

Segmental Chloride and Fluid Handling during Correction of Chloride-Depletion Alkalosis without Volume Expansion in the Rat

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Abstract. To determine whether chloride-depletion metabolic alkalosis (CDA) can be corrected by provision of chloride without volume expansion or intranephronal redistribution of fluid reabsorption, CDA was produced in Sprague-Dawley rats by peritoneal dialysis against 0.15 M NaHCO₃; controls (CON) were dialyzed against Ringer's bicarbonate. Animals were infused with isotonic solutions containing the same Cl and total CO₂ (tCO₂) concentrations as in postdialysis plasma at rates shown to be associated with slight but stable volume contraction. During the subsequent 6 h, serum Cl and tCO₂ concentrations remained stable and normal in CON and corrected towards normal in CDA; urinary chloride excretion was less and bicarbonate excretion greater than those in CON during this period. Micropuncture and microinjection studies were performed in the 3rd h after dialysis. Plasma volumes determined by ¹²⁵I-albumin were not different. Inulin clearance and fractional chloride excretion were lower ($P < 0.05$) in CDA. Superficial nephron glomerular filtration rate determined from distal puncture sites was lower ($P < 0.02$) in CDA (27.9 ± 2.3 nl/min) compared with that in CON (37.9 ± 2.6). Fractional fluid and chloride reabsorption in the proximal convoluted tubule and within the loop segment did not differ. Fractional chloride delivery to the early distal convolution did not differ but that out of this

segment was less ($P < 0.01$) in group CDA. Urinary recovery of ³⁶Cl injected into the collecting duct segment was lower ($P < 0.01$) in CDA (CON 74 ± 3 ; CDA $34 \pm 4\%$).

These data show that CDA can be corrected by the provision of chloride without volume expansion or alterations in the intranephronal distribution of fluid reabsorption. Enhanced chloride reabsorption in the collecting duct segment, and possibly in the distal convoluted tubule, contributes importantly to this correction.

Introduction

Chloride-depletion metabolic alkalosis (CDA)¹ is usually also associated with hypochloremia, hypokalemia, and sodium, chloride, potassium, and volume depletion. The relative contribution of the independent correction of each of these variables for the correction of alkalosis is controversial.

In a series of balance studies in dog and man, Schwartz and co-workers (1-4) showed that CDA produced by gastric drainage or nitrate loading could be corrected only by the administration of NaCl or KCl despite persisting potassium depletion. From these studies, Schwartz et al. (5) concluded that the provision of chloride is essential to the renal correction of CDA but the mechanism was unclear. In several of the aforementioned studies (1, 3), expansion of the extracellular fluid (ECF) volume after correction was calculated on the basis of chloride space (6).

In the dog that is rendered alkalotic by the administration of ethacrynic acid and a subsequent chloride-deficient diet, Cohen (7) showed that expansion of the ECF volume with isometric fluids (i.e., isotonic electrolyte solutions with an anion composition that approximated that of the plasma of the alkalotic animal) corrected metabolic alkalosis and he suggested that ECF volume expansion per se mediated the correction. He later sug-

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1. *Abbreviations used in this paper:* BW, body weight; CDA, chloride-depletion metabolic alkalosis (and experimental animals); CDS, collecting duct segment; CON, control animals; DCT, distal convoluted tubule; ECF, extracellular fluid; GFR, glomerular filtration rate; SNGFR, superficial nephron GFR; tCO₂, total CO₂; TF/UF, tubular fluid/ultrafiltrate; TGF, tubuloglomerular feedback; UF_{CL}, ultrafiltrate chloride concentration.

gested that the expansion of the ECF volume alters the intranephronal distribution of fluid and sodium reabsorption² to favor chloride reabsorption and correct CDA (8). In apparent contradiction of this hypothesis, Hulter et al. (9) showed that isometric volume expansion in dogs also corrects chronic hyperchloremic metabolic acidosis and suggested that the kidney selectively alters the anion composition of reabsorbate to correct the plasma acid-base composition. The dominance of volume homeostasis over acid-base balance has been most forcefully espoused by Schwartz and Cohen (10) who hypothesized that acid excretion by the kidney is determined by factors regulating the site and rate of sodium reabsorption and not by a homeostatic mechanism(s) that responds to systemic pH.

In balance studies in the rat, we (11) have shown that complete correction of CDA produced by peritoneal dialysis occurs within 24 h by the oral intake of isotonic solutions of sodium or choline with chloride and bicarbonate concentrations that would only maintain alkalosis; chloride is retained by the kidney. Also in this model, CDA persisted for at least 7 d if chloride was eliminated from the diet. Correction occurred despite negative sodium and potassium balance, weight loss, and an obligatory bicarbonate load. From a conceptual standpoint, changes in plasma anion composition that correct CDA can be produced by the administration of chloride, by shifts of these anions between the intracellular and extracellular compartments, or by the excretion of bicarbonate and the conservation of chloride affected either by redistribution of fluid reabsorption or by alterations of anion transport within the various segments of the nephron.

The purpose of the present series of experiments was to determine: (a) whether correction of CDA occurs during isometric intravenous infusions in the rat without volume expansion and, if so, (b) which nephron segments participate in the process, and (c) whether alterations in intranephronal fluid reabsorption are required. To accomplish these goals, micropuncture and microinjection studies were carried out in rats during correction of CDA by maintenance infusion of isotonic solutions with chloride and bicarbonate concentrations that approximate those of the plasma of chronic CDA rats.

Methods

An overview of our investigative approach follows. All animals were initially dialyzed. Infusion studies over 6 h determined the time course of correction in terms of plasma electrolytes and the maintenance infusion requirements necessary to avoid expansion of the ECF volume. Micropuncture and microinjection studies in the 3rd h after dialysis were then performed in control animals (CON) and experimental animals (CDA)

2. Because of preferential bicarbonate reabsorption in the proximal tubule and preferential chloride reabsorption in the thick ascending limb of the loop of Henle, shifts of fractional fluid reabsorption from the thick ascending limb of the loop of Henle to the proximal tubule would favor bicarbonate reabsorption and vice versa.

to determine fluid and chloride transport in the various nephron segments during correction.

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Cambridge, MA) ate regular rat chow (Ralston Purina Co., St. Louis, MO) and drank tap water ad lib. before all studies. On the day of dialysis, rats were anesthetized with an intraperitoneal injection of Inactin, 100 mg/kg body weight (BW). The animals were placed on a heated board and their body temperatures were maintained at 37°C. Warmed dialysate (15 ml/100 g BW) was infused into the peritoneal cavity through an 18-gauge Intracath. CON animals were dialyzed against a solution composed (in meq/liter) of Na, 150; K, 4; Cl, 114; HCO₃, 25; acetate, 15; and glucose, 15 g/liter. In CDA animals, chloride-depletion alkalosis was produced by a dialysate composed (in milliequivalents per liter) of Na, 150; K, 4; HCO₃, 154; and glucose, 15 g/liter. After 30 min, the dialysate was removed and the volume and concentrations of sodium, potassium, and chloride were determined.

To determine the effect of time, maintenance infusions, and surgical preparation on the changes induced by dialysis, rats were studied for 6 h after dialysis. In each experiment, two littermate rats which weighed 250–300 g were anesthetized. In the experimental rat, the femoral artery was catheterized with PE-50 tubing and an arterial blood sample obtained for hematocrit and plasma chloride and total CO₂ (tCO₂) concentration. This and subsequent blood samples were replaced quantitatively by volume with an erythrocyte suspension obtained from the littermate. Notwithstanding this procedure, blood samplings were held to a minimum. The littermate was bled by aortic puncture. Arterial pH and PaCO₂, and plasma sodium, potassium, chloride, and tCO₂ concentrations were determined on this sample for base-line values. The remainder of the blood was centrifuged and the supernatant plasma was removed. The erythrocytes were resuspended in a modified Krebs-Ringer's-bicarbonate solution which contained (in millimoles per liter) Na, 140; K, 4; Ca, 1; Mg, 0.5; Cl, 80; HCO₃, 40; SO₄, 0.5; acetate, 24; PO₄, 1.3; glucose, 5.6 and bovine serum albumin, 60 g/liter to a packed cell volume of 45%. The resultant suspension was gassed slowly with 95% O₂ and 5% CO₂ in a 37°C water bath. In the experimental rat, a tracheostomy and two PE-50 jugular venous catheters were then placed. Peritoneal dialysis was carried out as described. To correct for dialysis-related fluid shifts (12), 6% bovine serum albumin in the modified Krebs-Ringer's-bicarbonate solution was infused slowly in a volume of 1.0 ml/100 g BW for each 5.5% change in hematocrit (mean volume: 0.51 ml CON and 1.02 ml CDA). Group CDA rats were then infused with the aforementioned modified Krebs-Ringer's-bicarbonate without albumin at 0.5 ml/100 g BW/h throughout the experiment and group CON rats with a standard Krebs-Ringer's-bicarbonate solution with chloride of 100, acetate, 24 and HCO₃, 25 mmol/liter. The bladder was catheterized with PE-50 tubing. The left kidney was exposed through a subcostal incision and placed in a Lucite cup as for micropuncture and the left ureter was catheterized with PE-50 tubing at the hilum. When the peritoneal cavity was entered, an undetermined small volume of fluid leaked out routinely in both groups. This fluid, a remainder from hypertonic dialysis, was not replaced. Furthermore, surgical fluid losses (13) were also not replaced to avoid expansion of ECF volume. After this surgical preparation, which required ~60 min, serial arterial samples were obtained for determination of chloride concentrations. The experiment was terminated with an aortic puncture, and arterial pH and PaCO₂, and plasma sodium, potassium, chloride, and tCO₂ concentrations were determined in this sample.

In separate groups of CON and CDA rats studied in pairs, serial urinary chloride and tCO₂ excretions were determined during dialysis and preparative surgery and periodically thereafter. A bladder catheter

was placed before dialysis and secured by sutures to prevent leakage of dialysate. Otherwise, the protocol was as described above. Excretion rates obtained during the dialysis period before catheterization of the left ureter were halved to obtain single kidney values. Thereafter, excretion rates apply to the left kidney exposed for micropuncture. The body weights for these groups (CON, 279 ± 6 , $n = 9$; CDA, 279 ± 6 , $n = 9$) did not differ ($P = \text{NS}$).

Free-flow micropuncture studies. Animals were prepared and studied in a manner identical to that for the infusion studies with the following exceptions. Late proximal and early distal tubule puncture sites were selected by intravenous injections of 7.5% Food, Drug, and Cosmetic (FD and C) green (Keystone Aniline and Chemical Co., Chicago, IL). [*Methoxy- ^3H*]inulin, 250 $\mu\text{Ci}/\text{h}$, was infused in the maintenance infusion. The inulin was dialyzed against distilled water for 72 h before use (14). In the 3rd h after peritoneal dialysis, one to three late proximal and one to three early distal timed tubule fluid samples were obtained during two successive 30-min clearance periods. The volume, ^3H -activity, and chloride concentration were determined for each tubule fluid sample. At the midpoint of each clearance period, arterial samples were obtained for hematocrit and inulin and chloride concentrations. Urine was collected for determination of volume and sodium, potassium, chloride, and inulin concentrations. Final arterial sample was obtained for hematocrit and sodium, potassium, chloride, tCO_2 , and protein concentration. The body weights of these groups (CON, 281 ± 8 , $n = 11$; CDA, 276 ± 2 g, $n = 8$) were not different ($P = \text{NS}$).

Separate groups of CON and CDA rats were prepared in an identical manner and plasma volumes were determined by ^{125}I -albumin in the middle of the clearance period.

Microinjection studies. Animals were prepared in a manner identical to that for the micropuncture studies. After surgical preparation, tubule puncture sites were localized by the transit time of intravenous boluses of 0.05 ml 7.5% FD and C green, which correlate well with microdissection (15). The difference between early and late distal convoluted tubule (DCT) transit times was used to compare the mean loci of punctures between groups (16) and did not differ (CON, 21.0 ± 1.9 ; CDA, 21.2 ± 1.8 s; $P = \text{NS}$). In the 3rd h after dialysis, two 15-min clearance periods bracketed a 1-h period during which late DCTs were punctured. 8% polyfructosan was substituted for [^3H]inulin in the maintenance infusion.

Fluid for microinjections was prepared with sodium chloride labeled with ^{36}Cl (Amersham Corp., Arlington Heights, IL) and [*methoxy- ^3H*]inulin (New England Nuclear, Boston, MA). These isotopes were diluted with distilled water to yield radioactivities of 10 $\mu\text{Ci}/\text{ml}$ of ^{36}Cl and 50 $\mu\text{Ci}/\text{ml}$ of [^3H]inulin. 2 nl of 5% solution of FD and C green were added to 45 μl of injectate to tint the solution. The final chloride concentration of the injectate was 40 meq/liter. 8–10-nl droplets of the labeled solution were placed under oil in a Petri dish with a constant-volume pipette and aspirated into an oil-filled micropipette. The radioactivity of three pipettings was counted directly to determine the activity of ^3H and ^{36}Cl injected. Injections into the collecting duct segment (CDS) were made slowly enough to avoid retrograde flow. The often quite low tubule fluid flow rate in the late DCT in group CDA rats dictated an injection time of ~ 5 min. The same 5-min injection time was used for group CON rats. For the 15 min after the initiation of the injection, urine was collected into scintillation vials that contained 0.5 ml of distilled water and 10 ml Scintiverse (Fisher Scientific Co., Pittsburgh, PA). In preliminary studies, a collection of this duration was sufficient to recover all of the injected [^3H]inulin, and 30-s injections gave ^{36}Cl recovery (73%) in group CON similar to that obtained with 5-min injections. In random punctures, the microinjection pipette was left in place and a tubule fluid sample obtained from previously injected

or, alternatively, from freshly punctured late DCT by the introduction of a column of Sudan black-stained paraffin oil four to five tubule diameters in length. Collections either were spontaneous or required slight suction to maintain the oil block. Timed collections of tubule fluid in which chloride concentration and volume were determined permitted the calculation of tubule fluid flow rate and chloride delivery rate. The body weights of these groups (CON, 268 ± 7 , $n = 12$; CDA, 271 ± 5 g, $n = 20$) were not different ($P = \text{NS}$).

To examine further the independent influences of chloride load or fluid flow rate on ^{36}Cl recovery in group CDA, the chloride concentration of the injectate in several injections was increased to 100 peq/nl, which approximated the chloride delivery to the CDS in group CON (1,270 peq) during the 5-min injection. Attempts to double the volume of injectate into group CDA to approximate the late DCT flow rate in group CON were unsuccessful due to backflow.

Analytical techniques. Activities of ^{36}Cl and [^3H]inulin in tubule fluid, plasma, and urine were determined by liquid scintillation counting (Packard model 3325 Tri-Carb liquid scintillation spectrometer, Packard Instrument Co., Downers Grove, IL). Corrections were made for background activity and for crossover counts of ^{36}Cl into the ^3H channel in the appropriate samples. Quench corrections were obtained from an internal standard. Microinjection samples in which the recovery of [^3H]inulin was $<93\%$ or $>107\%$ were discarded.

The volume of tubule fluid samples was measured in constant-bore glass tubing with a microslide comparator (Gaertner Scientific Corp., Chicago, IL). Tubule fluid chloride concentration was determined by the second method of Ramsay et al. (17).

In the microinjection series, inulin concentration in urine and plasma was determined by the anthrone method (18). Sodium and potassium concentrations in urine and plasma were determined by flame photometry (model 243, Instrumentation Laboratory, Inc., Lexington, MA) and chloride concentration by electrometric titration (Buchler Instruments, Inc., Fort Lee, NJ). tCO_2 concentration in plasma and urine was determined manometrically (Harleco CO_2 apparatus, Dade Diagnostics Inc., Aquado, PR). Arterial pH and CO_2 tension were determined with a BMS3 MK2 blood gas analyzer (Radiometer A/S, Copenhagen). Protein concentration was determined by refractometry (model 10400A, American Optical Corp., Buffalo, NY).

Plasma volume was estimated with ^{125}I -bovine serum albumin by the method of Belcher and Harriss (19) with a correction for plasma trapping.

Calculations. Whole kidney glomerular filtration rate (GFR) and urinary excretions of sodium, potassium, and chloride were calculated according to standard expressions. The change in plasma volume based on serial hematocrits was calculated as previously described (20). Ultrafiltrate chloride concentration (UF_{Cl}) was obtained by multiplying plasma chloride concentration by the correction factor for plasma water (1.06), as previously suggested (21, 22). The various single nephron functional characteristics (including superficial nephron GFR (SNGFR), absolute and fractional reabsorptions, and deliveries of fluid and chloride from both proximal and distal puncture sites) and estimated reabsorptions of fluid and chloride in the loop segment were calculated as previously described (23). Distally determined SNGFRs were used to calculate load to the loop segment. The term "loop segment" refers to the entire nephron segment between the latest proximal and earliest distal surface convolutions. Recovery of nuclide in microinjection studies was expressed as counts recovered divided by counts injected multiplied by 100. Values given are mean \pm SEM. Statistical significance was assessed between groups with the unpaired t test and within groups with the paired t test (24). Significance was set at the 5% level.

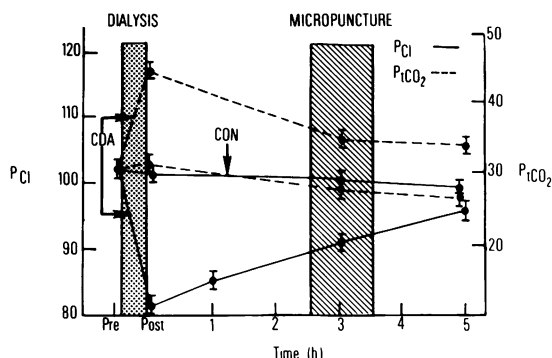


Figure 1. Changes in plasma chloride (P_{Cl} , milliequivalents per liter) and tCO_2 (P_{tCO_2} , milliequivalents per liter) concentration in CON and CDA groups before and during 6 h of infusion. The dialysis period is indicated by the stippled bar and the micropuncture period by the hatched bar.

Results

Plasma chloride concentration in group CON was unaffected by dialysis and the 6-h infusion (Fig. 1; Tables I and II). In group CDA, plasma chloride concentration decreased ($P < 0.001$) after dialysis and then increased during the isometric infusion toward ($P < 0.001$), but remained lower than ($P < 0.001$), the initial normal concentration at both 3 and 6 h after dialysis (Fig. 1). Serial urinary chloride concentrations and excretion rates were less in group CDA (Table III). Plasma tCO_2

concentration in group CDA increased reciprocally ($P < 0.001$) after dialysis and then decreased toward ($P < 0.001$), but at 3 and 6 h after dialysis remained higher than ($P < 0.01$), the initial concentration. The predialysis plasma tCO_2 in group CON that was slightly higher than the initial value obtained from littermates may be related to the initiation of maintenance infusions and the intervening surgical procedures. After dialysis, plasma tCO_2 concentration in group CON remained slightly above base line, probably because of the acetate in the dialysate, it then declined ($P < 0.05$) to normal values by 3 h and remained stable thereafter. This probably accounts for the slight bicarbonaturia in group CON. Nevertheless, serial urinary tCO_2 concentrations and excretions were greater in group CDA (Table III).

Mean arterial blood pressure did not differ between groups (Table II). Mean arterial hematocrits in both the infusion and micropuncture studies (Table IV) before and in the 1st, 3rd, and 6th h after dialysis did not differ between groups. Dialysis in group CDA induced greater volume contraction (at least in the micropuncture studies) that was successfully corrected to hematocrit levels not different from that in group CON in the 1st h. Subsequent serial hematocrits in both protocols did not change significantly within either group. 3 h after dialysis, hematocrits were higher ($P < 0.01$) than the initial hematocrits within both groups. On the basis of initial and 3rd-h hematocrits, plasma volume in the 3rd h after dialysis had decreased ($P < 0.001$) in both groups; the estimated decrease in group CON ($-11.9 \pm 2.0\%$) was greater ($P < 0.05$) than that in group CDA ($-7.4 \pm 1.0\%$). However, the plasma volumes in the 3rd h de-

Table I. Plasma Electrolytes and Arterial Blood Gases before and Six H after Dialysis

		Group		P value
		CON	CDA	
Plasma				
Na ⁺ (meq/liter)	initial*		143±1	
	6th h	142±2	142±1	NS
K ⁺ (meq/liter)	initial*		4.0±0.1	
	6th h	4.2±0.1	4.1±0.4	NS
Cl (meq/liter)	initial*		99±1	
	6th h	100±1	96±2	NS
tCO_2 (meq/liter)	initial*		25±1	
	6th h	25±1	33±1	<0.0001
Arterial				
pH	initial*		7.42±0.01	
	6th h	7.49±0.03	7.60±0.01	<0.001
PaCO ₂ (mmHg)	initial*		36.2±1.0	
	6th h	26.8±2.4	34.4±0.99	<0.005

* Data obtained from littermates.

Table II. Summary of Experimental Kidney Functions and Plasma Electrolytes 3 H after Dialysis in Microinjection Studies

		Group		P value
		CON (n = 12)	CDA (n = 20)	
Arterial pressure (mmHg)	pre PD	121±2	120±2	NS
	3rd h	116±2	118±2	NS
Plasma tCO ₂ (meq/liter)	pre PD	31±1	30±1	NS
	post PD	32±1	43±1	<0.01
	3rd h	27±0.9	33±1	<0.01
Plasma Cl (meq/liter)	pre PD	99±1	101±1	NS
	post PD	101±2	79±1	<0.01
	3rd h	103±1	90±1	<0.01
Inulin clearance (μl/min)		1153±81	901±70	<0.05
Urine flow rate (μl/min)		4.1±0.5	4.1±0.3	NS
Sodium excretion (neq/min)		561±159	199±39	<0.05
Potassium excretion (neq/min)		1062±125	1239±88	NS
Chloride excretion (neq/min)		418±101	16±2	<0.01
Fractional sodium excretion (%)		0.40±0.13	0.14±0.03	<0.05
Fractional chloride excretion (%)		0.30±0.07	0.02±0.01	<0.01

terminated by ¹²⁵I-albumin spaces (CON, 3.74±0.24; CDA, 3.77±0.22 ml/100 g BW; *P* = NS) and plasma protein concentrations (CON, 5.0±0.1 g/dl; CDA, 5.2±0.1; *P* = NS) did not differ.

Inulin clearance and urinary sodium and chloride excretions in the microinjection studies were significantly lower in group CDA compared with group CON in the 3rd h after dialysis (Table II). Plasma chloride and tCO₂ concentrations in the micropuncture and microinjection studies in the 3rd h after dialysis were similar to those in the infusion studies (Fig. 1). In the micropuncture studies of proximal and distal tubules during the 3rd h after dialysis, the experimental kidney inulin clearance in CDA (1,201±219 μl/min) was also lower, although not significantly so (*P* = NS), than that in CON (1,461±135). To determine whether GFR returns to control values when CDA is completely corrected, separate groups of rats (CON, *n* = 7; CDA, *n* = 6) were dialyzed in pairs, and upon awakening they were given low sodium chloride chow and drink containing Na, 150; Cl, 70; HCO₃, 40; and acetate, 40 meq/liter ad lib. 24 h later, single kidney inulin clearances were not different (CON, 1,640±437; CDA, 1,546±256 μl/min) when both plasma Cl and tCO₂ were not different from normal control values.

Mean SNGFR as determined from the latest proximal puncture sites did not differ between groups (Table V). However, mean SNGFR determined from earliest distal puncture sites was lower in group CDA compared with group CON.

Absolute and fractional fluid and chloride reabsorptions determined from proximal punctures did not differ (Table V). Superficial nephron fractional fluid and chloride reabsorptions up to the early DCT did not differ, whereas absolute fluid and chloride reabsorptions did. Because of a lower filtered load, absolute chloride delivery to the loop segment (1,873±192 peq/min) in group CDA was lower (*P* < 0.02) than that (2,402±170 peq/min) in group CON. The tubule fluid/ultrafiltrate (TF/UF) chloride ratios in the late proximal and early distal tubules did not differ (Table V).

In the loop segment, both absolute fluid (*n* = 15) and chloride (*n* = 9) reabsorptions were lower (*P* < 0.05 and <0.01, respectively) in group CDA, whereas fractional reabsorptions did not differ (Fig. 2).

Fluid delivery to the early DCT did not differ (CON, 5.9±0.6; CDA, 4.9±0.8 nl/min; *P* = NS) between groups. Similarly, chloride delivery to DCT did not differ (CON, 191±37; CDA, 110±18 peq/min; *P* = NS). However, chloride delivery out of the late DCT was less in group CDA (CON, 188±39; CDA, 72±8) primarily because of a lower tubule fluid flow rate (Table VI).

Urinary recovery of ³⁶Cl injected into the CDS was higher in group CON than in group CDA (Table VI). Urinary ³⁶Cl recoveries in group CDA in injectates that contained chloride of 100 peq/nl and thus approximated the chloride delivery in CON remained less than those in group CON. Neither chloride

Table III. Serial Urinary Chloride and $t\text{CO}_2$ Concentrations and Excretion Rates in the Experimental Kidney

Period	Urinary chloride			Urinary $t\text{CO}_2$		
	CON	CDA	<i>P</i> value	CON	CDA	<i>P</i> value
Dialysis	166±28*	41±14	<0.005	19±5	175±27	<0.0001
	344±85‡	233±59	NS	47±12	1320±305	<0.01
Surgery	106±15	4±1	<0.0001	45±11	206±9	<0.0001
	349±85	53±20	<0.01	158±58	2523±373	<0.01
2nd h§	53±10	4±1	<0.0005	47±14	145±17	<0.0005
	313±78	47±15	<0.01	332±128	1691±287	<0.01
4th h	21±5	3±1	<0.005	26±8	71±18	<0.05
	137±57	26±8	NS	205±100	600±187	NS
6th h	14±4	4±1	<0.05	19±5	57±15	NS
	48±14	25±9	NS	85±34	361±107	<0.05

* Concentration in milliequivalents per liter.

‡ Excretion rate in nanoequivalents per minute.

§ Time after dialysis.

delivery into the CDS (Fig. 3) nor tubule fluid flow rate in the late DCT (Fig. 4) correlated ($r = 0.04$ and $r = 0.01$, respectively; $P = \text{NS}$) with simultaneously determined ^{36}Cl urinary recovery.

Discussion

Acute chloride-depletion alkalosis in the rat can be produced by peritoneal dialysis against isotonic NaHCO_3 ; metabolic al-

kalosis persists for at least 7 d if chloride is excluded from the diet (11). In the present series of experiments, chloride was given intravenously in a concentration less than that in plasma immediately after dialysis and with bicarbonate in a concentration adequate to preserve the alkalosis in the absence of corrective homeostatic responses. Volume expansion was avoided and plasma volumes did not differ between groups. Indeed, slight but stable volume contraction was maintained in both groups.

Table IV. Summary of Serial Hematocrits

Group	Pre-dialysis	Post-dialysis	1st h	3rd h	<i>P</i> value (1st vs. 3rd h)	6th h	<i>P</i> value 3rd vs. 6th h
	%	%	%	%	%	%	%
Infusion experiments only							
CON (<i>n</i> = 11)	41.8±0.5	46.4±0.9	44.1±0.8	43.8±0.9	NS	44.6±1.0	NS
CDA (<i>n</i> = 10)	41.8±0.5	47.6±0.8	44.8±0.6	43.3±0.6	NS	43.2±0.6	NS
<i>P</i> value	NS	NS	NS	NS	NS	NS	NS
Micropercutaneous and microinjection experiments							
CON (<i>n</i> = 19)	41.8±0.4	44.8±0.5	43.4±0.6	44.1±0.8	NS	—	—
CDA (<i>n</i> = 28)	41.7±0.3	47.5±0.3	44.5±0.5	44.0±0.6	NS	—	—
<i>P</i> value	NS	<0.01	NS	NS	—	—	—

Table V. Summary of Single Nephron Fluid and Chloride Data from Late Proximal and Early Distal Micropunctures

	Group		P value
	CON	CDA	
Proximal			
SNGFR (nl/min)	36.9±2.1 (26)*	33.8±3.0 (23)*	NS
TF/UF _{Cl}	1.18±0.02	1.18±0.02	NS
Fluid reabsorption (%)	51.4±2.2	53.3±3.2	NS
Fluid reabsorption (nl/min)	18.0±1.4	16.8±2.2	NS
Chloride reabsorption (%)	39.4±2.7	37.7±3.8	NS
Chloride reabsorption (peq/min)	1,577±155	1,300±192	NS
Distal			
SNGFR (nl/min)	37.9±2.6 (15)*	27.9±2.3 (10)*	<0.02
TF/UF _{Cl}	0.30±0.03	0.25±0.04	NS
TF _{Cl} (peq/nl)	32±3	24±4	NS
Fluid reabsorption (%)	84.2±1.4	82.4±2.0	NS
Fluid reabsorption (nl/min)	32.0±2.4	23.0±1.8	<0.02
Chloride reabsorption (%)	95.2±0.7	95.5±0.9	NS
Chloride reabsorption (peq/min)	3,844±266	2,488±209	<0.001

TF_{Cl}, tubular fluid chloride concentration.

* Number of observations in parentheses.

Nevertheless, plasma chloride concentration increased and plasma bicarbonate decreased progressively. During correction, significantly greater bicarbonaturia in group CDA persisted throughout the 6 h of observation. Chloride conservation and low fractional sodium and chloride excretions were associated with a reduced GFR but without detectable alterations in fractional fluid or chloride reabsorption in the proximal tubule or the loop segment. However, fractional chloride uptake within the CDS and, perhaps, in the DCT was accelerated.

GFR in the experimental kidney and in superficial nephrons determined from distal puncture sites were lower in group CDA,

78 and 74% of control, respectively. At the same time, mean arterial blood pressure and measured plasma volumes, arterial hematocrits, and plasma albumin concentrations did not differ between groups CON and CDA. The estimated decrement in plasma volume was actually greater in group CON which should, if anything, lead to a lower GFR. Although previous studies in the dog did not show significant differences in plasma creatinine concentrations (1) or creatinine clearances (3) between the alkalotic and corrected state, in a micropuncture study, Mello Aires and Malnic (25) reported lower inulin clearance in maintained CDA despite inulin spaces that did not differ in control and alkalotic rats. Thus, in agreement with previous studies in the rat, our data show a decreased GFR in alkalotic as compared with control rats not explained by differences in plasma volumes and mean arterial blood pressures.

The mechanism of the reduced GFR was not specifically investigated in this study. Nevertheless, within group CDA, proximally determined mean SNGFR did not parallel the decrement in experimental kidney GFR and was higher ($P < 0.05$) than that determined from distal punctures; this suggests interruption of tubuloglomerular feedback (TGF). Considering the signal for TGF, Bell and Navar (26) have reported that luminal osmolality in the earliest distal convolution is inversely correlated with SNGFR while others have argued that either chloride transport dependent upon luminal Cl concentration at the macula densa (27) or absolute chloride reabsorption within the loop segment (28) acts as the TGF signal and is inversely correlated with SNGFR. We have previously shown in rats infused with NaHCO₃ that decreased chloride delivery to and

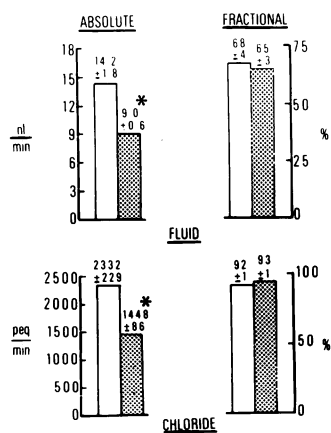


Figure 2. Absolute and fractional fluid and chloride reabsorption in the loop segment in CON (clear bars) and CDA (stippled bars) groups. *, $P < 0.05$; mean±SE is given over each bar.

Table VI. Summary of Late Distal Micropuncture and Microinjection Data

	Group		P value
	CON	CDA	
Tubule fluid flow rate (nl/min)	6.3±0.9 (23)*	2.9±0.4 (26)	<0.01
Cl concentration (peq/nl)	29.4±3.7	26.6±2.6	NS
Cl delivery to CDS (peq/min)	188±39	72±8	<0.01
³ H recovery (%) (40 peq/nl)‡	95.5±0.9 (11)	97.0±0.9 (20)	NS
³⁶ Cl recovery (%)	73.7±2.6	33.9±3.8	<0.01
³ H recovery (%) (100 peq/nl)‡	—	95.4±1.1 (8)	NS§
³⁶ Cl recovery (%)	—	41.5±7.7	NS§

* Number of observations.

‡ Chloride concentration of the injectate.

§ Compared with 40 peq/nl injectate within group CDA.

concomitantly decreased absolute chloride reabsorption in the loop segment, such as seen in group CDA, were associated with increased osmolality but decreased chloride concentration in early distal tubule fluid (23). In preliminary studies in rats handled identically to groups CON and CDA, we have found early distal tubule fluid osmolality higher ($P < 0.01$) in group CDA (142 ± 6 mosmol/kg) than that in group CON (101 ± 8 mosmol/kg). Since the plasma bicarbonate concentration was high and proximal tubule tubular fluid/plasma inulin and TF/UF chloride were not different from control, we assume that the bicarbonate concentration in tubule fluid delivered to the loop segment is higher than normal and, therefore, that the impermeance of bicarbonate in the thick ascending limb of Henle's loop (29, 30) probably accounts for these findings. If TGF is the mechanism by which GFR is reduced in CDA, our data would be most compatible with luminal osmolality as the signal, particularly since luminal chloride concentration and absolute chloride reabsorption in the loop segment (the other two proposed signals) were actually lower in CDA rats with a lower SNGFR.

The CDS avidly retained chloride in group CDA rats as shown by the reduced recovery of ³⁶Cl, which was unrelated to tubule fluid flow rate or chloride delivery. Although ³⁶Cl uptake by microinjection provides only a semiquantitative index of unidirectional chloride flux, these indices agree in direction with net fractional chloride reabsorptions in the CDS of 99% in group CDA and 92% in group CON.³ We have previously provided evidence that hypochloremia per se accelerates chloride uptake in this segment (16). To the extent that determinations from the late distal segment of superficial nephrons are representative of delivery of fluid and solute from deeper nephrons, the observations in the present study are consistent with our earlier

3. If chloride delivery to the CDS, as determined in the microinjection studies, is assumed to be the same for the 30,000 nephrons of a rat kidney, estimated absolute chloride reabsorption in the CDS is 5,640 neq/min less urinary chloride excretion of 418 neq/min and 2,160 neq/min less 16 neq/min for groups CON and CDA, respectively.

findings. In their study of maintained CDA, Mello Aires and Malnic (31) also showed that fractional chloride/inulin fluid/plasma ratios were decreased only in urine and suggested that metabolic alkalosis accelerates chloride reabsorption in the CDS. In direct micropuncture studies of the rat papilla, Diezi and co-workers (32) showed enhancement of chloride uptake in the chloride-deprived rat in the collecting duct and suggested that chloride transport in this segment is active. Although tubule fluid flow rate or chloride delivery did not correlate with ³⁶Cl uptake in the CDS, both of these variables were lower in the alkalotic rats. Since DuBose et al. (33) have shown that chloride load to the CDS is positively correlated with fractional chloride excretion in the urine, the lower chloride delivery in CDA may also serve to promote chloride conservation in the CDS.

The proximal convoluted tubule and the loop segment separately and together had similar fractional chloride reabsorptions in both CON and CDA groups, observations that are in essential agreement with previous micropuncture studies of maintained CDA. Mello Aires and Malnic (31) found no differences in late proximal and early distal fractional chloride reabsorptions between control and alkalotic rats. More recently, Cogan (34) also concluded that superficial proximal tubule chloride reabsorption is not accelerated during metabolic alkalosis. Taken together, our observations and those of others suggest that intrinsic changes in reabsorption within the proximal tubule and loop of Henle do not contribute importantly to enhanced chloride reabsorption during correction of hypochloremia in CDA.

Alterations in chloride reabsorption in response to various maneuvers have not been extensively studied in the DCT. However, Sheth and co-workers (35) have shown that the DCT is an important site for adjustments in chloride balance during volume expansion. Although the micropuncture data from the early and late DCT were obtained in separate groups of rats in this study, fractional reabsorption of delivered chloride in the DCT in group CDA (estimated by subtracting chloride delivery out of from delivery into the DCT) is 34.5% compared with 1.6% in group CON. Moreover, if the filtered chloride loads for

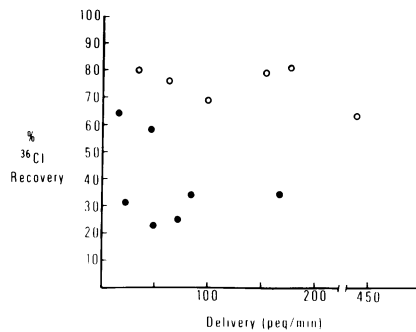


Figure 3. Relationship between urinary ^{36}Cl recovery and chloride delivery to the collecting duct segment in CON (O) and CDA (●) groups. The correlation coefficient (r) is 0.04.

groups CON and CDA are considered representative for both the micropuncture and microinjection series, fractional chloride delivery to the DCT did not differ (CON, $4.8 \pm 0.8\%$; CDA, $4.5 \pm 0.9\%$; $P = \text{NS}$), whereas chloride delivery out of the DCT did differ (CON, $4.8 \pm 1.0\%$; CDA, $2.9 \pm 0.3\%$; $P = 0.05$). Both of these estimates suggest enhanced fractional chloride uptake in the DCT. These observations for the CDS and the DCT are consistent with several earlier observations and proposals (36–39).

The mechanism(s) by which these adjustments for chloride reabsorption are made in the DCT is speculative. The variability in the absolute delivery of chloride to the DCT in control rats may have masked significant differences between the groups. Thus, we cannot separate the roles of delivery and enhanced intrinsic chloride uptake, but suspect that both may contribute.

Our data also show that alterations in the intranephronal distribution of fluid reabsorption are unnecessary during correction. Specifically, fractional fluid reabsorptions did not differ at the late proximal or early distal tubule or within the loop segment. Mello Aires and Malnic (25) also found no differences in late proximal or early distal tubular fluid/plasma inulin ratios although their animals were studied during infusion of 5% mannitol in isotonic NaHCO_3 at 5 ml/h. Thus, in different models of alkalosis in the rat, no redistribution of intranephronal fluid reabsorption has been detected and, at least in the rat, recovery from CDA can proceed without such adjustments.

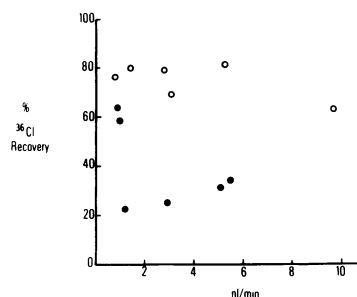


Figure 4. Relationship between urinary ^{36}Cl recovery and late distal tubule fluid flow rate in CON (O) and CDA (●) groups. The correlation coefficient (r) is 0.01.

Metabolic alkalosis may stimulate the production and excretion of other organic anions (40, 41), alter intestinal anion transport (42), and lead to shifts in anions between extracellular and intracellular spaces (43). Our studies do not address these possible extrarenal homeostatic mechanisms but on the basis of our estimates of plasma volume, net intercompartmental fluid shifts do not appear to participate detectably in the correction of plasma anion composition.

Arterial CO_2 tensions were lower than normal (36 ± 1 mmHg) in both groups CON (27 ± 2) and CDA (34 ± 1) and thus merit comment. We attribute the lower PaCO_2 to diaphragmatic stimulation by both peritoneal dialysis and mechanical retraction for exposure of the kidney for micropuncture. The effect of acute changes in PaCO_2 on chloride reabsorption has not been extensively studied. Warren et al. (39) showed that rats inspiring 12% CO_2 had no change in proximal TF/UF chloride ratios or in urinary chloride excretion until at least 3 h of exposure; they did not study hypocapnia. Jacobson (44) has shown that chloride reabsorption in the isolated perfused proximal tubule of the rabbit is enhanced when exposed to a CO_2 tension of 15 mmHg. It seems unlikely that a 7-mmHg difference in PaCO_2 can explain the correction of the plasma Cl and tCO_2 concentrations toward normal. Since group CON had the greater degree of hypocapnia, we would have expected progressive and greater increments in plasma chloride concentration, and a lower proximal TF/UF chloride ratio in group CON. Since none of these differences was seen and plasma chloride concentration remained stable in group CON, we suggest that the small difference in PaCO_2 does not contribute importantly to chloride reabsorption in this experimental setting.

In summary, acute chloride-depletion alkalosis in the rat can be corrected by the provision of chloride and without volume expansion or alterations in intranephronal fluid reabsorption. During correction, the renal contribution to correction of hypochloremia is achieved by enhanced fractional reabsorption of chloride in the collecting duct and probably the distal convoluted tubule and, in part, by a reduction in filtered chloride.

How might these observed differences in renal functions contribute to homeostasis—especially for sodium—in the maintenance phase of CDA? Since the delivery of excessive amounts of bicarbonate beyond the proximal tubule is likely to result in bicarbonaturia and sodium depletion, any reduction in GFR will decrease delivery of HCO_3^- out of the proximal tubule and thus tend to prevent such losses. Nevertheless, such filtration-mediated decrements in HCO_3^- delivery in maintenance CDA are counterbalanced by an elevated plasma bicarbonate. Since it is poorly reabsorbed in the loop of Henle (29, 30), any additional bicarbonate delivered out of the proximal tubule will be presented to distal sites, which have a limited capacity for reabsorption. We have previously shown that CDA, such as in the present study, is a potent stimulus to renin release (12), and, presumably would lead to enhanced aldosterone secretion which would, in turn, serve to accelerate bicarbonate reabsorption at sites beyond the loop segment. In addition, Stone et al. (45) have recently reported that decreased luminal chloride concen-

tration independently of sodium increases bicarbonate reabsorption in the medullary collecting duct. All of these mechanisms that maintain alkalosis and conserve sodium are not dependent on sodium or volume depletion but on chloride depletion alone. Thus, it is possible that CDA in the rat may not necessarily be associated with concomitant sodium depletion. Previous data (16, 32) as well as the present experiments show that chloride depletion enhances chloride uptake in the collecting duct and perhaps the distal convoluted tubule. Subsequent repletion of chloride, in this schema, would allow the distal nephron to restore acid/base balance without the necessity of volume expansion and, indeed, even in the presence of slight but significant volume contraction, although the precise signals that evoke these alterations in anion transport have not been established.

None of the aforementioned mechanisms excludes the possibility that, when concomitant volume depletion accompanies CDA, additional processes including the intranephronal redistribution of fluid reabsorption promote renal conservation of sodium and bicarbonate. Nevertheless, the notion that chloride depletion, per se, can alter renal function so as to maintain alkalosis and fluid volume brings us full circle to an earlier position of Gulyassy et al. (1) “. . . provision of chloride was the factor critical to the corrective process (for metabolic alkalosis).”

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