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Research Article

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ABSTRACT This free-flow micropuncture study examined the dependence of bicarbonate reabsorption in the rat superficial proximal convoluted tubule to changes in filtered bicarbonate load, and thereby the contribution of the proximal tubule to the whole kidney's response to such changes. The independent effects of extracellular fluid (ECF) volume expansion and of acidosis on proximal bicarbonate reabsorption were also examined.

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Metabolic acidosis was associated with very high fractional proximal total CO_2 reabsorptive rates of 0.82 to 0.91 over a wide range of SNGFR and ECF volumes. At a single level of SNGFR, end-proximal total CO_2 concentration progressively decreased from 5.6±0.5 to 1.6±0.2 mM as arterial pH fell from 7.4 to 7.1. Expansion of ECF volume in the acidotic rats did not inhibit the ability of the proximal tubule to lower end-proximal total CO_2 concentrations to minimal levels.

In conclusion, bicarbonate reabsorption in the superficial proximal convoluted tubule is highly load-dependent (75–90%) in normal and acidotic rats. No inhibitory effect of ECF volume per se on proximal bicarbonate reabsorption, independent of altering the filtered bicarbonate load, could be discerned. Acidosis enabled the end-proximal luminal bicarbonate concentration to fall below normal values and reduced distal bicarbonate delivery.

INTRODUCTION

The filtered load of bicarbonate is known from clearance studies to be a major determinant of renal bicarbonate reabsorption. Pitts and Lotspeich (1) in 1946 suggested that the two components of the filtered bicarbonate load, the glomerular filtration rate (GFR)[1] and the ultrafiltrate bicarbonate concentration, affected whole kidney bicarbonate reabsorption in the dog in somewhat different ways. Bicarbonate reabsorption was found to saturate as a function of concentration but was linearly related to GFR.

More recent clearance studies have proposed that the extracellular fluid (ECF) volume status of the animal is an additional determinant of whole kidney bicarbonate reabsorption. Purkerson et al. (2) found that the point at which the bicarbonate reabsorptive capacity of the rat kidney would saturate as a function of bicarbonate concentration could be modified and even

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[1] *Abbreviations used on this paper*: B_o, arterial hematocrit obtained after anesthesia but before surgery; ECF, extracellular fluid; GFR, glomerular filtration rate; KWt, kidney weight; SNGFR, single nephron glomerular filtration rate; tCO_2, total carbon dioxide content.

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abolished by limiting ECF expansion. Kurtzman (3) and Cohen (4, 5) in dogs and Slatopolsky et al. (6) in man confirmed the effect of an increase in ECF volume to suppress whole kidney bicarbonate reabsorption independent of the filtered bicarbonate load.

Systemic arterial pH has also been suggested to play an independent role in modifying bicarbonate reabsorption by some (7, 8), though not all investigators (9, 10). Acidosis may act, therefore, to enhance hydrogen ion secretion even when the filtered load and ECF volumes are constant.

Changes in proximal tubule bicarbonate transport were assumed in these clearance studies to be principally responsible for the observed changes in whole kidney bicarbonate reabsorption when the filtered bicarbonate load was altered. This assumption was based on the micropuncture observation that under volume-contracted conditions (hydropenia), the superficial proximal convoluted tubule is responsible for approximately 90% of the bicarbonate reabsorbed (11–13). However, Levine et al. (14) have reported that an increase in single nephron glomerular filtration rate (SNGFR) above hydropenic levels caused no increase in absolute bicarbonate reabsorption in the superficial proximal convoluted tubule. Such a finding might suggest that during hydropenia the proximal convoluted tubule had already been saturated, or reached a transport maximum (T_m), and could not further augment bicarbonate reabsorption as the filtration rate is increased. A constancy of absolute bicarbonate reabsorption in the superficial proximal convoluted tubule raises the possibility that the increase in whole kidney bicarbonate reabsorption observed with increasing GFR is mediated by nephron segments other than the superficial proximal convoluted tubule (e.g., juxtamedullary nephrons and distal tubule). Such segments can have a large capacity for reabsorbing bicarbonate, as much as 50% of the filtered bicarbonate load under certain circumstances (carbonic anhydrase inhibition).[2]

A similar question concerns the site of action whereby ECF expansion reduces whole kidney bicarbonate reabsorption. It has been proposed that the suppression of bicarbonate reabsorption by increased back-leak of bicarbonate induced by ECF expansion occurs in the proximal tubule (15–17). Finally, the site of enhancement of whole kidney bicarbonate reabsorption by progressive acidosis has also not been systematically studied. Split droplet experiments have suggested, however, that the minimal pH to which the proximal convoluted tubule lumen can be acidified is reduced in systemic acidosis (7).

[2] Cogan, M. G., D. A. Maddox, D. G. Warnock, E. T. Lin, and F. C. Rector, Jr. 1979. The effect of acetazolamide on bicarbonate reabsorption in the proximal tubule of the rat. *Am. J. Physiol.* In press.

The purpose of this free-flow micropuncture study was to examine the dependence of bicarbonate reabsorption in the proximal tubule on isohydric alterations in the GFR. We wished to assess the degree to which the superficial proximal convoluted tubule participates in the whole kidney response to increases in GFR. We also wished to ascertain the extent to which the relationship between proximal bicarbonate reabsorption to GFR is altered either by ECF volume status or by acidosis.

METHODS

Animals

55 male Munich-Wistar rats (Simonsen Laboratories, Gilroy, Calif.) averaging 216±4 g in weight were studied.

Group I (normal pH: hydropenia–plasma repletion–aortic constriction). 12 animals comprised this group. They were maintained on Purina rat chow pellets (Ralston Purina Co., St. Louis, Mo.) and tap water ad-lib before anesthetization for micropuncture surgery. The kidney was micropunctured twice: after the preparatory surgery was complete (hydropenia period) and after the plasma lost during the surgical preparation was restored with homologous, isoncotic plasma (plasma repletion period), as described below. One-half of these animals were studied a third time while the renal perfusion pressure was reduced by constriction of the abdominal aorta (group I_A).

Group II (normal pH: plasma expansion). Seven rats on a normal diet were studied after a 5% body wt homologous, isoncotic infusion of plasma. Micropuncture was not performed during hydropenia.

Group III (normal pH: plasma expansion (+K^+)–bicarbonate Ringer's expansion). Eight rats were studied identically to Group II except that sufficient crystalline KCl was added to the 5% body wt plasma infusion to achieve a concentration of 60 meq/liter. A second period of micropuncture was also performed in seven of the rats after a 10% body wt infusion of bicarbonate Ringer's solution with supplemental potassium containing: NaCl, 105 mM; $NaHCO_3$, 25 mM; Na_2HPO_4, 4 mM; KCl, 25 mM; $MgSO_4$, 1 mM; and $CaCl_2$, 1.8 mM.

Group IV (normal pH: hydropenia–bicarbonate Ringer's expansion). Six rats on a normal diet were studied during hydropenia and after a 10% body wt infusion of bicarbonate Ringer's solution (same composition as used in group III).

Group V (mild acidosis: hydropenia–plasma repletion). Six rats were studied after ingestion of a meal containing 0.5 g casein. They were anesthetized 2 h after receiving the meal, by which time the meal had been consumed but before ingested organic acids could be excreted (18), to study the effect of acute acidosis on proximal bicarbonate reabsorption. The diet was 40 ml/d for 10–14 d of a standard electrolyte-deficient diet (18) supplemented with 6 meq/d of NaCl and 1.5 meq/d of KCl in two divided feedings, plus free access to tap water. A quantitative urine collection was made for determination of the average overnight sodium excretion rate before the study. The rats were micropunctured during hydropenia and after the plasma volume loss resulting from surgery was replaced (plasma repletion period) as in the group I animals.

Group VI (mild acidosis: NaCl expansion). Five rats on a normal diet were studied after a 10% body wt isotonic NaCl infusion (plus 20 meq/liter KCl). No micropuncture was performed during hydropenia.

Group VII (severe acidosis: hydropenia–isohydric expansion). 11 animals were maintained for 1–5 d on the standard

electrolyte-deficient diet as in group V, supplemented with 3 meq/d of NaCl, 1.5 meq/d of KCl, and 6 meq/d of NH_4Cl. Isotonic NH_4Cl drinking water was provided. 90 min before anesthetization the animals were gavaged with 5 ml of 0.5 M NH_4Cl. All rats were studied during hydropenia and five were studied a second time after isohydric expansion (10% body wt) with a modified Ringer's solution: NaCl, 124 mM; $NaHCO_3$, 6 mM; Na_2HPO_4, 4 mM; KCl, 5 mM; $MgSO_4$, 1 mM; and $CaCl_2$, 1.8 mM.

Micropuncture protocol

All animals had free access to food and water before anesthetization with Inactin, 100–120 mg/kg i.p. Rectal temperature was continuously monitored (model 73A, Yellow Springs Instrument Co., Yellow Springs, Ohio) and maintained at 37°C using a heated micropuncture table. A PE-50 catheter was inserted into the left femoral artery for blood pressure monitoring (model P23Db, Gould-Statham Instruments, Hato Rey, Puerto Rico) and for blood sampling. Immediately after arterial catheter insertion, a blood sample of 180 μl was withdrawn for the determination of the hematocrit, referred to as the B_0 hematocrit. The B_0 hematocrit has been previously shown to be equal to the hematocrit in the calm awake rat (19, 20). This and all subsequent blood samples were quantitatively replaced with whole blood obtained on the day of study from another Munich-Wistar rat maintained on the same diet as the rat under study. PE-50 catheters were then inserted into both jugular veins for infusions and a tracheostomy was performed. Incisions were sutured closed to minimize evaporative losses. The left kidney was then exposed through an abdominal approach, stabilized, and bathed continuously with an isotonic NaCl drip maintained at 37°C. Excess bathing fluid was drained away from the peritoneal cavity using a parafilm trough placed under the kidney. The ureter was cannulated with PE-50 tubing. Oil was placed over all exposed abdominal surfaces to diminish evaporative losses. A priming dose of 15–20 μCi of [^{14}C]inulin (New England Nuclear, Boston, Mass.) was then given, followed by a sustaining infusion of 60 μCi/h in an isotonic Ringer's solution (groups I–IV) or a solution of 145 meq/liter NaCl and 5 meq/liter KCl (groups V–VII) at 0.87 ml/h.

After an equilibration period of 45–60 min, the first period of micropuncture (hydropenia period) was begun in groups I, IV, V, and VII. Bowman's space and end-proximal tubules were punctured for determination of inulin and total CO_2 concentrations. The last loops of proximal tubules were located by their typical appearance next to the efferent arteriolar "star" vessels and by the relatively thick capillaries that border them. The loops were confirmed as being the last accessible proximal convolutions by the failure of a small injected oil droplet to reappear in any subsequent proximal loop on the surface of the kidney. Collections were made from two to three end-proximal tubules and from Bowman's space using sharpened glass pipettes having an outside tip diameter of 6–9 μm. After an injection of an oil block 5–6 tubule diameters in length, 3- to 4-min exactly timed collections from these tubules were made. Unless the flow into the pipette was such that no or only slight suction was necessary to maintain the oil block in position, the sample was discarded. The oil used was paraffin oil stained with Sudan black. The oil was also equilibrated with a Hepes buffer solution, consisting of 100 mM Hepes with 48 mM $NaHCO_3$ and bubbled with 6.7% CO_2 gas to achieve a P_{CO_2} of 60–70 mm Hg. This value of P_{CO_2} was chosen because of the recent reports by two laboratories that the in vivo P_{CO_2} in the proximal tubule is as much as 15–40 mm Hg higher than the corresponding arterial P_{CO_2} (21, 22). The measured P_{CO_2} of the buffered solutions with which the blocking oil was equilibrated was 63.0±3.2 mm Hg (n = 14).

During the period that the tubule fluid was obtained, two to three consecutive, timed urine collections were made for estimates of whole kidney GFR and sodium, potassium, and total CO_2 excretory rates. The urine was collected in constant bore, calibrated glass vessels with water-equilibrated paraffin oil on each side. Three to four blood samples of 180 μl each were interspersed throughout the period for the determination of the hematocrit and the sodium, potassium, protein, and inulin concentrations. An additional blood sample of 270 μl was taken for determination of the arterial pH and P_{CO_2}. The mean of the tubule fluid or urine collection data was used to represent each period.

Plasma infusions. After the first hydropenic study was completed in groups I and V, or an equivalent time had elapsed in groups II and III, a plasma infusion was begun. This infusion consisted of isoncotic plasma obtained on the same day from another Munich-Wistar rat that had been on the same diet as the rat under study. The amount of plasma given for plasma repletion (groups I and V) was equal to that lost subsequent to the micropuncture surgery, as reflected by the rise in hematocrit from B_0 (before neck and abdominal surgery) to that in the postsurgical, hydropenic period (19, 20). Sufficient plasma was given in a 20–40-min period to restore the hematocrit as close as possible to the B_0 hematocrit. The amount of plasma needed has been previously determined to be ≅0.18% body wt of plasma for each volume percent change in hematocrit (19). Therefore, an average of 1.3% body wt of plasma was needed to restore the average hematocrit rise of 7.4% in group I, as seen in Table I. A 5% body wt plasma infusion was given to expand groups II and III at 250 μl/min. A sustaining plasma infusion of 5 μl/min in groups I and V and 40 μl/min in groups II and III was then instituted to prevent the hematocrit from rising again. After an equilibration period of 30–45 min, a second fresh set of end-proximal tubules and Bowman's spaces were punctured with the concomitant blood and urine collections.

To further evaluate the relative importance of plasma volume and bicarbonate delivery to bicarbonate and water reabsorption, six of the group I animals underwent another period of study after the two periods detailed above. In these rats the abdominal aorta superior to the left renal artery was constricted to achieve a femoral arterial and renal perfusion pressure of 75–80 mm Hg to reduce GFR towards the hydropenic level. The sustaining plasma infusion was continued. Thus, the plasma volume status of the animal was maintained in the repleted state of the second period, but SNGFR was reduced to levels found in the plasma volume contracted state of the first period. Fresh end-proximal tubules and Bowman's spaces were punctured in this third period.

Colloid-free infusions. After the plasma expansion period in group III, the hydropenia period in groups IV and VII, or an equivalent time in group VI, isotonic solutions were infused at 250 μl/min until 10% body wt was attained, followed by a sustaining infusion of 100 μl/min. After an equilibration period of 30–45 min, micropuncture was performed along with blood sampling and urine collections.

At the termination of the experiment, the kidney was excised, blotted, and weighed.

Analytical

The volume of the collected tubule fluid and Bowman's space samples was determined by injecting them into constant bore capillary glass tubing with a known volume per length and by measuring the length occupied. The samples were

in contact with the Hepes buffer-equilibrated oil at all times. A 20-nl aliquot was taken for total CO_2 measurement and the remaining sample remeasured and then mixed in a 1:4 mixture of acetic acid:aquasol (New England Nuclear) for scintillation counting (mark II, Nuclear-Chicago, Chicago, Ill.). Total CO_2 (tCO_2) measurements of tubule fluid, plasma, and urine were performed by microcalorimetry (picapnotherm), as described (23). Within the physiologic pH range, the tCO_2 in a sample represents bicarbonate plus the dissolved CO_2 gas. Urine and plasma sodium and potassium concentrations were measured by flame photometry (model 343, Instrumentation Laboratory, Inc., Lexington, Mass.) and [^{14}C]inulin activity by scintillation counting. Blood pH and P_{CO_2} were measured by a Corning model 165 blood gas analyzer (Corning Medical, Corning Glass Works, Medfield, Mass.). Plasma protein concentration was determined by refractometry.

Calculations

The SNGFR and whole kidney GFR were estimated from the single nephron and whole kidney inulin clearances, respectively. The absolute proximal water reabsorption was the difference in SNGFR and end-proximal flow rates. Similarly, the absolute tCO_2 reabsorption (APR_{tCO_2}) was the difference in the filtered tCO_2 (SNGFR × Bowman's space [tCO_2]) and the tCO_2 delivered out of the proximal tubule (end-proximal flow rate × end-proximal [tCO_2]). The fractional reabsorption of water or tCO_2 (FPR_{tCO_2}) was the absolute reabsorption divided by the SNGFR or filtered tCO_2, respectively. All results are factored by kidney weight (KWt) to help compensate for differences caused by animal size.

Several animals had insufficient surface glomeruli for puncture in the second period. The Bowman's space [tCO_2] of the second period was then estimated by adding the difference in the arterial plasma [tCO_2] (corrected for changes in plasma protein) in the two periods to the Bowman's space [tCO_2] of the first period. Arterial [tCO_2] was calculated from the arterial pH and P_{CO_2} using the Henderson-Hasselbalch equation, using a pK_a of 6.1 and a CO_2 solubility coefficient of 0.03. Validation for this estimation was subsequently obtained by comparing the values for plasma water [tCO_2] obtained using blood gas measurements to the corresponding values obtained using microcalorimetry. A ratio of unity was obtained (1.003±0.015, n = 49). Furthermore, the plasma water [tCO_2] has been shown to bear a constant relationship over a wide range of concentrations to the ultrafiltrate [tCO_2]. The ratio of the Bowman's space [tCO_2] to the corresponding arterial plasma water [tCO_2] measured by microcalorimetry is 1.05±0.01 (n = 53).

The logarithmic mean [tCO_2] (log mean [tCO_2]) in the tubule was calculated using the [tCO_2] for Bowman's space ([tCO_2]$_{BS}$) and for the end-proximal tubule ([tCO_2]$_{EP}$) by the formula (24):

$$\text{log mean } [tCO_2] = \frac{[tCO_2]_{BS} - [tCO_2]_{EP}}{\ln[tCO_2]_{BS} - \ln[tCO_2]_{EP}}$$

Results are expressed as the mean±SEM. Statistical significance was assesed using the paired t test for results obtained in the same animal or the unpaired t test for comparisons between groups.

RESULTS

Arterial blood composition (Table I)

Hematocrits were returned to the presurgical B_0 values by plasma repletion in groups I and V. Hematocrits fell below the B_0 value in the other groups because of volume expansion. Infusions with plasma tended to raise plasma protein concentrations, whereas colloid-free expansions markedly reduced plasma protein concentrations. The large increase in protein concentration in group III by plasma infusion (7.7±0.2 g/dl) was corrected toward the plasma replete value by Ringer infusion (5.7±0.2 g/dl). Plasma potassium concentrations were within the normal range in all groups after infusions with the exception of group II in which hypokalemia (3.5±0.1 meq/liter) developed after isoncotic plasma expansion. This abnormality was corrected in group III by addition of potassium to the plasma infusion (4.3±0.1 meq/liter). Arterial pH was normal in groups I–IV while acidosis was mild in groups V (postprandial) and VI (NaCl dilution) and severe in group VII (NH_4Cl loading). Arterial P_{CO_2} values were relatively similar among the groups except for group VII, which was slightly lower because of partial respiratory compensation for the metabolic acidosis.

Whole kidney filtration and excretion rates (Table II)

GFR increased above hydropenia levels in all groups after infusion. Sodium excretion rate after plasma repletion in group I (757±161) was similar to the estimated awake sodium excretion (800 neq/min·kidney) of a rat on a chow diet receiving an inulin infusion (25). Likewise, the sodium excretion in group V after plasma repletion (2,534±391 neq/min) was similar to the measured overnight sodium excretion of 1,912±147 neq/min. Confirming the existence of ECF expansion in groups II–IV, VI, and VII, sodium and estimated chloride excretion in each was considerably greater than plasma replete values. The mean estimated chloride excretion rates ($U_{Na}V + U_KV - U_{tCO_2}V$) were (in neq/min): group II, 6,447; group III, 8,993 and 7,454; group IV, 4,884; group VI, 7,586; and group VII, 7,596, compared to 2,357 in the normal group I plasma-repleted animals. Also confirming ECF expansion was the fact that bicarbonaturia developed in groups II–IV, whereas it did not in the plasma repleted group I animals. No acidotic animal developed bicarbonaturia.

Proximal bicarbonate and water reabsorption in normal rats (Table III)

Plasma Repletion

Group I (normal pH: hydropenia–plasma repletion–aortic constriction). In hydropenia these animals had a normal arterial pH of 7.38±0.01 and P_{CO_2} of 38.0±1.1 mm Hg. Bowman's space [tCO_2] was 27.2±0.8 mM. The tubule fluid to plasma water (TF/P) inulin concentration ratio in those Bowman's space samples having

TABLE I
Arterial Blood Composition

Group	Period	Hematocrit	Plasma [protein]	Plasma [K^+]	pH	P_{CO_2}
		Vol %	*g/dl*	*meq/liter*		*mm Hg*
Normal pH						
I (n = 12)	Hydropenia	53.4±0.8* (B_o = 46.0±0.7)	5.0±0.1	4.9±0.2	7.38±0.01	38.0±1.1
BWt = 242±12 g	Plasma repletion					
KWT = 0.98±0.04 g	(≅1.3 BWt)	45.9±0.6	5.2±0.1	4.0±0.1	7.41±0.01	35.1±1.2
	P <‡	0.001	0.025	0.001	0.005	0.025
II (n = 7)	Plasma expansion					
BWt = 216±4 g	(5% BWt)	36.4±0.5§ (B_o = 48.9±1.0)	7.4±0.2§	3.5±0.1¶	7.39±0.01	39.6±0.7‖
KWt = 0.98±0.04 g						
III (n = 8)	Plasma expansion					
BWt = 210±3 g	$+K^+$ (5% BWt)	36.2±0.2§ (B_o = 47.6±1.2)	7.7±0.2§	4.3±0.1	7.41±0.02	39.1±1.9
KWt = 0.89±0.03 g	Bicarbonate ringer expansion (10% BWt)	36.2±0.9§	5.7±0.2‖	4.1±0.1	7.45±0.01‖	33.6±1.6
	P <‡	NS	0.001	NS	NS	0.05
IV (n = 6)	Hydropenia	52.0±0.5 (B_o = 47.3±1.1)	5.1±0.1	4.3±0.1‖	7.38±0.01	37.9±0.8
BWt = 213±6 g	Bicarbonate ringer expansion (10% BWt)	44.9±0.9	3.7±0.1§	4.1±0.2	7.42±0.01	35.8±0.8
KWt = 0.89±0.04 g	P <‡	0.005	0.001	NS	0.005	NS
Acidosis						
V (n = 6)	Hydropenia (mild acidosis)	52.5±1.3 (B_o = 45.1±1.4)	4.2±0.2¶	4.5±0.1	7.27±0.01§	35.6±1.4
BWt = 201±14 g	Plasma repletion					
KWt = 0.84±0.07 g	(≅1.3% BWt)	45.7±1.4	4.5±0.2‖	4.1±0.3	7.34±0.02¶	34.0±0.8
	P <‡	0.001	0.025	NS	0.05	NS
VI (n = 5)	NaCl expansion					
BWt = 212±6 g	(10% BWt)	46.3±1.0 (B_o = 48.4±0.8)	3.6±0.1§	4.6±0.2‖	7.30±0.03§	34.5±1.4
KWt = 0.93±0.03 g						
VII (n = 11)	Hydropenia					
BWt = 204±5 g	(severe acidosis)	51.8±1.3 (B_o = 49.2±1.1)	5.3±0.1	5.2±0.2	7.05±0.02§	29.2±1.0§
KWt = 0.93±0.02 g	Isohydric expansion					
	(10% BWt)	48.0±3.0	3.5±0.1§	4.1±0.1	7.17±0.02§	28.5±1.5¶
	P <‡	0.025	0.025	0.001	0.005	NS

BWt, body weight.
* Mean±SEM.
‡ P values for second period compared to first period in same group.
§ $P < 0.001$ for value compared to that in group I during the same period.
‖ $P < 0.05$ for value compared to that in group I during the same period..
¶ $P < 0.01$ for value compared to that in group I during the same period.

sufficient volume for [^{14}C]inulin counting was 1.03 ±0.01 (n = 25).

After plasma repletion, SNGFR rose by 50% from 33.4±1.5 to 51.6±1.6 nl/min·g KWt ($P < 0.001$). Since the [tCO_2] in Bowman's space remained constant (Fig. 1A) the filtered load of tCO_2 (SNGFR × Bowman's space [tCO_2]) also rose by 50% from 906±57 to 1,412±82 pmol/min·g KWt (Fig. 1B). Absolute proximal reabsorption of tCO_2 increased as well (Fig. 1D) but by only 30%, from 808±47 to 1,081±57 pmol/min·g KWt ($P < 0.001$). The fractional reabsorption thus declined from 0.90±0.01 to 0.77±0.02 ($P < 0.001$, Fig. 1C).

In spite of the substantial increase in SNGFR, water reabsorption rose only slightly after plasma repletion

TABLE II
Whole Kidney GFR and Urinary Excretion Rates

Group	Period	GFR	$U_{Na}V$	U_KV	$U_{tCO_2}V$
		ml/min·gKWt	*neq/min*	*neq/min*	*nmol/min*
Normal pH					
I	Hydropenia	1.10±0.04*	93±22	487±61	<10
	Plasma repletion (≅1.3 BWt)	1.42±0.03	757±161	1,600±128	<10
	P <‡	0.001	0.001	0.001	NS
II	Plasma expansion (5% BWt)	1.56±0.08§	4,514±713‖	2,456±298¶	523±134‖
III	Plasma expansion (5% BWt)	1.21±0.06§	5,230±765‖	4,111±324‖	358±100‖
	Bicarbonate ringer expansion (10% BWt)	1.12±0.07‖	5,577±997‖	2,545±166‖	668±150‖
	P <‡	NS	NS	0.01	NS
IV	Hydropenia	0.96±0.07	86±25	396±94	<10
	Bicarbonate ringer expansion (10% BWt)	1.42±0.11	2,879±508‖	2,082±365	77±36¶
	P <‡	0.025	0.005	0.025	0.05
Acidosis					
V	Hydropenia (mild acidosis)	1.07±0.05	244±90¶	560±76	<10
	Plasma repletion (≅1.3% BWt)	1.33±0.08	2,534±391‖	932±99§	<10
	P <‡	0.001	0.001	NS	NS
VI	NaCl expansion (10% BWt)	1.27±0.09	4,515±1,023‖	3,071±366‖	<10
VII	Hydropenia (severe acidosis)	0.79±0.07‖	552±155§	873±176	<10
	Isohydric expansion (10% BWt)	0.99±0.06‖	3,093±681‖	813±80§	<10
	P <‡	NS	0.025	NS	NS

$U_{Na}V$, sodium excretion rate; U_KV, potassium excretion rate; $U_{tCO_2}V$, tCO_2 excretion rate.
* Mean±SEM.
‡ P values for a second period compared to first period in same group.
§ $P < 0.01$ for value compared to that in group I during the same period.
‖ $P < 0.001$ for value compared to that in group I during the same period.
¶ $P < 0.05$ for value compared to that in group I during the same period.

from 16.3±0.8 to 19.0± 1.1 nl/min·g KWt ($P < 0.005$). Thus, fractional reabsorption of water declined from 0.49±0.02 to 0.37±0.01 ($P < 0.001$).

To examine whether the changes in tCO_2 reabsorption induced by plasma repletion were reversible, the filtered load of tCO_2 was reduced by aortic constriction in 6 of the 12 rats after the animals had been plasma volume repleted and with the maintenance plasma infusion continued. Thus, the plasma volume was continued in the repleted state of the second period, while the constriction reduced the filtered load of tCO_2 to that observed in the first period. The results are shown in Table III (group I_A) and in Fig. 2. Bowman's space [tCO_2] remained relatively constant during aortic constriction (Fig. 2A). Aortic constriction reduced the filtered tCO_2 load to values somewhat lower than obtained in hydropenia. Proportional to the changes in filtered load, absolute proximal reabsorption of tCO_2 fell from 1,039±101 to 595±73 pmol/min·g KWt ($P < 0.001$) after aortic constriction, also slightly below the hydropenic level (Fig. 2D). Thus, the fractional reabsorption of tCO_2 returned from the suppressed value during plasma repletion of 0.79±0.03–0.90±0.01 ($P < 0.005$), indistinguishable from the hydropenic level of 0.91 ±0.01. Likewise, the end-proximal [tCO_2] returned to 4.9±0.5 from 9.3±1.5 mM (Fig. 2C). Finally, fractional water reabsorption during aortic constriction returned to the higher hydropenic level of 0.50±0.03 from the reduced plasma repleted value of 0.36±0.02 ($P < 0.001$). Thus, aortic constriction returned the tCO_2 and water proximal reabsorptive rates to hydropenic levels by reducing the filtered load of tCO_2 and water, even though the plasma volume remained higher than had existed in hydropenia.

ISOHYDRIC ECF EXPANSION

Group II (plasma expansion). When compared to the plasma replete group I animals, no significant reduction in either absolute or fractional tCO_2 and water reabsorption was observed in this group. The filtered tCO_2 load (1543±122 pmol/min·g KWt) and absolute tCO_2 reabsorption (1,220±107 pmol/min·g KWt) were slightly, but not significantly higher than in group I. Fractional tCO_2 reabsorption (0.79±0.02) was similar to group I as was absolute and fractional water reabsorption, averaging 21.3±1.3 nl/min·g KWt and 0.41±0.01,

TABLE III

Effect of Isohydric ECF Expansion on Glomerular Filtration, Proximal Reabsorption, and Distal Delivery of tCO_2 and H_2O

Group	Period	Bowman's space [tCO_2]	SNGFR	Filtered tCO_2	End-proximal [tCO_2]	End-proximal flow rate	Distal delivery tCO_2	Absolute proximal reabsorption tCO_2	Fractional proximal reabsorption tCO_2	Absolute proximal reabsorption H_2O	Fractional proximal reabsorption H_2O
		mM	*nl/min·g KWt*	*pmol/min·g KWt*	*mM*	*nl/min·g KWt*	*pmol/min·g KWt*	*pmol/min·g KWt*		*nl/min·g KWt*	
I	Hydropenia	27.2±0.8*	33.4±1.5	906±57	5.6±0.5	17.1±1.0	98±12	808±47	0.90±0.01	16.3±0.8	0.49±0.02
	Plasma repletion	27.7±0.9	51.6±1.6	1,412±82	10.3±0.9	31.8±1.1	329±36	1,081±57	0.77±0.02	19.0±1.1	0.37±0.01
	P <‡	NS	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.005	0.001
I_A	Hydropenia	27.0±1.5	30.1±1.9	819±85	4.9±0.5	15.6±1.5	76±9	744±77	0.91±0.01	14.3±0.7	0.48±0.02
	Plasma repletion	27.8±1.6	48.9±2.6	1,322±129	9.3±1.5	30.7±1.1	283±50	1,039±101	0.79±0.03	17.1±1.7	0.36±0.02
	P <‡	NS	0.001	0.005	0.025	0.001	0.01	0.01	0.01	NS	0.01
	Aortic constriction	25.6±1.5	25.3±2.1	658±79	4.9±0.5	12.6±0.8	63±10	595±73	0.90±0.01	12.7±1.6	0.50±0.03
	P <‡	0.025	0.001	0.001	0.025	0.001	0.005	0.001	0.005	0.05	0.001
	P <§	0.05	0.05	0.025	NS	0.05	NS	0.025	NS	NS	NS
II	Plasma expansion (5% BWt)	29.4±1.2	52.3±2.7	1,543±122	10.2±0.8	31.0±1.6	323±36	1,220±107	0.79±0.02	21.3±1.3	0.41±0.01
III	Plasma expansion $+K^+$ (5% BWt)	27.2±0.8	54.8±2.5	1,502±90	8.9±0.8	31.6±1.5	283±29	1,212±92	0.81±0.02	23.3±1.7	0.42±0.02
	Bicarbonate ringer expansion (10% BWt)	25.4±1.2	58.2±4.3	1,488±167	8.5±0.7	38.2±2.3¶	330±42	1,158±139	0.78±0.02	20.7±3.1	0.35±0.03
	P <‡	0.05	NS	NS	NS	0.01	NS	NS	NS	NS	0.025
IV	Hydropenia	25.9±0.9	30.3±2.2	780±46	4.9±0.4	15.3±1.8	74±9	711±42	0.91±0.01	15.0±0.7	0.50±0.03
	Bicarbonate ringer expansion (10% BWt)	26.7±1.1	52.6±2.3	1,396±35	9.0±1.0	37.2±2.0	337±45	1,057±24	0.76±0.03	16.0±1.5¶	0.32±0.02¶
	P <‡	NS	0.001	0.001	0.01	0.001	0.005	0.001	0.005	NS	0.005

* Mean±SEM.

‡ P values for second period compared to first period in same group.

§ P values for aortic constriction period compared to hydropenia period.

¶ $P < 0.05$ for value compared to that in Group I during the same period.

respectively. Plasma volume expansion was thus consistent with a high rate of tCO_2 reabsorption.

Group III (plasma expansion-bicarbonate Ringer's expansion). To obviate the fall in plasma potassium concentration observed in the group II animals, potassium was added to the plasma. The potassium concentration was, therefore, kept in the normal range (4.3±0.1 meq/liter). No significant differences between

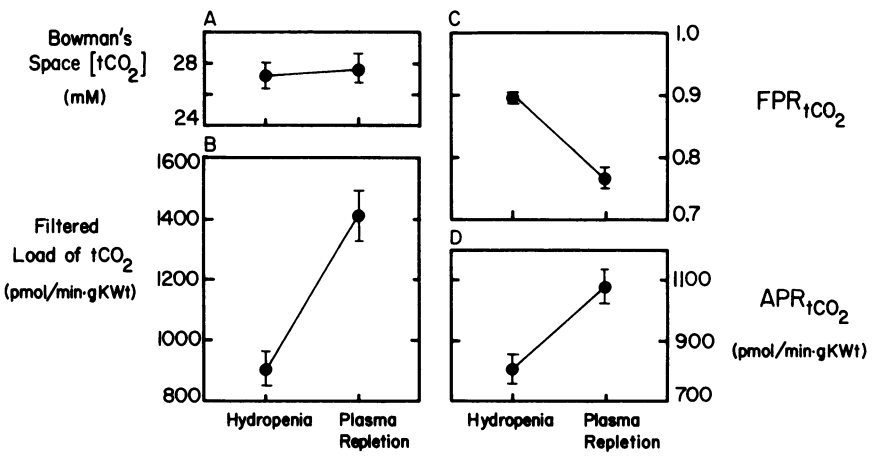

FIGURE 1 Filtration and reabsorption of tCO_2 and water for normal group I animals. Shown are mean±SEM for Bowman's space [tCO_2] (A), filtered load of tCO_2 (B), and fractional (C) and absolute (D) proximal reabsorption of tCO_2.

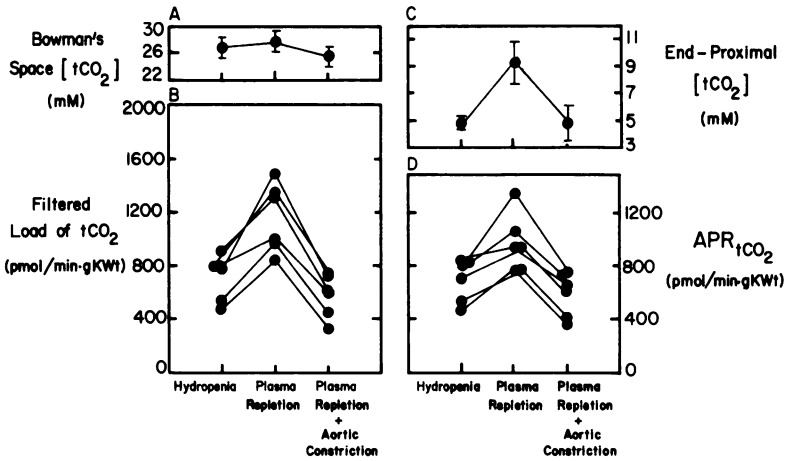

FIGURE 2 Filtration and reabsorption of tCO_2 for group I_A animals (n = 6) in hydropenia, plasma repletion, and plasma repletion with aortic constriction. Shown are mean±SEM or individual points for each animal for Bowman's space [tCO_2] (A), filtered load of tCO_2 (B), end-proximal [tCO_2] (C), and absolute proximal reabsorption of tCO_2 (D).

groups II and III were observed in absolute or fractional tCO_2 reabsorption. The similarity between the results of groups I and II does not appear to be caused by counter-balancing effects of a stimulus to reduce bicarbonate reabsorption (ECF expansion) and of one to increase reabsorption (hypokalemia). Another stimulus to independently enhance tCO_2 reabsorption might have been the hyperproteinemia, by increasing peritubular oncotic pressure. Bicarbonate Ringer's expansion (10% body wt) was, therefore, employed to reduce the plasma protein level toward that obtained in the plasma replete rats. As shown in Table III, even though the SNGFR was slightly higher than had been obtained in the first period, the filtered load and absolute reabsorption of tCO_2 was unchanged so that fractional reabsorption remained high at 0.78±0.02. Absolute and fractional water reabsorption fell slightly. Thus, mild hypokalemia and hyperproteinemia were excluded as substantially affecting the high fractional tCO_2 reabsorptive rates (≅80%) seen in groups II and III. ECF expansion per se could not be demonstrated to be an independent determinant for tCO_2 reabsorption.

Group IV (hydropenia–Ringer's expansion). Colloid-free infusion of 10% body wt bicarbonate Ringer's solution also increased absolute proximal tCO_2 reabsorption, from 711±42 to 1,057±24 pmol/min·g KWt. Filtered tCO_2 loads were comparable to those of group I. Fractional proximal tCO_2 reabsorption of 0.76±0.03 after expansion was thus indistinguishable from the plasma replete value. In contrast to the plasma infusions used in groups I–III, water reabsorption did not significantly increase in group IV (15.0±0.7 to 16.0±1.5 nl/min·g KWt) because of the colloid-free nature of the infusion. Therefore, an almost 50% increment in tCO_2 reabsorption occurred without a significant change in water reabsorption.

The results of tCO_2 reabsorption as a function of filtered load are shown in Fig. 3. Colloid infused animals are shown in closed circles and colloid-free infused animals in open circles. No evidence of reabsorptive saturation is discernible at these filtered loads.

Effect of acidosis on proximal tCO_2 reabsorption (Table IV)

Group V (mild acidosis: hydropenia–plasma repletion). Bowman's space [tCO_2] was somewhat lower in the acidotic rats than in the normal pH group I rats at 21.4±0.9 mM ($P < 0.001$) though SNGFR after plasma repletion was similar in each group. Absolute proximal tCO_2 reabsorption also increased in the group V rats, from 619±60 to 859±74 pmol/min·g KWt ($P < 0.025$), while fractional reabsorption fell from 0.91±0.01 to 0.82±0.02 ($P < 0.025$). Closed squares represent the plasma replete acidotic animals in Fig. 3. The end-proximal tubule [tCO_2] was lower, however, in both the hydropenic (3.5±0.3 mM) as well as the plasma volume replete state (6.5±0.8 mM) such that the distal delivery of tCO_2 in the acidotic rats was only about two-thirds in each period of that found in the normal pH group I rats.

Group VI (mild acidosis: NaCl expansion). NaCl infusion caused a rise in SNGFR accompanied by a fall in Bowman's space tCO_2 such that the filtered tCO_2 load (808±84 pmol/min·g KWt) remained similar to the hydropenic values. The absolute reabsorption of tCO_2 was also close to hydropenic levels (represented by open diamond symbols in Fig. 3), since these acidotic

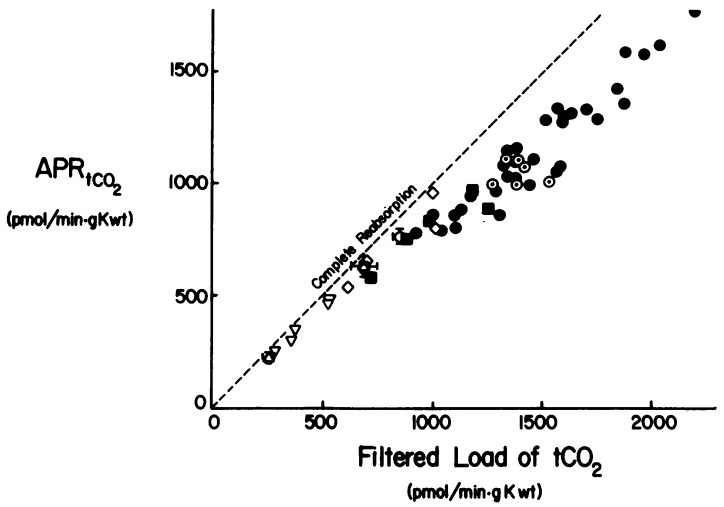

FIGURE 3 The relationship of absolute proximal tCO_2 reabsorption of the filtered tCO_2 load. Animals infused with plasma are represented by closed symbols: groups I–III, circles (●), and group V, squares (■). Animals infused with colloid-free solutions are represented by open symbols: group IV, circled dot (⊙); group VI, diamonds (◇); and group VII, inverted triangles (▽). Mean ±SEM for all animals studied in hydropenia is represented by an open circle for group I (○), open square for group V (□), and open upright triangle for group VII (△). The line describes complete reabsorption of the filtered tCO_2 load.

animals were able to lower the end-proximal [tCO_2] to 2.9±0.8 mM. Fractional tCO_2 reabsorption thus remained high at 0.89±0.3 in spite of massive ECF expansion with a colloid-free solution.

Group VII (severe acidosis: hydropenia–isohydric expansion). NH_4Cl loading dropped the Bowman's space [tCO_2] to 9.3±0.6 mM. SNGFR was similar to group I during hydropenia. A high fractional tCO_2 reabsorptive rate of 0.89±0.02 was maintained since the end-proximal [tCO_2] fell to 1.6±0.2 mM ($P < 0.001$). Isohydric expansion caused a small increment in SNGFR and Bowman's space [tCO_2] (26), but no diminution in fractional tCO_2 reabsorption, which remained at 0.90±0.02. Distal tCO_2 delivery in these expanded acidotic rats was less than half of that found in volume contracted normal rats. Hydropenic and expanded group VII animals are represented in Fig. 3 by upright (mean±SEM) and inverted triangles, respectively.

TABLE IV
Effect of Acidosis on Proximal Reabsorption of Total CO_2

Group	Period	Bowman's space [tCO_2]	SNGFR	End-proximal [tCO_2]	Distal delivery tCO_2	Absolute proximal reabsorption tCO_2	Fractional proximal reabsorption tCO_2
		mM	*nl/min·g KWt*	*mM*	*pmol/min·g KWt*	*pmol/min·g KWt*	
V	Hydropenia (mild acidosis)	21.4±0.9*‡	33.0±3.1	3.5±0.3§	66±10	619±60	0.91±0.01
	Plasma repletion (≅1.3% BWt)	22.8±0.9‖	46.2±3.9	6.5±0.8§	194±35§	859±74§	0.82±0.02
	P <¶	NS	0.005	0.025	0.025	0.025	0.025
VI	NaCl expansion (10% BWt)	17.6±0.8‡	45.4±2.9	2.9±0.8‡	92±30‖	704±66‖	0.89±0.03‖
VII	Hydropenia (severe acidosis)	9.3±0.6‡	30.1±1.7	1.6±0.2‡	31±6‡	248±24‡	0.89±0.02
	Isohydric expansion (10% BWt)	12.2±1.1‡	33.9±1.8‡	1.8±0.4‡	35±13‡	375±46‡	0.90±0.02‡
	P <¶	0.005	NS	NS	0.05	0.05	NS

* Mean±SEM.
‡ $P < 0.001$ for value compared to that in group I during the same period.
§ $P < 0.05$ for value compared to that in group I during the same period.
‖ $P < 0.01$ for value compared to that in group I during the same period.
¶ P values for second period compared to first period in same group.

The effect of acidosis on the end-proximal [tCO_2] is shown in Fig. 4. The end-proximal [tCO_2] is plotted as a function of the Bowman's space [tCO_2]. Open symbols represent volume contracted (hydropenic) animals. These animals had a similar SNGFR. It can be seen that the ability to lower the end-proximal [tCO_2] is enhanced as metabolic acidosis becomes more severe. An increase in SNGFR by plasma volume repletion or by isohydric expansion with plasma (closed circle) or colloid-free solution (circled dot) in normal animals causes a more marked increase in end-proximal [tCO_2] than in acidotic animals (closed square, diamond, and inverted triangle). ECF volume expansion does not prevent the reduction in end-proximal [tCO_2] during acidosis as best demonstrated in group VII (triangles).

DISCUSSION

Load-dependence of proximal bicarbonate reabsorption in the plasma-repleted rat. A state of marked volume contraction is created by micropuncture surgery because of plasma volume loss, which is as much as 18% in adult rats (19, 20). When the plasma volume is restored by an infusion of homologous, isoncotic plasma, the elevated hematocrit returns to a value similar to that which had been obtained in the awake state and diminished salt excretion returns to levels more appropriate to the dietary history of the animal (25). Though this plasma repletion method can in no way be said to return the animal to "normal", the physiology of filtration and reabsorption is most probably closer to the awake state than has been previously reported in plasma volume contracted, hydropenic animals (11–13). For instance, the SNGFR of 51.6±1.6 nl/min·g KWt after plasma repletion is reasonably close to that found in superficial nephrons of the awake Wistar rat of 48.7±4.5 nl/min as determined by the ferrocyanide infusion technique (27).

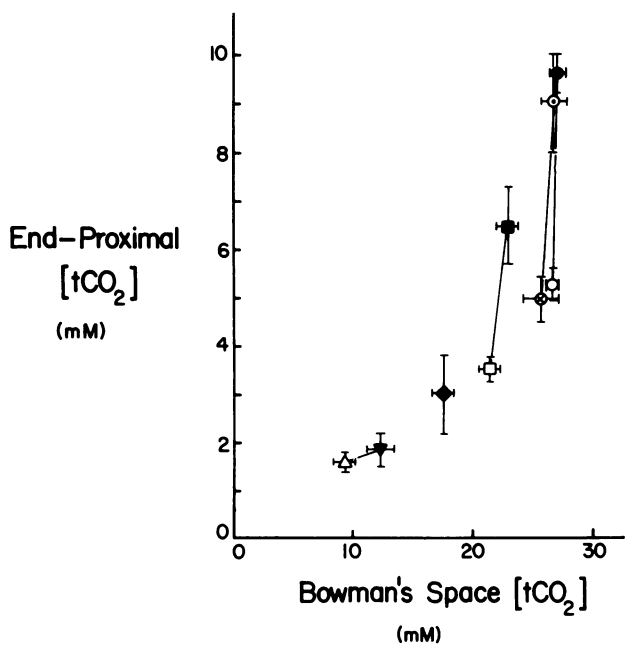

FIGURE 4 The relationship of the end-proximal [tCO_2] to the Bowman's space [tCO_2]. Mean±SEM for all hydropenia animals is shown in open symbols: groups I and IV, circle (○); group V, square (□); and group VII, upright triangle (△). The group I aortic constriction period is shown by a circle with central cross (⊗). Infusion periods are represented by closed symbols: groups I–III, circle (●); group IV, circle with central dot (⊙); group II, square (■); group VI, diamond (◆); and group VII, inverted triangle (▼).

When the surgically induced plasma volume losses were replaced, a 50% increase in the filtered bicarbonate load resulted in an increase in absolute proximal bicarbonate reabsorption of 30%. The modest decrease in fractional bicarbonate reabsorption from 0.90±0.01 to 0.77±0.02 during the transition from the plasma volume contracted to the volume-replete state may have been the result of: (*a*) intrinsic reabsorptive transport properties of the proximal tubule that fail to completely keep up with higher filtered loads; or (*b*) a suppressive effect of the higher ECF volume. ECF volume is moderately higher in the plasma-replete state (about 1.3% body wt) and it has been suggested that increased bicarbonate-rich back-leak into the tubule lumen is promoted by higher ECF volumes (15–17). The aortic constriction experiments were performed to test whether an augmented back-leak could be exposed as the filtered load, but not the ECF volume, was returned to hydropenic levels. The fractional tCO_2 reabsorption returned to 90%. A higher end-proximal [tCO_2] than obtained in hydropenia as a result of net bicarbonate back-flux was not observed. Although the modest increment in ECF volume during plasma repletion may well have enhanced back-leak when compared to hydropenia, it is apparent that the tubular bicarbonate transport process was efficient enough to maintain high net fractional reabsorption.

Effect of ECF expansion on proximal bicarbonate reabsorption. More profound volume expansion was employed to examine whether the lack of an apparent effect by a higher ECF volume status in the previous aortic constriction experiments was caused by insufficient expansion. The experiments were designed to keep reasonably constant those factors that might independently alter bicarbonate reabsorption, such as arterial pH and P_{CO_2}, plasma bicarbonate, potassium, and protein concentrations, and parathyroid hormone levels. That the protocols employed were successful in achieving ECF expansion can be inferred from the low hematocrits, the large natriuresis and estimated chloruresis, and development of bicarbonaturia after infusions. Isohydric expansion with 5% body wt plasma (group II), three times more plasma than used in plasma

repletion studies, caused no reduction in fractional tCO_2 reabsorption compared to plasma-repleted rats. Even when the mild reduction in potassium concentration and rise in plasma protein concentration were corrected by potassium addition or bicarbonate Ringer's expansion, respectively (group III), fractional tCO_2 reabsorption remained at 80%. Finally, colloid-free isohydric expansion with Ringer's solution to 10% body wt (group IV) produced no significant decrement in fractional tCO_2 reabsorption. Of interest in this last group is that this colloid-free infusion effected a large increase in absolute proximal tCO_2 reabsorption even when absolute water reabsorption did not significantly change (28–30). This ability to disjoin changes in bicarbonate reabsorption from those in water reabsorption in the proximal tubule stands in contrast to some other studies (14, 31).

In conclusion, these results suggest that the superficial proximal tubule assumes a primary responsibility for mediating the increase in whole kidney bicarbonate reabsorption as GFR increases (glomerulo-tubular balance). No independent effect of the ECF volume in diminishing proximal bicarbonate reabsorption as a function of filtered bicarbonate load occurs when the ECF is expanded over the plasma-replete level. The slope of this relationship, as seen in Fig. 3, is thus unchanged by ECF expansion.

Our results are in disagreement with the micropuncture findings of Levine et al. (14) who found that Ringer or hyperoncotic albumin infusions increase SNGFR without altering absolute proximal bicarbonate reabsorption. The reasons for the disparity between our study and that of Levine et al. are at present unclear, because similar degrees of volume expansion were induced.

It is necessary to reconcile the present free-flow micropuncture results, which fail to show a clear suppressive effect by ECF expansion on proximal bicarbonate reabsorption, to previous clearance studies that demonstrated a decrease in whole kidney fractional bicarbonate reabsorption produced by ECF volume expansion (2–6, 32, 33), even when filtered bicarbonate load was unaltered (5). Several explanations are possible. First, in the clearance studies, most of the effects of ECF expansion were demonstrated in alkalotic animals. It is possible that the expansion effect, even if caused by alterations in proximal tubule transport, may be only apparent during metabolic alkalosis. Second, volume expansion was often accomplished using colloid-free solutions in these clearance studies (2–6, 32, 33). Thus, it may be necessary that two factors be present, the higher filtered bicarbonate loads and the depression of absolute water reabsorption evoked by the use of colloid-free infusions (28–30), which act in concert to depress proximal bicarbonate reabsorption. Finally, inhibition of whole kidney bicarbonate reabsorption by ECF expansion may be attributable to effects on nephron segments other than the superficial proximal convoluted tubule, such as juxtamedullary nephrons or the distal tubule and collecting duct. In this regard it is noteworthy that the expanded animals in the present experiments (groups II–IV) developed bicarbonaturia even though distal delivery from the superficial proximal tubule was not increased over the nonexpanded, plasma-repleted animals without bicarbonaturia. The mechanism by which ECF volume expansion suppresses whole kidney bicarbonate reabsorption is, therefore, not clarified by these studies, but the effect is not evident in the rat superficial proximal convoluted tubule at a normal arterial pH.

Mechanism of load-dependence of proximal bicarbonate reabsorption. An important question remains regarding the mechanism by which increases in filtration rate enhance absolute reabsorption. The proximal tubule avidly reabsorbs bicarbonate thereby lowering the luminal bicarbonate concentration. This lower concentration might then become rate-limiting for reabsorption toward the end of the tubule. As flow rate increases, however, more bicarbonate would be presented to the transport elements along the tubule. The luminal bicarbonate concentration averaged along the tubule would also increase. In stationary split droplet experiments, hydrogen ion secretion increases as the luminal buffer concentration rises (7). Thus, to the extent that the hydrogen ion secretory process were sensitive to the luminal bicarbonate concentration that it "sees", this higher integrated mean concentration would evoke a higher net reabsorptive rate.

The absolute rate of proximal bicarbonate reabsorption would then depend on the mean luminal bicarbonate concentration along the tubule, or more precisely, the logarithmic mean, since bicarbonate reabsorption appears to follow first-order kinetics (7, 34, 35). A similar model by which amino acid reabsorption would be flow dependent, secondary to changes in luminal concentration, has been recently proposed (36, 37). As illustrated in Fig. 5, the isohydric rise in SNGFR evokes an increase in mean luminal tCO_2 concentration from hydropenia (open symbols) to plasma replete or expanded levels (closed symbols) which correlate with the observed increase in proximal tCO_2 reabsorption. The calculated mean concentrations for the hydropenic groups may be over-estimates if a steady-state bicarbonate concentration is reached before the end of the proximal tubule. In any event, the rise in absolute bicarbonate reabsorption that accompanies an increase in filtered bicarbonate load may be mediated by the higher mean luminal bicarbonate concentration.

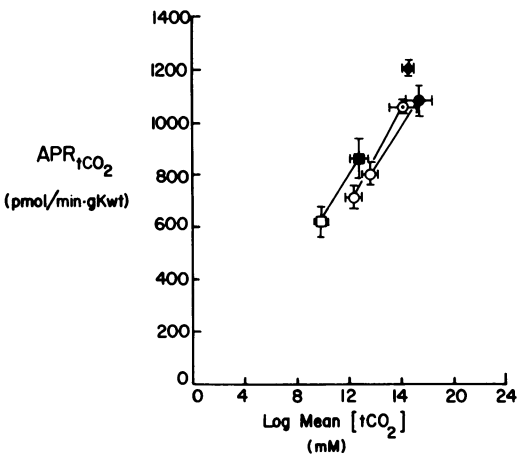

FIGURE 5 Absolute proximal tCO_2 reabsorption as a function of the logarithmic mean luminal [tCO_2] in the proximal tubule. Mean±SEM for the hydropenia periods are represented by open symbols and infusion periods by closed symbols for group I, circles (●); groups II–III, diamond (◆); group IV, circled dot (⊙); and group V, squares (■).

A related question concerns the mechanism underlying the small decline in fractional reabsorption as load increases. This may be caused by the fact that the transport elements in the proximal tubule are arranged in series. Axial flow systems are known to induce nonlinearity in load-dependent transport as flow increases (38).

Effect of acidosis on proximal bicarbonate reabsorption. The hydropenic end-proximal [tCO_2] decreased from 5.6±0.5 to 1.6±0.2 mM as arterial pH fell from 7.38 to 7.05. The proximal tubule, therefore, is capable of maintaining a high fractional tCO_2 reabsorptive capacity of 0.90 during acidosis (Table IV). The reduction in distal bicarbonate delivery frees the distal nephron to expend hydrogen ion secretion on net acid production rather than on bicarbonate reclamation. Plasma repletion or ECF expansion also had a lesser effect in raising the end-proximal [tCO_2] (Fig. 4, solid symbols) and in increasing absolute distal tCO_2 delivery (Table IV) than was the case in nonacidotic rats. Although some investigators have found that acidosis had little effect or even an inhibitory effect on maximal bicarbonate reabsorptive capacity (9, 10, 39), our results are consistent with Giebisch et al. (7) who found in split droplet experiments that acidosis lowers the minimal pH to which the tubule lumen can be acidified.

The mechanism by which metabolic acidosis enhances luminal acidification is not clear. One way may be simply by limiting the tCO_2 filtered load. Another could be that if the end-proximal [tCO_2] in volume contraction represents a steady-state concentration of a balanced pump-leak system (40), it may be that acidosis directly augments or resets the pump activity responsible for hydrogen secretion or diminishes the backward leak. For instance, hydrogen ion secretion may be limited by a maximum transepithelial pH gradient as occurs in the distal nephron (41). Systemic acidosis may then serve to allow the luminal bicarbonate to be titrated to lower than normal values (e.g., <5 mM) in the presence of a constant transepithelial pH gradient. Isohydric volume expansion in group VII (Fig. 4, triangles) demonstrated that this ability to lower the luminal bicarbonate concentration by systemic acidosis is little affected by changes in ECF volume.

In summary, the reabsorption of bicarbonate in the superficial convoluted tubule of the rat is highly load-dependent in normal and acidotic rats. No effect of the ECF volume status of the animal could be discerned in suppressing proximal bicarbonate reabsorption. Acidosis enhances fractional bicarbonate reabsorption and limits distal bicarbonate delivery, since the end-proximal bicarbonate concentration falls to lower levels than occurs in animals with a normal arterial pH. The ability to generate steep bicarbonate concentration gradients is not impaired by expansion of extracellular fluid volume.

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