Colonic electrolyte transport in health and in congenital chloride diarrhea.

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Research Article

Congenital chloride diarrhea (CCD) is a rare autosomal recessive disorder, characterized by watery stools with C1- concentration around 150 meq/liter. We have perfused the colon of three patients and their three healthy siblings with different salt solutions containing 36C1- to determine the nature of the colonic defect in CCD. In the controls, net absorption of Na+ and C1- occurred against steep concentration gradients. The influx (lumen-to-plasms flux) of C1- was twice the effux. Omission of HCO3- from the perfusate caused a clear decrease in C1- efflux which suggests a coupling of C1- efflux to HCO3- influx. In CCD, net Na+ absorption occurred normally when HCO3- was present in the lumen. However, Na+ absorption was always impaired when the luminal contents were acid, a situation that prevails in CCD. Net K+ secretion was clearly increased. Both influx and efflux of C1- were practically absent. Only slight net secretion occurred along a steep gradient. Net appearance of HCO3- was not observed, in contrast to controls. These findings and earlier studies of ileal function in CCD are best explained by a defect in the C1-/HCO3- exchange mechanism, which operates in both directions in the normal ileum and colon.

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Colonic Electrolyte Transport in Health

and in Congenital Chloride Diarrhea

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A B S T R A C T Congenital chloride diarrhea (CCD) is a rare autosomal recessive disorder, characterized by watery stools with Cl^- concentration around 150 meq/liter. We have perfused the colon of three patients and their three healthy siblings with different salt solutions containing $^{36}Cl^-$ to determine the nature of the colonic defect in CCD.

In the controls, net absorption of Na^+ and Cl^- occurred against steep concentration gradients. The influx (lumen-to-plasma flux) of Cl^- was twice the efflux. Omission of HCO_3^- from the perfusate caused a clear decrease in Cl^- efflux, which suggests a coupling of Cl^- efflux to HCO_3^- influx.

In CCD, net Na^+ absorption occurred normally when HCO_3^- was present in the lumen. However, Na^+ absorption was always impaired when the luminal contents were acid, a situation that prevails in CCD. Net K^+ secretion was clearly increased. Both influx and efflux of Cl^- were practically absent. Only slight net secretion occurred along a steep gradient. Net appearance of HCO_3^- was not observed, in contrast to controls.

These findings and earlier studies of ileal function in CCD are best explained by a defect in the Cl^-/HCO_3^- exchange mechanism, which operates in both directions in the normal ileum and colon.

INTRODUCTION

Since Gamble, Fahey, Appleton, and MacLachlan and Darrow first described congenital chloride diarrhea (CCD)[1] in 1945, a total of 20 cases has accumulated in

[1] *Terms and abbreviations used in this paper:* Efflux, plasma-to-lumen flux; influx, lumen-to-plasma flux; CCD, congenital chloride diarrhea; ECF, extracellular fluid. PEG, polyethylene glycol.

the literature from outside Finland (1-10). In Finland we have observed 21 cases.[2] This autosomal recessive (4) disease is manifested by hydramnios, presumably due to intrauterine diarrhea and, from the first day of life, by large watery stools with a high Cl^- concentration (above 60 meq/liter in the newborn, and above 100 meq/liter later). These features constitute the diagnostic criteria of CCD. Solid stools and lower Cl^- concentrations have been observed only in chronic dehydration.[2] The diarrhea results in excessive neonatal loss of weight with dehydration and jaundice. Hyponatremia and hypochloridemia develop rapidly and, later, hypokalemia and metabolic alkalosis. Unless they receive proper treatment, most patients die in the first few weeks of life (4) from hypokalemia and dehydration, but some survive with chronic dehydration and variable general retardation. The appropriate treatment, lifelong oral replacement of the fecal loss of electrolytes and water, enables the children to develop normally.[2]

The nature of the basic intestinal defect is unclear. Launiala, Perheentupa, Pasternack, and Hallman showed that the defect must be in the distal ileum and the colon (11). Recently, three reports of ileal perfusion studies in single patients have appeared. Turnberg (12) found increased secretion of Cl^- and postulated that ileal Cl^-/HCO_3^- exchange (13) was working in reverse, i.e. secreting Cl^- and absorbing HCO_3^-. Bieberdorf, Gorden, and Fordtran (14) observed absence of active transfer of Cl^-; passive movement occurred according to the concentration gradient. Pearson et al. (7) reported that no Na^+ or Cl^- movement took place in the ileum. They also perfused the sigmoid, the only part of the colon remaining after operations done on their patient, and the same defect appeared to be present. Lauras et al. (10) introduced $^{82}Br^-$ into the rectum of two patients and found almost none of it in the serum, in contrast to controls. They concluded that absorption of Cl^- was decreased.

To elucidate the nature of the colonic defect in CCD and to add to our knowledge of normal transport, we have performed colonic perfusions with $^{36}Cl^-$ in three CCD patients and their three healthy siblings. In the controls Cl^- efflux was found to be dependent on luminal HCO_3^- concentration, suggesting a coupling between the efflux of Cl^- and influx of HCO_3^-. In CCD both influx and efflux of Cl^- were greatly reduced. Previous findings for the ileum (14) and the present ones for the colon in CCD are best explained by absence of active Cl^-/HCO_3^- exchange in the intestine in this disease.

METHODS

Patients. A. S. was 10.0 yr old. She was first seen by us at the age of 8 days; the diagnosis of CCD was made at 9 mo, and substitution therapy was instituted. During the 1st yr of life her fluid balance was unstable, with periods of alkalosis and once of anuria and hyperphosphatemia. In the last few years she has been doing well and is attending school normally, receiving 5 meq $Cl^-/kg \cdot day$ as a NaCl-KCl solution (Na/K = 5/1). This was continued during the study. Her height was 127.5 cm, weight 26 kg, serum Na^+ 140, K^+ 3.7, HCO_3^- 24, and Cl^- 96 meq/liter. Her stool volume was 1.1–1.5 liter/day, and the electrolyte concentrations were Na^+ 58–65, K^+ 33–44, and Cl^- 100–148 meq/liter.

R. Ko. was 9.5 yr old. She was first admitted to our hospital at the age of 4 days because of abdominal distention, and was found to have hypoelectrolytemia. She did well only when on electrolyte substitution therapy. The diagnosis of CCD was finally made at the age of 8 mo. She had since received a continuous KCl supplement and developed normally. Her present dose of KCl, 2 meq/kg·day, was continued during the period of this study. Her height was 144 cm and weight 33 kg, serum Na^+ concentration 142, K^+ 4.2, HCO_3^- 25, and Cl^- 102 meq/liter. Her stool volume was 0.7–1.1 liter/day and the electrolyte concentrations were Na^+ 37–71, K^+ 51–92, and Cl^- 129–156 meq/liter.

J-P. K. was a 6.5-yr-old boy, whose CCD was recognized at birth and substitution therapy started immediately. He had been doing perfectly well, receiving the same solution as A. S., at 3 meq $Cl^-/kg \cdot day$. His height was 114 cm and weight 25 kg, serum Na^+ 143, K^+ 4.0, HCO_3^- 24, and Cl^- 101 meq/liter. His stool volume was 1.0–1.2 liter/day, and the electrolyte concentrations were Na^+ 50–81, K^+ 14–38, and Cl^- 75–144 meq/liter.

Controls. Three healthy siblings of the patients, aged 6.8, 7.8, and 8.4 yr, were studied in the same way.

Perfusions. The procedure was explained in detail to the parents and the children before hospitalization, and all the children were in perfect electrolyte balance and cheerful. They were on a semisolid diet during the period of the study. The colons were perfused with a series of electrolyte solutions (called perfusates in the text). A two-lumen X-ray-positive rubber tube was inserted into the cecum via the nose. The perfusion site aimed at was just beyond the ileocaecal valve (Fig. 1). Its actual location was checked by fluoroscopy before and after each perfusion. The location varied between the cecum and the mid-part of the ascending colon.

Perfusions were started at 9 a.m., after a light breakfast, and emptying of the rectum in the controls with Microlax (Pharmacia Fine Chemicals, Uppsala, Sweden), a 5-ml enema that contains 90 mg of Na-citrate and 9 mg of Na-
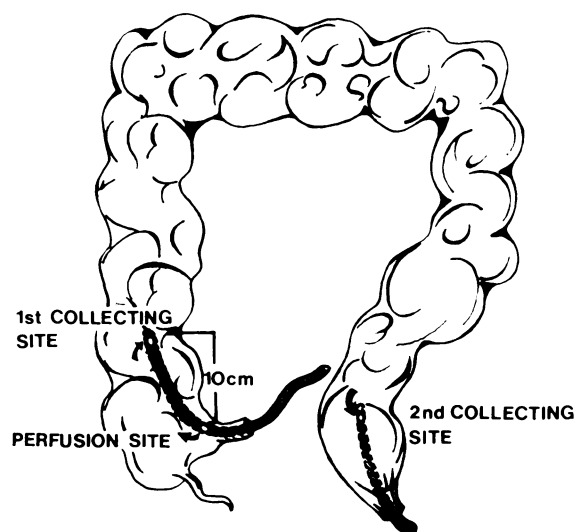

FIGURE 1   The perfusion system.

laurylsulfoacetate. Perfusions were done every 2nd day. Two solutions were used on the same day; the child had a 1-h rest and a light meal between the perfusions. The sequence of the perfusates was randomized. The perfusate was pumped at 7.5 ml/min. Collection was started after a steady flow from the rectum had been established. This rinsing period lasted about 30 min. The fluid entering the test segment proper was sampled at the first collecting site, 10 cm beyond the perfusion site, by continuous siphoning or gentle suction (approximately 0.3 ml/min) and the fluid leaving the segment was collected by drainage through a rectal tube (second collecting site). Thus the aim was to use the entire colon, save a 10-cm mixing segment, as a test segment. With every solution, collections were made in periods of 20 min for 2–3 h. The electrolyte composition of the perfusates used is given in Table I; mannitol was added when necessary to achieve isosmolality. All perfusates contained polyethylene glycol (PEG) 5 g/liter. The Cl^- perfusates contained 0.5 μCi/liter $^{36}Cl^-$ (The Radiochemical Centre, Amersham, England). The HCO_3^--containing perfusates were bubbled with O_2-CO_2 (95:5) for 30 min before and during the perfusions. All perfusates were kept in a water bath at 37°C.

Analytical methods. Electrolyte concentrations were measured by standard methods, PEG by a modification of the

TABLE I

Ion Composition of the Perfusates

Per-fusate	Na^+	K^+	Cl^-	HCO_3^-	Other
			meq/liter		
1	140	10	125	25	
2	140	10	60	25	Sulfate
3	140	10	0	25	Sulfate
4	140	10	125	0	Sulfate
5	0	0	120	0	Choline
6	0	0	0	0	0.3 M mannitol

ABSORPTION
meq/h   ml/h
30 — 300   CONTROLS

20 — 200   Na⁺

Cl⁻

10 — 100   H₂O

0 —

0·   60   120

luminal   Cl⁻ ,meq/liter

10 — 100
SECRETION

ABSORPTION
meq/h   ml/h
30 — 300   CHLORIDE DIARRHEA

20 — 200

Na⁺

10 — 100   H₂O

0 —

0   60   120

Cl⁻

luminal   Cl⁻ ,meq/liter

10 — 100
SECRETION

	N D	35.9	44.8	N D	6.4	3.1
Cl⁻influx						
Cl⁻efflux (meq/h)	N D	20.3	21.8	N D	8.5	2.8

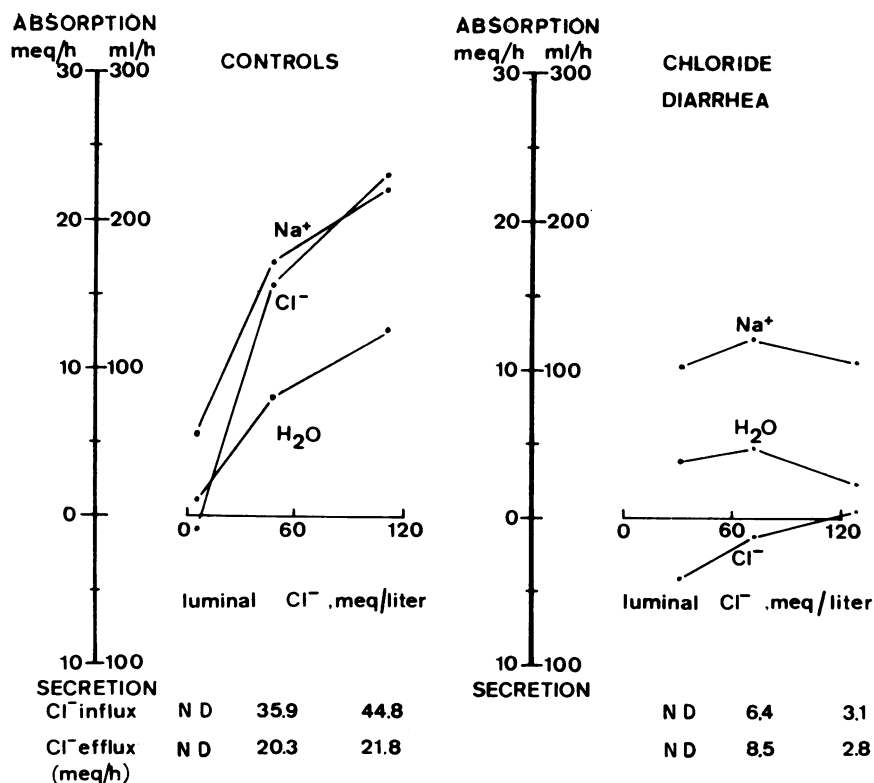FIGURE 2 Mean net ion and water movement in sequential perfusions of the colon with solutions of different Cl⁻ concentrations, in three controls and three patients. The mean Cl⁻ concentration of the fluid in the test segments is given on the horizontal scale. The mean concentrations of Na⁺, K⁺, and HCO₃⁻ were 130, 11, and 22 meq/liter in the controls, and 129, 14, and 12 meq/liter in the patients, respectively. The mean unidirectional Cl⁻ fluxes are given at the bottom. They were not determined (ND) for the Cl⁻-free perfusates.

method of Hyden (15) and total HCO₃⁻ with a Natelson microgasometer (Model 600, Scientific Industries, Inc., Mineola, N. Y.). pH was measured with a pH meter 26[3] and Pco₂ with a Pco₂ electrode Type E5036[3] coupled to an acid-base analyzer PHM 71.[3] pH, Pco₂, and total HCO₃⁻ were measured immediately from samples taken with a syringe without air contact at the end of each 20-min collecting period.

Calculations. Calculations were done from a set of consecutive 20-min periods in which electrolyte and PEG concentrations were steady at both collection sites. This was obtained after four to five periods in the controls and after five to six periods in the CCD patients. Ion net absorption and secretion were calculated from the perfusion rate and the changes in the concentrations of PEG and electrolytes in the test segment (16). Unidirectional Cl⁻ fluxes were calculated according to Curran and Solomon (17). In the control child T. Ko. cecal collections failed and the composition of the perfusate was used in the calculations instead of the fluid entering the test segment. This does not entail a substantial error, because admixture of fluid from the ileum was small (0.9 ml/min) in the controls, in contrast to the CCD patients. Mean concentrations of electrolytes in

the colon were calculated arithmetically from the concentrations in the fluid entering and leaving the test segment.

RESULTS

The data of the individual subjects are given in Table II and some means for the groups in Figs. 2 and 3.

Ileal effluent

From the difference in composition between the perfusate and the fluid entering the test segment, the electrolyte concentrations and flow rate can be calculated for the fluid being added to the perfusate in the mixing segment. This fluid closely resembles the ileal effluent, because the mixing segment was only 10 cm long, and its secretory and absorptive contributions thus were minimal. In eight perfusions of the controls, the mean flow rate was 0.9 (range 0.2–1.9) ml/min and the mean concentrations of Na⁺ 108.3 (94.1–125.7), K⁺ 8.8 (2.3–19.7), Cl⁻ 67.9 (60.1–104.0), and HCO₃⁻ 25.3 (5.6–97.0) meq/liter. These figures agree with previous observations (18, 19). In 12 perfusions of the CCD pa-

ABSORPTION
meq/h ml/h
30 ┬ 300   CONTROLS
            Cl⁻
20 ┼ 200   Na⁺
            H₂O
10 ┼ 100
0 ┼
            0   25
            luminal HCO₃⁻. meq/liter
10 ┴ 100
SECRETION

ABSORPTION
meq/h ml/h
30 ┬ 300   CHLORIDE
            DIARRHEA
20 ┼ 200
            Na⁺
10 ┼ 100
            H₂O
0 ┼
            0   Cl⁻   25
            luminal HCO₃⁻. meq/liter
10 ┴ 100
SECRETION

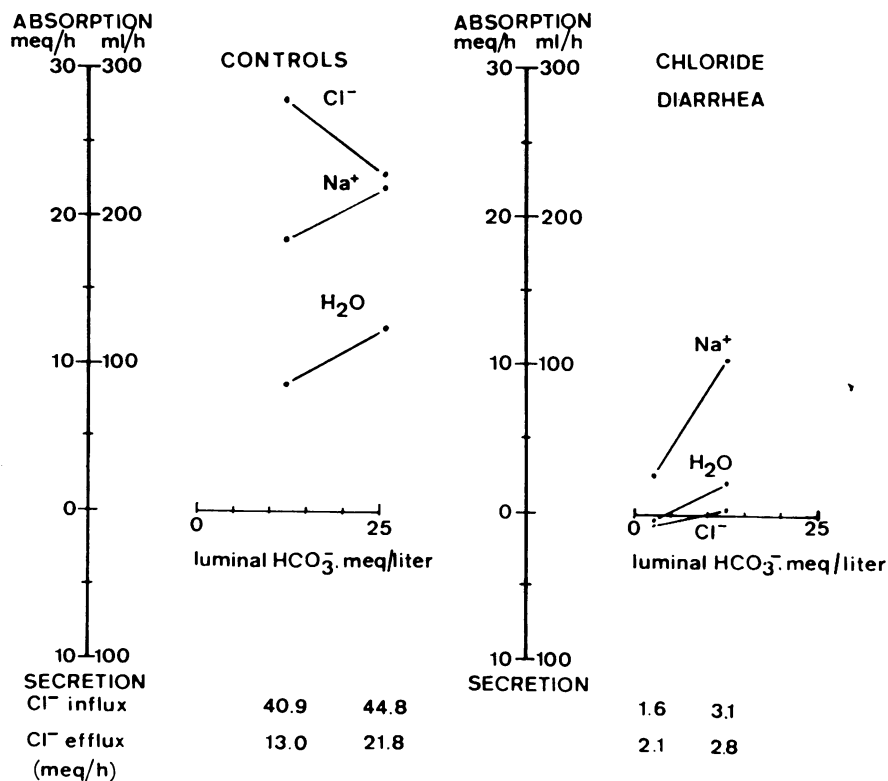|			
Cl⁻ influx	40.9	44.8	1.6 3.1
Cl⁻ efflux (meq/h)	13.0	21.8	2.1 2.8

FIGURE 3 Mean net ion and water movement in perfusions of the colon with solutions of different HCO_3^- concentrations in three controls and three patients. The mean HCO_3^- concentration of the fluid in the test segment is given on the horizontal scale. The mean concentrations of Na^+, K^+, and Cl^- were 130, 11, and 103 meq/liter in the controls and 130, 11, and 127 meq/liter in the patients, respectively. The mean unidirectional Cl^- fluxes are given at the bottom.

tients, the mean rate was 1.8 (1.2–2.6) ml/min and the concentrations Na^+ 100.0 (95.8–134.1), K^+ 7.3 (1.8–19.8), Cl^- 118.0 (85.0–145.0), and HCO_3^- 3.4 (0.8–15.6) meq/liter. The marked difference in the concentrations of Cl^- and HCO_3^- between the controls and patients is compatible with the view that a defect in the ileal Cl^-/ HCO_3^- exchange in CCD (7, 12, 14) is the cause of the increased effluent volume.

Transport of Cl⁻ and HCO₃⁻

Controls. On perfusion with the extracellular fluid (ECF)-like perfusate 1, the mean net Cl^- absorption was 23.0 meq/h. This was the net result of an efflux and an influx, the influx being twice as great as the efflux. When the luminal Cl^- concentration was reduced, the concentrations of Na^+, K^+, and HCO_3^- remaining constant, net Cl^- absorption decreased, because of a decrease in influx (Fig. 2). However, net absorption still continued at Cl^- concentrations as low as 10 meq/liter, obtained with the mannitol perfusate (Table II).

When HCO_3^- was omitted from the ECF-like perfusate, there was a clear decrease in Cl^- efflux (Fig. 3).

When Na^+ and K^+ were further omitted (choline-Cl^- perfusate) the efflux was unchanged, but there was a marked reduction in influx, the influx and efflux being now of the same magnitude (compare perfusates 5 and 6, Table II).

In perfusions with mean Cl^- concentrations of 110 and 47 meq/liter in the segment, there was only a slight net change (− 0.1 and − 1.5 meq/h, respectively) in the HCO_3^- content (perfusates 1 and 2, Table II). When all Cl^- was omitted (perfusate 3), there was a clear net disappearance of HCO_3^- (4.4 meq/h). The mean luminal HCO_3^- concentration was similar in all instances. In all these perfusions pH decreased and P_{CO_2} increased.

When the ECF-like perfusate without HCO_3^- was used, there was a clear net appearance of HCO_3^- (9.1 meq/h; perfusate 4, Table II).

Chloride diarrhea. In the CCD patients Cl^- movement was greatly reduced (Fig. 2). With the ECF-like perfusate 1 there was practically no net movement of Cl^- at all. Clearly, not only influx but also efflux was minimal. Reduction of Cl^- concentration in the perfusate caused net secretion of Cl^-. In contrast to controls,

TABLE II

Net Movement* of Water‡ and Ions in Colonic Perfusions in Three Controls and Three Patients with Congenital Chloride Diarrhea with Six Different Solutions. Unidirectional Fluxes of Cl⁻ are also Given, as well as Mean Concentrations of the Ions in the Test Segment, and pH and P_{CO_2} at the Entrance and Exit of the Test Segment

Na⁺/K⁺/Cl⁻/HCO₃⁻ concentrations in perfusate	Subjects	n§	H₂O	[Na⁺]	Na⁺	[K⁺]	K⁺	[Cl⁻]	Cl⁻ Net	Cl⁻ Influx	Cl⁻ Efflux	[HCO₃⁻]	HCO₃⁻	pH Ent	pH Ex	P_{CO_2} Ent	P_{CO_2} Ex
			ml/h	meq/liter	meq/h	meq/liter	meq/h	meq/liter	meq/h	meq/h		meq/liter	meq/h			mm Hg	
Controls																	
1. 140/10/125/25	K. S.	3	−277±8	119	−42.7±1.6	15.3	+0.1±0.4	97	−42.6±3.7	63.0	20.4	23.0	−4.0±0.3	7.3	6.8	45	104
	T. Ko.	5	−38±10	136	−9.1±1.1	10.4	−0.1±0.2	116	−13.2±0.8	33.0	19.8	27.6	+1.7±1.0	7.7	7.5	33	37
	J. K.	4	−61±10	134	−12.2±0.5	11.7	+0.9±0.3	116	−13.2±1.0	38.4	25.2	27.4	+1.9±0.4	7.7	7.6	30	48
	Mean		−125	130	−22.0	12.5	+0.3	110	−23.0	44.8	21.8	26.0	−0.1				
2. 140/10/60/25	K. S.	3	−205±27	119	−33.6±2.7	10.6	−1.5±0.5	43.1	−29.4±2.1	42.0	12.6	17.0	−3.4±1.6	7.1	6.8	49	90
	T. Ko.	4	−18±9	133	−10.1±1.8	9.6	−0.7±0.1	51.2	−6.8±1.0	28.9	22.1	24.2	−0.5±0.4	7.5	7.3	30	39
	J. K.	3	−20±4	133	−8.0±1.6	11.6	+0.6±0.2	45.7	−10.8±1.1	36.9	26.1	24.2	−0.5±0.3	7.5	6.9	31	92
	Mean		−81	129	−17.2	10.6	−0.5	47	−15.7	35.9	20.3	21.8	−1.5				
3. 140/10/0/25	K. S.	3	−15±1	126	−7.8±1.6	12.5	+1.6±0.1	2.7	−0.6±1.2			13.9	−3.8±1.3	7.2	6.9	26	47
	T. Ko.	4	−5±9	136	−5.1±1.0	10.7	+0.6±0.1	1.3	+1.3±1.0			20.9	−4.9±0.1	7.7	6.8	23	65
	J. K.	3	+17±5	132	−3.7±0.5	11.4	+1.1±0.2	13.0	+1.7±0.2			18.6	−4.4±0.7	7.8	6.7	24	120
	Mean		−11	132	−5.5	11.5	+1.1	6	+0.8			17.8	−4.4				
4. 140/10/125/0	K. S.	3	−222±8	121	−38.4±3.2	9.1	−2.5±0.1	80	−45.6±3.5	57.5	11.9	15.7	+7.3±0.2	6.4	7.3	32	62
	T. Ko.	3	−12±4	133	−8.6±0.8	10.2	−0.5±0.1	104	−19.4±0.6	31.8	12.4	11.2	+11.2±1.4	7.0	7.0	15	65
	J. K.	3	−27±12	133	−8.5±1.4	10.6	+0.4±0.1	102	−18.7±0.5	33.4	14.7	11.0	+9.0±1.4	7.1	7.3	15	48
	Mean		−87	129	−18.5	10.0	−0.9	95	−27.9	40.9	13.0	12.6	+9.1				
5. 0.15 M choline-Cl⁻	K. S.	3	+49±11	32.0	−2.7±1.0	2.4	+0.9±0.2	97.4	−1.6±0.9	10.9	9.3	15.7	+3.8±1.8	7.5	7.3	24	37
	J. K.	3	+2±8	6.3	−1.6±0.5	1.1	+0.3±0.1	95.9	−6.7±1.0	16.5	9.8	18.3	+4.0±0.5	7.3	7.4	40	42
	Mean		+25	19	−2.2	1.8	+0.6	97	−4.2	13.7	9.5	17.0	+3.9				
6. 0.3 M mannitol	K. S.	3	+3±11	21.1	−5.2±1.9	2.0	+0.8±0.1	8.9	−5.2±1.5			8.9	−0.6±0.7	7.2	6.9	22	42
	J. K.	3	−48±20	21.6	−7.1±0.4	2.0	+0.7±0.7	11.8	−4.2±0.1			9.3	−1.3±0.1	7.4	7.2	29	45
	Mean		−23	21	−6.2	2.0	+0.8	10.4	−5.0			9.1	−1.0				

* Mean ±SD of net movements during consecutive 20-min perfusion periods are given.
‡ A minus sign denotes absorption, a plus sign secretion. Influx means lumen-to-blood movement, efflux blood-to-lumen movement.
§ Number of 20-min perfusion periods used in the calculations.

TABLE II—(Continued)

Na$^+$/K$^+$/Cl$^-$/HCO$_3^-$ concentrations in perfusate	Subjects	n§	H$_2$O ml/h	[Na$^+$] meq/liter	Na$^+$ meq/h	[K$^+$] meq/liter	K$^+$ meq/h	[Cl$^-$] meq/liter	Cl$^-$ Net meq/h	Cl$^-$ Influx meq/h	Cl$^-$ Efflux meq/h	Influx [HCO$_3^-$] meq/liter	HCO$_3^-$ meq/h	pH Ent	pH Ex	PCO$_2$ Ent mm Hg	PCO$_2$ Ex mm Hg
Congenital chloride diarrhea																	
1. 140/10/125/25	A. S.	3	+7±15	132	−4.8±1.9	13.4	+0.3±0.4	131	−1.6±2.4	2.7	1.1	11.1	−2.0±0.2	7.0	6.8	47	52
	R. Ko.	3	−6±8	115	−15.2±0.7	17.4	+8.2±0.1	133	+3.3±0.8	0.0	3.3	11.8	−5.9±1.0	7.1	5.7	61	107
	J-P. K.	3	−65±17	136	−11.4±2.0	13.0	+2.6±0.3	131	−2.6±1.7	6.5	3.9	13.7	−8.7±0.7	7.4	6.7	38	54
	Mean		−22	128	−10.5	14.6	+3.7	132	−0.3	3.1	2.8	12.2	−5.5				
2. 140/10/60/25	A. S.	3	−111±8	132	−16.6±1.0	11.2	−0.3±0.3	73.1	−6.6±0.7	14.0	7.4	8.7	−3.1±0.4	7.5	6.4	38	79
	R. Ko.	2	−10±3	128	−10.0±0.6	13.1	+4.5±0.1	81.0	+9.4±0.9	4.4	13.8	12.3	−7.5±0.7	7.2	5.8	53	70
	J-P. K.	4	−38±5	129	−9.5±1.3	12.1	+1.9±0.2	78.6	+1.4±0.6	2.0	3.4	13.5	−8.6±0.9	7.3	6.4	42	66
	Mean		−46	130	−12.0	12.1	+2.0	77	+1.4	6.4	8.5	11.5	−6.4				
3. 140/10/0/25	A. S.	3	−12±3	130	−3.1±0.1	11.8	+0.9±0.0	31.5	+2.1±0.8			9.1	−0.3±0.7	7.6	6.1	28	103
	R. Ko.	3	−47±1	129	−15.8±2.1	13.2	+4.4±0.9	48.6	+8.4±1.6			9.2	−9.5±1.4	7.1	5.7	53	70
	J-P. K.	3	−48±1	130	−10.7±0.7	11.0	+1.9±0.3	19.2	+3.0±2.8			14.1	−7.3±0.7	7.3	6.5	41	71
	Mean		−36	130	−9.9	12.0	+2.4	33	+4.5			10.8	−5.2				
4. 140/10/125/0	A. S.	3	+6±3	131	−1.3±0.5	14.4	+1.2±0.2	122	+1.0±1.4	3.2	4.2	2.8	+0.7±0.1	5.4	5.3	62	89
	R. Ko.	5	+22±13	130	−2.0±1.9	11.0	+3.2±0.4	123	+1.7±1.6			2.9	0.0±0.1	6.2	6.3	40	42
	J-P. K.	4	−14±21	133	−4.8±2.1	11.3	+2.5±0.8	120	−0.8±1.0	0.8	0.0	3.0	−0.2±0.1	6.5	6.3	35	47
	Mean		+5	131	−2.7	12.2	+2.3	122	+0.5	1.6	2.1	2.9	+0.2				
5. 0.15 M choline-Cl$^-$	A. S.	3	+18±1	24.6	+7.1±1.4	4.6	+1.7±1.1	110	+0.6±0.7	3.0	3.6	3.6	+0.2±0.2	6.0	5.4	67	110
	R. Ko.	3	+29±9	27.3	−2.0±2.1	4.1	+1.9±0.5	114	+2.6±1.0	0.0	2.6	4.0	+0.3±0.1	6.5	6.6	39	38
	J-P. K.	3	−31±14	38.1	−9.1±1.4	3.8	+1.4±0.1	116	−3.0±1.8	3.4	6.4	3.7	0.0±0.1	6.7	6.5	28	42
	Mean		+6	30	−1.3	4.1	+1.7	114	−0.7	2.1	4.2	3.8	+0.2				
6. 0.3 M mannitol	A. S.	3	+18±9	18.6	+1.1±1.2	3.1	+1.1±0.1	18.0	+0.3±2.1			3.8	+0.3±0.2	6.3	6.3	62	72
	R. Ko.	3	+50±4	19.4	+4.3±1.7	4.6	+3.2±0.3	21.0	+8.8±2.3			2.6	+1.0±0.4	6.6	6.2	40	54
	J-P. K.	3	+68±12	24.4	+4.5±1.1	3.2	+2.3±0.1	17.9	+4.9±1.8			3.0	+0.7±0.4	6.6	6.4	32	47
	Mean		+45	21	+3.3	7.0	+2.2	19	+4.7			3.1	+0.6				

absence of HCO_3^- did not alter the Cl^- movement (Fig. 3). Perfusion with the mannitol solution gave the same slight net secretion of Cl^- as was seen with the other Cl^--free perfusate 3 (Table II). The mean serum $^{36}Cl^-$ activities measured at the end of each perfusion (and corrected for previous activity) were 266 cpm/ml (range 75–728 cpm/ml, $n = 8$) in the controls, and 43 cpm/ml (range 13–83 cpm/ml, $n = 7$) in the CCD patients. This also demonstrates impaired influx of Cl^-.

When ECF-like perfusates of varied Cl^- concentration (perfusates 1 to 3, Table II) were used, there was a net disappearance of HCO_3^- of similar magnitude (5.2–6.4 meq/h) in all perfusions. This was greater than in the controls, although the mean luminal HCO_3^- concentration was only half of that in the controls (10.8–12.2 and 17.8–26.0 meq/liter, respectively). pH fell clearly with a rise of Pco_2. The patients had no net appearance of HCO_3^- in the HCO_3^--free Cl^--containing perfusates 4 and 5, in contrast to the controls (Table II), although they had a much steeper plasma-to-lumen gradient for HCO_3^- (because of less flow of HCO_3^- to the segment from the ileum). Changes in pH and Pco_2 were only slight.

Na^+ transport

Controls. In perfusions with the ECF-like perfusate there was a mean net Na^+ absorption of 22.0 meq/h, slightly less than the mean net Cl^- absorption. Lowering of Cl^- concentration gave a clear decrease in net Na^+ absorption (Fig. 2). The slight net absorption of Na^+ seen with the Cl^--free perfusate 3 was accompanied by a net disappearance of HCO_3^- (Table II). Omission of HCO_3^- from the ECF-like perfusate did not alter the net Na^+ movement (Fig. 3). At a mean Na^+ concentration of 21 meq/liter or even lower in the test segment there was still a net Na^+ absorption (solutions 5 and 6, Table II). Net water movement showed a strong correlation with net Na^+ movement (ΔH_2O (ml/h) $= 7.0 \times \Delta Na^+$(meq/h) $- 32.5$; $r = 0.99$, $P < 0.001$).

Chloride diarrhea. The net Na^+ absorption was not decreased from the ECF-like perfusate 1. It was of the same magnitude as in two of the controls (Table II). Thus the Na^+-absorbing system was unimpaired.

However, in three respects net Na^+ absorption in the patients contrasted sharply with that of the controls. (*a*) Reduction of Cl^- concentration in the perfusate did not alter it (Fig. 2). (*b*) Omission of HCO_3^- from the perfusate caused a marked decrease in it (Fig. 3). (*c*) It did not take place from Na^+ concentrations below 27 meq/liter (solutions 5 and 6, Table II). In the last two situations the fluid entering the test segment was more acid than in the controls, and the test situation was thus different (mean pH 6.4 in the patients and 7.1 in the

controls). Such unusual acidity prevails in the colon in patients with CCD, in whom the ileal effluent has a lower HCO_3^- concentration and lower pH than in the controls.

K^+ transport

Controls. In most perfusions with solutions containing K^+ at 10 meq/liter only slight net movement of K^+ took place; presumably this concentration was close to the equilibrium concentration. With K^+-free perfusates and less than 2 meq/liter of K^+ in the segment, net secretion was constantly observed.

Chloride diarrhea. In the patients net K^+ secretion was clearly increased. This was seen in all perfusions, and was most marked in patient R. Ko. In the perfusions with 10 meq/liter K^+ concentration, the mean increment was 1.3 meq/liter (± 1.7 SD) in the controls and 4.8 meq/liter (± 2.7 SD) in the patients.

DISCUSSION

Colonic electrolyte transport in the controls. The control subjects were siblings of our patients. Because CCD is an autosomal recessive disease, they may have been heterozygotes and may thus have had impaired intestinal electrolyte transport. They were healthy, and showed no signs of impaired intestinal function. Their perfusion results closely resembled those obtained in fasting adults (19, 20). Their normality remains unconfirmed, however, because there are no reports of similar studies in normal children or even in nonfasted adults. Because of the lability of the electrolyte balance in CCD, perfusions in fasting could not be considered.

From an ECF-like perfusate we observed net absorption of Na^+ and Cl^- against steep concentration gradients, and slight net appearance of HCO_3^-. A "coupling" between the movements of Na^+ and Cl^- was evident from two observations. Net absorption of both Cl^- and Na^+ was diminished when the luminal Cl^- concentration was reduced. Omission of Na^+ from the perfusate (choline-Cl^- perfusate) also reduced Cl^- influx. As the unidirectional fluxes of Na^+ were not measured, this coupling could not be further characterized.

With the ECF-like perfusate, the influx of Cl^- was twice as great as the efflux. The influx was reduced when the luminal concentration of Cl^- was diminished. Omission of HCO_3^- from the perfusate, on the other hand, clearly reduced the Cl^- efflux. This requirement of luminal HCO_3^- for the efflux of Cl^- suggests that this efflux is coupled with the influx of HCO_3^-. This could be explained if the Cl^-/HCO_3^- exchange that is suggested in the colon (19, 20) takes place in both directions. Evidence for a similar bidirectional exchange has been obtained for the ileum (13).

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Intestinal electrolyte transport in CCD. Studies by Bieberdorf et al. (14) of ileal function in CCD showed that Na^+ transport is intact but active transfer of Cl^- is lacking. Passive movement of Cl^- along the electrochemical gradient was documented. Our finding that the ileal effluent had higher Cl^- and lower HCO_3^- concentrations than in the controls is consistent with these observations.

We have documented a distinct difference in colonic function between our controls and patients. The possible heterozygosity of the controls does not seem to leave the nature of the colonic defect in doubt; if they were heterozygotes the difference between CCD and the truly normal state could only be larger.

The present studies of colonic function in CCD revealed a pattern similar to that found in previous studies of the ileum (14). Na^+ transport was intact, but net absorption of Na^+ was dependent on luminal presence of HCO_3^-, in contrast to the controls. Obviously, electroneutrality requires that absorption of Na^+ is associated with corresponding absorption of an anion or equivalent secretion of another cation. Thus in the controls net Na^+ and Cl^- movements were closely linked. In CCD, as we have shown, Cl^- did not move, and Na^+ absorption was balanced by net disappearance of HCO_3^- and net secretion of K^+. This explains the suppression of Na^+ absorption on omission of luminal HCO_3^-. The disappearance of HCO_3^- may be due to its absorption, or to secretion of H^+, or to both of these events simultaneously. The rise of Pco_2 that accompanied this disappearance of HCO_3^- suggests that H^+ secretion was at least partly responsible. This would imply the existence in the colon of Na^+/H^+ exchange, a mechanism already postulated for the ileum (13, 14). The possibility of a rise in Pco_2 through absorption of HCO_3^- and back-diffusion of CO_2 cannot be excluded. Whatever the mechanism(s), it leads to accumulation of H^+ and exhaustion of HCO_3^- in the luminal contents and stops the net absorption of Na^+.

A clearly increased secretion of K^+ was noted in all patients. The marked elevation of K^+ concentration in the anal effluent suggests increased luminal electronegativity, as colonic secretion of K^+ is at least partly due to passive diffusion along the electrochemical gradient (20, 21). Increased intracolonic potential difference has been documented in two CCD patients (7, 12). Enhanced aldosterone activity increases potential difference and net secretion of K^+ into the human colon (22–25), and such enhanced activity has been recorded in CCD (5, 7, 10, 11, 26). Urinary aldosterone excretion was 43.4 $\mu g/m^2 \cdot$ day in R. Ko. and 23.2 $\mu g/m^2 \cdot$ day in J-P. K. (normal < 12 $\mu g/m^2 \cdot$ day). This may mediate the increased K^+ secretion observed. It is an interesting possibility that cation exchange is increased in CCD, which

secretes K^+ along with H^+ in exchange for Na^+. Evidence for the presence of such a mechanism has been observed for frog colon (27).

The primary defect in the colon, as in the ileum, is one of Cl^- transport. With the ECF-like perfusate no transfer of Cl^- was seen, but both influx and efflux were practically absent. Only with steep gradients was a slight net secretion of Cl^- observed. Alteration of luminal Cl^- concentration did not affect the net change of luminal HCO_3^- content. Net appearance of HCO_3^- was not seen with the HCO_3^--free Cl^- containing perfusates, in contrast to controls. If HCO_3^- was secreted, but disappeared because of the greater acidity of the colonic contents in the patients, the rise of Pco_2 should have been larger than in the controls. This never occurred. Thus no evidence for secretion of HCO_3^- was obtained in the patients.

Nature of the basic defect. As Cl^- transport is impaired in both ileum and colon, it is logical to assume that the same defect is present in both. A hypothesis to account for the defect has to explain the difference in Cl^- movement between the two parts of the intestine; while the unidirectional fluxes in the ileum were almost normal (14), they were practically absent from the colon. A defect in permeability to Cl^- would explain the colonic findings, but is incompatible with the ileal ones.

Could absence of active Cl^-/HCO_3^- transfer, such as was proposed by Bieberdorf et al. for the ileum (14), explain our findings for the colon? Evidence has been obtained that Cl^-/HCO_3^- exchange occurs in the normal human colon (19, 20); this exchange has features of active transport (20, 21, 28, 29). The permeability of the intestine is known to decrease in the distal direction (18, 30–32). Conceivably, although an essential part of the Cl^- fluxes may take place through independent passive diffusion in the ileum, such movement may virtually fail to occur in the poorly permeable colon. The carrier-mediated Cl^-/HCO_3^- exchange may thus be necessary for any Cl^- flux in the colon. A defect in this mechanism would then explain the observed absence of active Cl^- transport in the ileum and all Cl^- movement in the colon. The apparent absence of HCO_3^- secretion in the colon is consistent with this theory.

Bieberdorf et al. (14) observed a slight increase in downhill Cl^- secretion on elevation of luminal HCO_3^- concentration to 80 meq/liter in the ileum of their CCD patient. This suggest that in that patient the Cl^-/HCO_3^- exchange mechanism was not totally lacking, but was capable only of passive function at a very high luminal HCO_3^- concentration.

In conclusion, present knowledge of intestinal transport in CCD is compatible with the notion that the basic defect is impairment or absence of a Cl^-/HCO_3^- exchange mechanism, which operates in both directions

in the normal ileum and colon. Such a defect explains the clinical findings in CCD: large watery stools with high Cl⁻ concentration and very low HCO₃⁻ concentration and pH. The passive permeability of the proximal intestine to Cl⁻ explains the success of oral replacement therapy.

ACKNOWLEDGMENTS

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