to fluid production is complex and that there is no clear correlation between the con-

centration of vibrios in the feces or small bowel and the severity of the disease. The main events associated with colonization by the vibrio and fluid secretion probably occurred several hours before study and even before admission to the hospital. Furthermore, vibrio quantitative counts performed in our laboratory in small intestinal fluid obtained during acute human cholera bore no relationship to the severity of the disease, the duration of diarrhea, or the magnitude of net fluid flux in the area of small bowel from which the samples were obtained (25).

On the second and third day after admission, fluid secretion was greater in three patients than on the first day. It is possible that intestinal fluid production may have been transiently depressed after admission, after the initial period of hypotension, metabolic acidosis, hemoconcentration, and tissue hypoxia. However, Carpenter, Greenough, and Sack (27) have shown in acute experi-

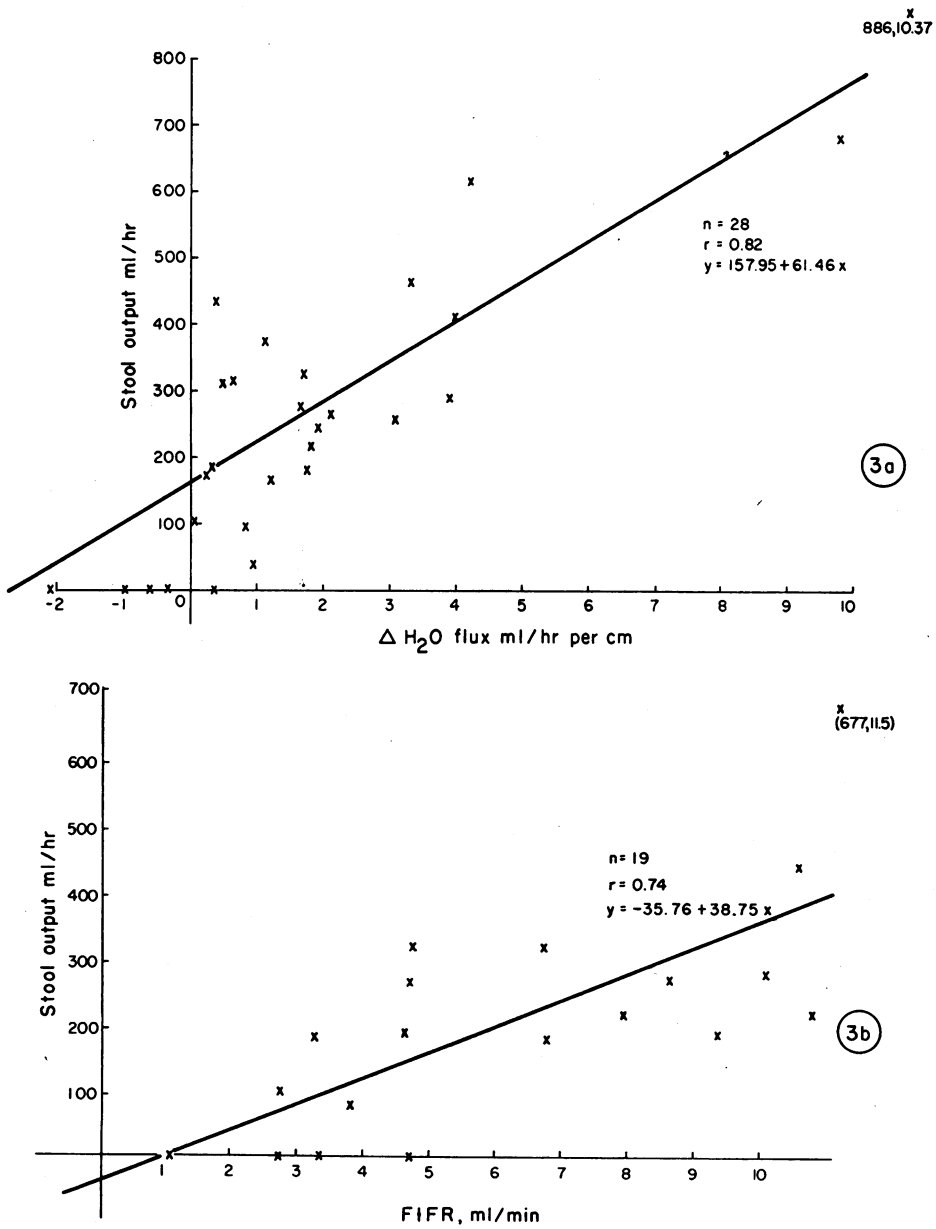


FIGURE 3 Correlation between FIFR, stool output, and net fluid transport rates in the jejunum in acute cholera. 3*a* Stool output and net fluid transport rate ($\Delta H_2O$) ($P = < 0.001$). 3*b* Stool output and FIFR ($P = < 0.01$). 3*c* $\Delta H_2O$ and FIFR ($P = < 0.05$).

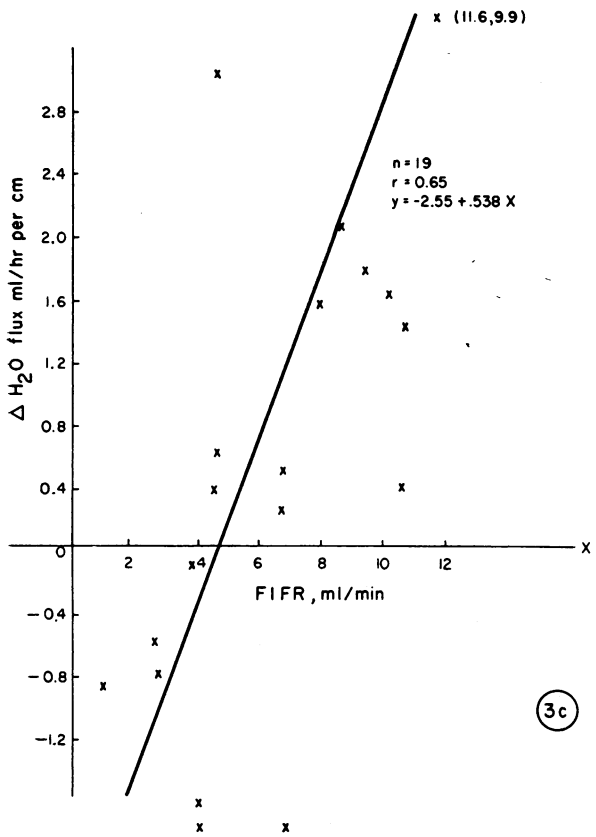


FIGURE 3 (*Continued*)

ments that intestinal fluid production did not diminish even after reduction of superior mesenteric arterial blood flow (SMA) to less than 30% of base line in the cholera-infected dog. Alternatively, the augmented response on the second and third day may result from the unknown factors controlling toxin production by the vibrio (28, 29).

All patients had a return to net fluid absorption in the jejunum by the sixth day after adminission. The recovery time for jejunal net fluid absorption was generally similar to the clinical disease although in at least two patients jejunal secretion could still be demonstrated after diarrhea had ceased.

Net jejunal fluid absorption was higher in late convalescent patients than in our control subjects ($P = <$ 0.05) but similar to the normal subjects of Fordtran, Levitan, Bikerman, and Burrows (11) and Whalen, Harris, Geenen, and Soergel (13). This may reflect a biased choice of "normal" subjects. Control subjects in Calcutta frequently have defects in *d*-xylose absorption and mucosal morphology (as our subjects did) although these same features might be expected to have occurred in cholera subjects from the same community as well. However, it is evident from the studies that acute human cholera infection resulted in no permanent change in the direction of net intestinal fluid and electrolyte transport by the small intestine.

Cholera is only one of many causes of acute diarrhea which occur throughout the world. The fluid loss in other types of diarrheal disorders may be produced by secretory processes similar to those in cholera. In this respect, the study of cholera, both in man and the animal models, may help in understanding the diarrhea associated with *Escherichia coli* (30), staphylococci (31), or other toxin-producing microorganisms.

TABLE V

*Mean Values for Stool Output, FIFR, and Net Fluid and Electrolyte Transport Rates in Cholera Patients and Control Subjects**

| Small intestine: region of study | No. of studies | Stool output | FIFR | Net fluid and electrolyte transport rates | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | $H_2O$ | Na | K | Cl | $HCO_3$ |
| | | *ml/hr* | *ml/hr* | *ml/hr per cm* | *μmoles/hr per cm* | *μmoles/hr per cm* | *μmoles/hr per cm* | *μmoles/hr per cm* |
| Jejunum | | | | | | | | |
| Acute | 26 | 286 ±214 | 6.2 ±3.2 | +1.90 ±3.00 | +344 ±399 | +10 ±18 | +332 ±374 | +39 ±49 |
| Early convalescence | 15 | — | 3.6 ±1.3 | −0.94 ±1.22 | −128 ±173 | −7 ±9 | −105 ±150 | −26 ±48 |
| Late convalescence | 5 | — | 2.3 ±1.1 | −1.53 ±0.88 | −219 ±120 | −7 ±3 | −171 ±99 | −41 ±27 |
| Control subjects | 7 | — | 2.2 ±1.3‡ | −0.58 ±0.48 | −68 ±56 | −5 ±5 | −64 ±59 | −34 ±24 |
| Ileum | | | | | | | | |
| Acute | 10 | 286 ±140 | 8.9 ±3.7 | +1.26 ±2.27 | +247 ±516 | +6 ±22 | +113 ±432 | +149 ±122 |
| Early convalescence | 4 | — | — | +0.89 ±1.77 | +98 ±519 | +4 ±41 | −13 ±323 | +126 ±249 |
| Late convalescence | 5 | — | 0.8§ | −0.12 ±0.78 | −167 ±110 | −7 ±5 | −153 ±88 | −8 ±15 |
| Control subjects | 9 | — | 0.7‡ | −0.77 ±1.20 | −87 ±71 | −3 ±9 | −110 ±135 | +18 ±61 |

+ = net secretion into intestinal lumen; − = net absorption from lumen.
* Mean values ±SD.
‡ Data of Whalen, Harris, Geenen, and Soergel (13).
§ n = 2.

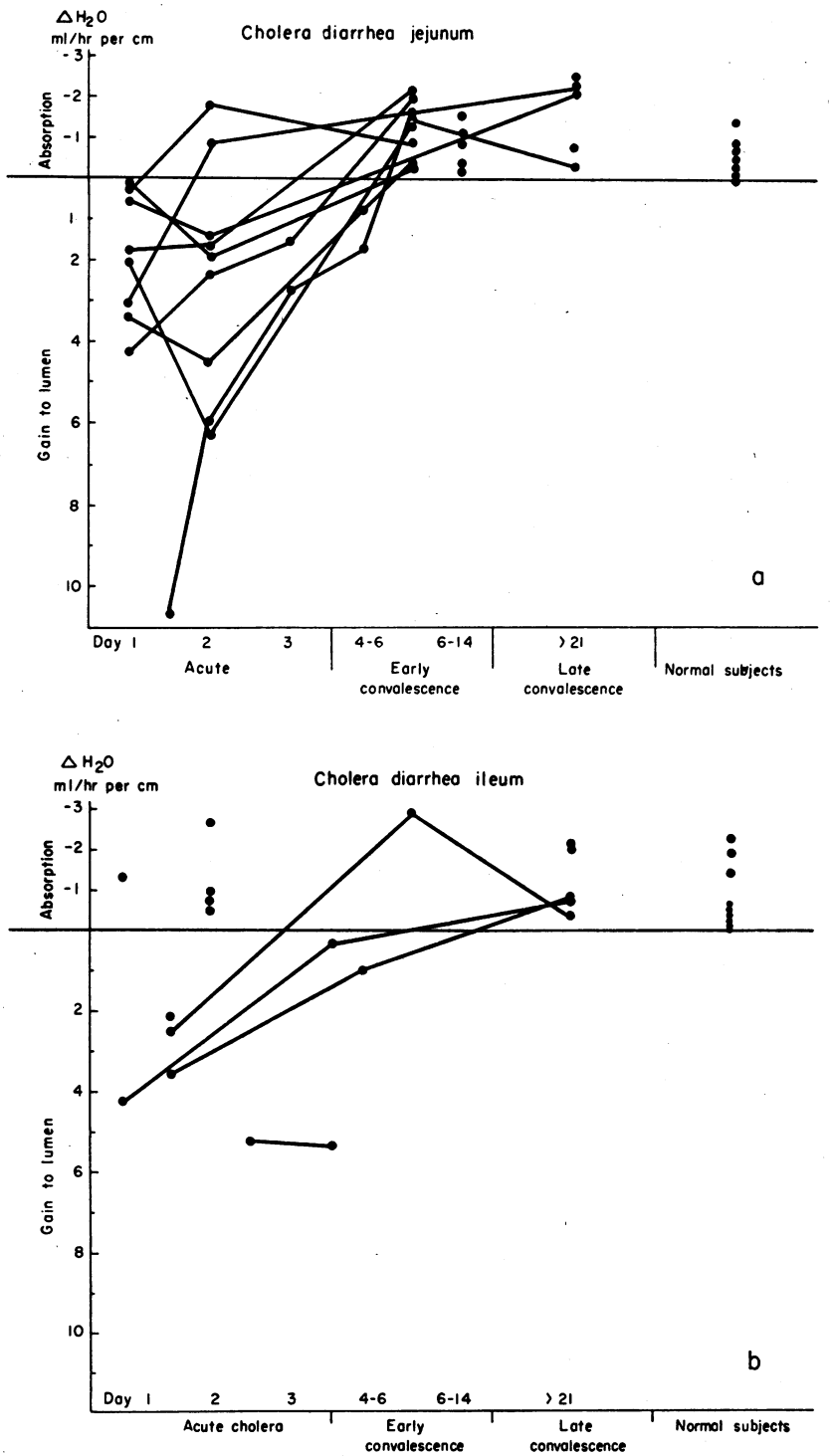


FIGURE 4 The rate of recovery of net fluid absorption in (*a*) the jejunum and (*b*) the ileum in cholera diarrhea.

## APPENDIX

*The accuracy of the marker perfusion technique for studying fluid and electrolyte transport in severe diarrheal disease.* 1. BSP (32, 10) and $Co^{58}$–Vitamin $B_{12}$ (33) were employed as poorly absorbable markers in all the perfusion studies. Although polyethylene glycol (PEG) is the most commonly employed marker in studies of this type, its accurate determination in intestinal fluid samples which contained abundant mucus proved difficult so that BSP was the preferred marker in all perfusion studies. The validity of these markers in perfusion studies of normal human subjects and animals has already been established. In order to confirm their reliability under conditions of acute diarrhea two studies were performed. (*a*) Six perfusions were performed in acute cholera using an isotonic perfusion solution incorporating both $Co^{58}$–Vitamin $B_{12}$ (5 $\mu$Ci/liter) and BSP (5–15 mg/100 ml) as markers. In the 48 30-min samples obtained from the study, there was a highly significant correlation ($r = 0.986$) in the concentration of the two markers at P and D. Both markers indicated essentially the same net fluid transport and the intercept of the regression line was indistinguishable from zero. (*b*) Eight additional studies were performed utilizing the rapid injection of a 1.0 ml bolus of concentrated BSP (50 mg/100 ml) together with $Co^{58}$–Vitamin $B_{12}$ (2–4 $\mu$Ci/ml) into a constant saline perfusion (10 ml/min) at P. By serial sampling at 2-min intervals from D, it was possible to measure the volume of the study segment by a dye dilution curve technique (34). No significant difference was present in the total area under the curve for the two markers (area BSP $= 162 \pm 33$; area $Co^{58}$–Vitamin $B_{12} = 159 \pm 26$, $n = 8$) under these conditions.

2. In order to ensure representative sampling of fluid passing down the study segment from P to D, the "staggering time" or delay required for sampling D after P was determined by calculation of the mean transit time in six patients with acute cholera, nine in early convalescence, and three control subjects. The same dye dilution curve technique as in 1 (*b*) was used. The mean transit time for the 30 cm jejunal segment was not significantly different in acute cholera (9.1 min $\pm$SD 1.6, $n = 10$) from that encountered in convalescent (9.2 min $\pm$SD 2.0, $n = 10$) and normal control subjects (9.6 min $\pm$SD 1.5, $n = 6$). On the basis of these findings, the same staggering time of 10 min was employed for all acute and convalescent jejunal studies. In the ileum mean transit time in the acute phase was 9.1 min $\pm$SD 1.5 and in convalescence 9.8 min $\pm$SD 2.1, ($n = 6$). Therefore, 10 min staggering time in the sampling of D after P was employed for all ileal studies.

3. The duration of equilibration employed at the onset of the perfusion study (60 min plus 20 min for tube clearance) was the same for all studies. Two lines of evidence suggest that reasonable equilibration of perfusion fluid with intestinal contents had been achieved within this period. (*a*) Observations in six acute cholera patients indicated that no detectable concentration of the $Co^{58}$–Vitamin $B_{12}$ marker contaminated the first 30 min sample of the perfusion study period when this marker had been used to measure FIFR immediately before the perfusion study. (*b*) Analysis for inequality of variances of the 24 jejunal and 10 ileal acute cholera studies in which fluid movement ($\Delta H_2O$) was calculated for each of the four 30-min periods indicated that variability in each collection period was similar and there was no significant difference in the variances at the 5% level ($F_{max} = 2.3$) (35).

4. The precision of this perfusion method was studied in a manner similar to that employed by Cooper, Levitan, Fordtran, and Ingelfinger (12). 11 patients with acute cholera were studied with a perfusion tube having an additional tube port (×) opening 50 cm proximal to 1. A constant perfusion through × of isotonic saline containing $Co^{58}$–Vitamin $B_{12}$ was started at a slow rate (0.5–1.0 ml/min) to label "contaminating" intestinal fluid entering the study segment from above. The equilibration phase of a standard perfusion study was commenced 30 min later and a standard perfusion study using BSP as the marker then performed. The marker concentration ratios for $Co^{58}$–Vitamin $B_{12}$: BSP in the eight 30-min samples obtained from P and D were calculated and a mean value obtained for each study. The per cent deviation of the mean value for each set of eight samples from the mean of the entire 11 studies was used to estimate precision. In these collections there was an average 11.8% deviation from the mean. This is comparable to a mean value of 13.1% obtained by Cooper, Levitan, Fordtran, and Ingelfinger (12) using 20-min collection periods and a large oral "contaminating" bolus and of 8.3% when "contamination" was induced by slow constant infusion of the second marker in a group of normal subjects.

5. The error arising from inadequate mixing in the mixing segment was estimated in nine acute cholera patients in whom FIFR had been measured immediately before the perfusion study. The data from each study was used to calculate the expected $Co^{58}$–Vitamin $B_{12}$ concentration at P, $[Co^{58}\ \text{Vit. } B_{12}]_{Pexp}$ if mixing were complete. $[Co^{58}\ \text{Vit. } B_{12}]_{Pexp} = [Co^{58}\ \text{Vit. } B_{12}]_D \times ([BSP]_P)/([BSP]_D)$ and to compare this value with the actual measured concentration $[Co^{58}\ \text{Vit. } B_{12}]_P$ at P. If incomplete mixing occurs, $[Co^{58}\ \text{Vit. } B_{12}]_P < [Co^{58}\ \text{Vit. } B_{12}]_{Pexp}$ as noted by Whalen, Harris, Geenen, and Soergel (13) and the mixing error is given a negative sign. The mixing error, expressed as a percentage of FIFR for the nine acute cholera studies, was $-1.3\% \pm$SD 18.01. The mean FIFR in all acute cholera studies was 6.2 ml/min in jejunum and 8.9 ml/min in the ileum. The effect of the calculated mixing error on net fluid movement can therefore be predicted to be $-0.1 \pm 1.4$ ml/hr per cm in jejunum and $-0.1 \pm 1.9$ ml/hr per cm in ileum. The mixing error can be assumed to be less during the recovery phase and during convalescent studies when the FIFR is reduced towards normal rates.

6. In order to be certain that no progressive change occurred during the study and that no one period was different from another, the differences between the average responses for each of the four 30-min collection periods were analyzed. The effects of inequality of variance and of correlation between errors in a two way system were measured (36). For this analysis $F = 1.12$ ($n = 34$) which was strong evidence that the expected values of the observations were equal during all four sampling periods.

The marker perfusion technique proved to be an accurate method for measuring intestinal fluid transport under conditions in which net fluid secretion was occurring. In acute cholera diarrhea, the precision of the method was similar to the studies of normal human subjects by Cooper, Levitan, Fordtran, and Ingelfinger (12) in which "contamination" of the upper small intestine was produced with an oral fluid load. The mixing error in the jejunum tended to slightly underestimate net fluid absorption and overestimate fluid gain to the lumen. However, the mean measured fluid loss for all acute jejunal studies ($+1.9 \pm 3.0$ ml/hr per cm) was significantly different from any possible effect of incomplete mixing with intestinal fluid ($P = < 0.001$). No

actual measurement was made of the mixing error in acute ileal studies; but since the mean FIFR in the seven ileal studies was similar to the jejunal FIFR, it is likely that the ileal mixing error would have been similar to that in the jejunum. The jejunal FIFR in convalescent cholera (2.26 ±1.1 ml/min) was similar to that determined by Whalen, Harris, Greenen, and Soergel (13) (2.16 ±1.32 ml/min) for normal human subjects. They found a mixing error of − 0.3 ±0.8 ml/hr per cm for jejunum and −0.1 ±0.3 ml/hr per cm in the ileum in 11 normal subjects. Although the mixing error was not specifically measured in our convalescent cholera or control subjects, a similar degree of accuracy to this would seem probable. The errors in measuring Na, K, Cl, and $HCO_3$ transport rates were of the same order of magnitude as those for water since the electrolyte concentrations of the perfusion solutions were similar to that of the "endogenous" intestinal fluid.

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