# Effect of Carbonic Anhydrase Inhibition on Superficial and Deep Nephron Bicarbonate Reabsorption in the Rat

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ABSTRACT The nephron segment responsible for the acetazolamide-insensitive fraction of renal bicarbonate reabsorption has not been clearly delineated. This study compares superficial and deep nephron bicarbonate reabsorption before and after acetazolamide at two dose levels (20 and 50 mg/kg per h) in mutant Munich-Wistar rats employing both cortical and papillary micropuncture and microcalorimetry. Systemic acid-base balance and right whole kidney glomerular filtration rate were similar in all groups examined. The effects of the two doses of acetazolamide were indistinguishable and resulted in a significant increase in whole kidney bicarbonate excretion that compared favorably with the fraction delivered out of the left papillary tip. Acetazolamide inhibited superficial proximal bicarbonate reabsorption by 80.0%, whereas reabsorption up to the deep loop of Henle was decreased by only 52% ($P < 0.001$). Bicarbonate reabsorption that was insensitive to acetazolamide occurred in the superficial and deep loop of Henle and between the distal tubule and base collecting duct. Because water reabsorption in these segments could serve to generate transepithelial bicarbonate concentration gradients favorable for reabsorption, we attempted to minimize water abstraction by combined administration of mannitol and acetazolamide. During this condition a significant increase in bicarbonate delivery up to the deep loop of Henle was noted (52 vs. 65%), whereas superficial nephron reabsorption was not altered. Furthermore, an outwardly directed bicarbonate concentration gradient from the deep loop of Henle to vasa recta was demonstrated during acetazolamide ($\Delta tCO_2 = 20.9 \pm 3.3$ mM), but was abolished during combined mannitol and acetazolamide administration ($\Delta tCO_2 = 3.5 \pm 0.9$ mM). It is concluded that carbonic anhydrase inhibition results in a disparate effect on nephron bicarbonate reabsorption when juxtamedullary and superficial nephron segments are compared. Our findings suggest that a mechanism for residual bicarbonate reabsorption during acetazolamide administration may be passive reabsorption driven by favorable transepithelial concentration gradients.

## INTRODUCTION

Recent in vivo and in vitro microperfusion and free-flow micropuncture studies have demonstrated that carbonic anhydrase inhibition, either by systemic administration or luminal application of the inhibitor, results in an 80–90% reduction in proximal tubule bicarbonate reabsorption (1–5). This marked degree of inhibition in the proximal tubule contrasts sharply with the widely observed effects of acetazolamide on whole kidney bicarbonate reabsorption. In this regard, Cogan and associates (2) have recently demonstrated that despite an 80% reduction in absolute and fractional proximal total $CO_2$ ($tCO_2$)[1] reabsorption after systemic administration of acetazolamide, whole kidney fractional excretion averaged only 25%. Moreover, data from this (3) and other laboratories (1, 2, 4) have clearly demonstrated that bicarbonate reabsorption in the proximal tubule is highly dependent on carbonic anhydrase. However, the large acetazolamide-insensitive fraction of bicarbonate reabsorbed beyond the

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[1] *Abbreviations used in this paper*: BW, body weight; $FEtCO_2$, fractional excretion of $tCO_2$; GFR, glomerular filtration rate; In, inulin; P, plasma; $PaCO_2$, systemic arterial $PCO_2$; $tCO_2$, total $CO_2$; TF, tubule fluid; $TF/P_{In}$, TF to $P_{In}$ ratio; $[TF/P]_{tCO_2/In}$, fractional delivery of $tCO_2$; VR, vasa recta.

proximal tubule far exceeds the amount that can be accounted for by the uncatalyzed hydration reaction (2, 6). To account for the residual 20% reabsorbed by the superficial proximal tubule, and the large fraction reabsorbed elsewhere, several possible mechanisms have been proposed. These various hypotheses include: (*a*) carbonic acid recycling (7), (*b*) direct bicarbonate ion reabsorption (8, 9), or (*c*) incomplete carbonic anhydrase inhibition in other nephron segments (10).

It appears appropriate, however, to define the nephron segment or segments responsible for the acetazolamide-insensitive fraction of bicarbonate reabsorption more clearly before such diverse mechanisms can be substantiated. Although it has been suggested that the site(s) for this process might include juxtamedullary proximal tubules, the loops of Henle, or more distal segments (6), a segmental examination of bicarbonate reabsorption in both superficial and deep nephrons has not been available previously.

The present study was designed, first, to compare the effect of carbonic anhydrase inhibition on bicarbonate reabsorption in accessible superficial and deep nephron segments. This was accomplished by employing free-flow micropuncture techniques and microcalorimetry in the surgically exposed papilla and superficial cortex of the mutant Munich-Wistar rat before and after administration of acetazolamide at two dose levels. Such an approach was deemed necessary to determine the nephron segment or segments responsible for the acetazolamide-insensitive fraction of bicarbonate reabsorption and to indirectly examine the possibility of incomplete inhibition of the enzyme in these segments. Second, the mechanism responsible for reabsorption of bicarbonate beyond the proximal tubule was investigated by evaluating the effects of concomitant administration of acetazolamide and mannitol in an attempt to minimize water abstraction and thus concentration gradients favorable for bicarbonate diffusion out of the tubule lumen. Further information was obtained in this regard by observing the effect of these two diuretics on the concentration gradient for bicarbonate between the nephron segment in question and the peritubular capillary or vasa recta.

We have observed, for the first time, an important contribution by the deeper nephron segments in the reabsorption of the acetazolamide-insensitive fraction of bicarbonate which is, in part, a function of the degree of water abstraction, and the luminal concentration of bicarbonate achieved by this process.

## METHODS

Studies were performed on a thermostatically controlled (37°C) micropuncture table after 100 mg/kg i.p. Inactin anesthesia (BYK, Hamburg, West Germany) on young mutant Munich-Wistar rats (Timco Breeding Laboratories, Houston, TX) weighing 65–129 g (mean wt = 109.5±2 g). All rats were allowed free access to tap water and standard rat chow until the time of anesthesia. Surgical exposure of the papilla was accomplished as previously described (11). The left kidney was placed in a lucite cup and stabilized by 3% agar in saline in such a manner that micropuncture of the accessible segments of both the superficial cortex and exposed papilla could be accomplished (12). The kidney was continuously bathed with water-equilibrated mineral oil maintained at 37°C. Care was taken to assure that the renal papilla was covered by an oil layer at all times. The kidney was illuminated by a small fiber optic light source (Dolan-Jenner Industries, Inc., Woburn, MA) mounted on a Brinkmann micromanipulator (Brinkmann Instruments, Inc., Westbury, NY) to allow illumination of either papilla or cortex as desired. After jugular vein cannulation, surgical losses were replaced with Ringer's bicarbonate ($Na^+$ = 140, $Cl^-$ = 110, $HCO_3^-$ = 25, and $K^+$ = 5 meq/liter) equal to 1% body weight (BW) over 15 min and an infusion of 1.0% BW/h was begun and continued until initiation of the micropuncture protocol (1–1.5 h). Preparation of the control rats in this manner resulted in a stable hematocrit (45.1±0.8%) and plasma protein concentration (5.1±0.2 g/dl) so that the degree of volume depletion characteristic of the "hydropenic" model was avoided.

All animals were then infused with the above Ringer's solution to which [$^3$H]methoxy inulin (New England Nuclear, Boston, MA) was added at a rate of 150 μCi/h.

Proximal tubule transit times and localization of superficial nephron segments was accomplished by observing the passage of lissamine green dye as previously described (12, 13). Transit times > 13 s or a mean arterial blood pressure < 100 mm Hg were cause for rejection at this point.

1 h after initiation of the inulin infusion and at least 30 min after the last lissamine green dye injection, whole kidney clearance periods (30 min) and micropuncture were begun. Urine for whole kidney glomerular filtration rate (GFR) and bicarbonate excretion rates was obtained from the right untouched kidney collected via the urinary bladder into preweighed vials containing mineral oil. Acid-base status of the rat was monitored and carefully maintained exactly as previously described (14, 15). Micropuncture of the desired cortical or papillary nephron segment was accomplished with sharpened micropipettes containing Sudan-colored mineral oil exactly as previously described in detail (12, 13). Descending or ascending vasa recta samples were collected in some of the groups noted below by the technique described by Lacy and Jamison (15) employing 8–11-μm-tip-diam pipettes pulled with an especially rapid taper to decrease the risk of clotting. Collection rates were controlled to allow osmotic equilibration as suggested by these investigators (15, 16).

*Group I (controls) (n = 23).* 23 rats served as controls while receiving a continued infusion of Ringer's bicarbonate plus inulin at 1% BW/h. After a suitable control period these rats were divided into one of two experimental groups (II and III) noted below. Tubule fluid in this and each subsequent group was obtained from the superficial late proximal tubule, superficial late distal tubule, the bend of Henle's loop (ascending and descending limbs near the bend), and the base and tip of the papillary collecting tubule as described previously (12).

*Group II (acetazolamide-low dose) (n = 11).* After obtaining data in the control period, 11 rats received acetazolamide (Lederle Laboratories, Div. American Cyanamid Co., Pearl River, NY) as a bolus (20 mg/kg) and as a maintenance infusion (20 mg/kg per h) in 275 mM $NaHCO_3$ and 25 mM KCl to replace urinary electrolyte losses. 30 min after completing the acetazolamide bolus, micropuncture samples were again obtained and two clearance periods initiated.

*Group III (acetazolamide-high dose) (n = 12).* 12 rats from group I received acetazolamide as a bolus (50 mg/kg) and a sustaining infusion (50 mg/kg per h) in the same manner and with the same maintenance solution noted for group II. Micropuncture and clearance data were then obtained as in group II. Vasa recta plasma was obtained in five rats in this group as noted above.

*Group IV (mannitol) (n = 7).* 7 rats received 12.5% mannitol at 1.2 ml/h plus [$^3$H]methoxy inulin in Ringer's bicarbonate at 1.2 ml/h. Urinary electrolyte losses were replaced with Ringer's bicarbonate. After a 1-h equilibration period, micropuncture and clearance data were obtained exactly as noted for group I. These animals served as controls for group V.

*Group V (acetazolamide-high dose plus mannitol) (n = 11).* 11 rats received both 12.5% mannitol (as in group IV) and acetazolamide 50 mg/kg bolus and 50 mg/kg per h. Urine electrolyte losses were carefully replaced and volume status maintained by infusion of 275 mM $NaHCO_3$ and 25 mM KCl plus Ringer's bicarbonate.

*Analytical techniques.* Tubule fluid $tCO_2$ concentration was determined by microcalorimetry (17) immediately after collection as previously described (14). Vasa recta plasma $tCO_2$ concentration was similarly determined after centrifugation of the collection pipette as previously described for stellate vessel plasma (14), and carefully evaluated for contamination by collecting duct fluid according to the methods of Gelbart and associates (16). The following equation was employed and samples with values > 5% discarded: $(VR_{In} - P_{In})/(CD_{In}) \times 100$, where VR is vasa recta, In is inulin, P is plasma, and CD is collecting duct. [$^3$H]methoxy inulin activity was counted in a liquid scintillation counter (model 4 60C-Packard Tri-Carb, Packard Instrument Co., Inc., Downers Grove, IL). Arterial and urine pH, $P_{CO_2}$ and $P_{O_2}$ were determined with a Corning Blood Gas Analyzer (model 165-2, Corning Medical, Corning Glass Works, Medfield, NY). $Na^+$, $K^+$, and $tCO_2$ concentrations in plasma and urine were determined with a flame photometer (Corning Medical model 435) or $CO_2$ analyzer (Corning model 965). Plasma protein was estimated by refractometry.

Plasma $tCO_2$ concentration was corrected for plasma water and Donnan equilibrium from measured serum solids. We employed a water correction factor of 0.93 ml/ml plasma for plasma water, and 1.05 for the Gibbs-Donnan correction factor (16, 18).

The results are expressed as mean values±the respective SE for each group and analyzed by the *t*-test for unpaired or paired data as appropriate.

## RESULTS

*Whole kidney data.* Systemic arterial blood gases and electrolytes, and right whole kidney pH, GFR, and bicarbonate excretion are summarized for all groups in Table I. Values for control rats are compatible with previous results from our laboratory (14). The

TABLE I
*Systemic Acid-Base and Right Kidney Data*

| pHa | $PaCO_2$ | $tCO_2p$ | $Na^+p$ | $K^+p$ | pHu | $tCO_2u$ | $\dot{V}$ | $U_{tCO_2}V$ | GFR | $FEtCO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| *units* | *mm Hg* | *mM* | *meq/liter* | *meq/liter* | *units* | *mM* | *μl/m* | *μeq/m* | *ml/m* | *%* |
| Group I, control (23) | | | | | | | | | | |
| 7.36 | 43.6 | 24.7 | 141.3 | 4.8 | 5.90 | 1.5 | 2.72 | 0.02 | 0.720 | 0.06 |
| ±0.01 | ±2.0 | ±0.9 | ±1.6 | ±0.06 | ±0.15 | ±0.5 | ±0.37 | ±0.09 | ±0.085 | ±0.04 |
| Group II, acetazolamide, 20 mg/kg per h (11) | | | | | | | | | | |
| 7.28 | 49.2 | 21.3 | 140.5 | 5.25 | 7.91* | 225.5* | 20.9* | 4.90* | 0.736 | 31.3* |
| ±0.03 | ±2.8 | ±2.3 | ±1.5 | ±0.05 | ±0.01 | ±7.2 | ±2.23 | ±0.50 | ±0.054 | ±2.0 |
| Group III, acetazolamide, 50 mg/kg per h (12) | | | | | | | | | | |
| 7.38 | 43.4 | 23.4 | 142.5 | 4.7 | 7.86* | 231.3* | 25.20* | 5.90* | 0.679 | 37.6* |
| ±0.01 | ±2.2 | ±0.6 | ±0.97 | ±0.1 | ±0.03 | ±2.7 | ±1.04 | ±0.26 | ±0.040 | ±2.1 |
| Group IV, mannitol (7) | | | | | | | | | | |
| 7.30 | 46.8 | 24.2 | 135.1 | 4.0 | 6.50 | 4.4 | 15.5* | 0.03 | 0.750 | 0.56 |
| ±0.02 | ±2.5 | ±1.2 | ±1.7 | ±0.07 | ±0.15 | ±2.0 | ±1.2 | ±0.01 | ±0.110 | ±0.38 |
| Group V, mannitol and acetazolamide, 50 mg/kg per h (11) | | | | | | | | | | |
| 7.21 | 57.0* | 22.7 | 136.1 | 5.33 | 7.66‡§ | 141.4‡§ | 36.8‡§ | 5.25‡ | 0.550 | 43.6‡ |
| ±0.02 | ±2.2 | ±0.8 | ±3.3 | ±0.58 | ±0.03 | ±11.6 | ±2.5 | ±0.50 | ±0.048 | ±4.1 |

$K^+p$, plasma $K^+$; $Na^+p$, plasma $Na^+$, pHa, systemic arterial pH; pHu, urine pH; $tCO_2p$, plasma $tCO_2$; $tCO_2u$, urine $tCO_2$; $U_{tCO_2}V$, absolute excretion of $tCO_2$.

* $P < 0.001$ vs. group I.

‡ $P < 0.001$ vs. group IV.

§ $P < 0.001$ vs. group III.

results in all controls were indistinguishable and have been combined. Plasma electrolytes remained unchanged, and except for the expected effect on systemic arterial $P_{CO_2}$ ($PaCO_2$), arterial blood gases were unaltered after acetazolamide or acetazolamide plus mannitol. The effect of acetazolamide on erythrocyte carbonic anhydrase and the resulting hypercapnia is compatible with the results of others (2). Urine pH, [$tCO_2$], flow rate, absolute bicarbonate excretion, and fractional bicarbonate excretion all increased significantly after acetazolamide administration (groups II, III, V vs. group I, $P < 0.001$). The effect of higher doses of acetazolamide (group III) on these same parameters was not different than in group II ($P > 0.05$). The group receiving mannitol displayed a much higher urine flow rate ($P < 0.001$) than controls, whereas GFR, fractional excretion of $tCO_2$ ($FEtCO_2$)%, and absolute excretion of $tCO_2$ remained constant ($P > 0.05$). Mannitol plus acetazolamide (group V) increased urine pH, [$tCO_2$], bicarbonate excretion, flow rate, and fractional bicarbonate excretion significantly when compared with mannitol alone. Mannitol plus acetazolamide increased the $FEtCO_2$% to 43.6±4.1%, which was significantly greater than the effect of lower doses of acetazolamide alone ($P < 0.01$) (group II), but was not different than group III ($P > 0.05$).

*Micropuncture data.* The micropuncture findings are summarized in Table II and Fig. 1 for each nephron segment in each group. The values for tubular fluid to $P_{In}$ ratio ($TF/P_{In}$), [$tCO_2$], and the fractional delivery of $tCO_2$([$TF/P]_{tCO_2/In}$%) in control rats in the late proximal tubule and distal tubule are compatible with previous reports from this and other laboratories (2, 14, 19). The bicarbonate concentration in the juxtamedullary loop of Henle was significantly greater than in the superficial late proximal tubule ($P < 0.001$), but the fractional delivery to each of these segments was similar (12.1±2.8 vs. 9.0±0.9, $P > 0.05$). Therefore, the apparent increase in bicarbonate concentration between these two segments can be accounted for by water abstraction ($TF/P_{In}$ = 2.28±0.07 to 9.38±0.84, $P < 0.001$), presumably in the descending limb of Henle. Despite the similar [$tCO_2$] in the superficial proximal tubule and distal tubule, the fraction delivered to the distal tubule was significantly less than that delivered out of the proximal tubule (3.73±0.89 vs. 12.1±2.8%, $P < 0.001$). Although only a small fraction of filtered bicarbonate was delivered to the base collecting duct (0.12±0.06%), significant reabsorption between base and tip was noted (0.10±0.03%). Furthermore, the fraction delivered out of the tip of the collecting duct of the left kidney compared favorably with the fractional excretion by the right untouched kidney (0.02±0.01 vs. 0.06±0.04%, respectively).

Acetazolamide administration (group II, 20 mg/kg) resulted in a marked increase in the $tCO_2$ concentration in each nephron segment (31.1, 50.3, 39.5, 178.3, and 209.4 mM for the superficial proximal convoluted tubules, loop of Henle, superficial distal convoluted tubules, base of the papillary collecting duct, and tip of the papillary collecting duct, respectively); ($P < 0.001$ vs. controls). Therefore, the fractional delivery to each segment was significantly greater (superficial proximal convoluted tubules = 80.0±3.6, loop of Henle = 51.4±3.5, superficial distal convoluted tubules = 47.3±10.5, base of the papillary collecting duct = 26.6±4.3, and tip of the papillary collecting duct = 31.8±2.4%) than the respective controls. However, in each segment, the effect of the higher dose of acetazolamide (50 mg/kg, group III) did not differ from the lower dose (group II). Interestingly, however, the fraction delivered to the loop of Henle, when compared with the corresponding delivery out of the late proximal tubule (in both groups II and III), was significantly less (80.0±3.6 vs. 51.4±3.5 and 79.5±3.8 vs. 53.1±3.8%, respectively, $P < 0.001$). Significant reabsorption of bicarbonate was not demonstrated between the base and tip collecting duct in either group II (26.6 vs. 31.8%, $P > 0.05$) or group III (23.6 vs. 28.3%, $P > 0.05$).

Mannitol alone (group IV) appeared to have little effect on bicarbonate reabsorption when compared to controls (group I). A small but significantly greater delivery of $tCO_2$ to the loop, base, and tip collecting duct was noted, however. A significant effect of mannitol on water reabsorption was noted, as expected. Interestingly, the effect of mannitol on deep nephron segments was more dramatic than the effect on superficial segments (compare $TF/P_{In}$ values group I vs. IV).

The effect of mannitol plus acetazolamide (50 mg/kg per h) (group V, Table II) was similar to the effect of acetazolamide alone (group III) in the superficial proximal tubule and distal tubule of cortical nephrons. However, a marked increase in fractional $tCO_2$ delivery was noted in the juxtamedullary loop of Henle (64.6±4.4 vs. 53.1±3.8%, $P < 0.001$), when compared with the effect of acetazolamide (50 mg/kg) alone (group III). Although the fractional delivery to the base collecting duct was also greater in group V (23.6±1.8 vs. 39.6±3.8%, $P < 0.005$), the delivery out of the tip (30.8±1.5%) did not differ from the delivery in group III (28.3±3.4%). Again, however, reabsorption along the papillary collecting duct was not observed (39.6 vs. 30.8%, $P > 0.05$).

The effect of mannitol (12.5%) alone (group IV) and mannitol plus acetazolamide (group V) on the concentration of $tCO_2$ in vasa recta plasma and loop of Henle tubule fluid, and the corresponding transepithelial gradient (corrected for plasma water) is summarized in Table III. The values obtained for $tCO_2$ concentration in the vasa recta of either group did not

TABLE II
*Micropuncture Data*

| | $TF/P_{In}$ | | | | | $[tCO_2]$ | | | | | $[TF/P]_{tCO_2/In}$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LP | LOH | DT | BCD | TCD | LP | LOH | DT | BCD | TCD | LP | LOH | DT | BCD | TCD |
| | | | | | | | | *mM* | | | | | % | | |
| Group I, controls | | | | | | | | | | | | | | | |
| Mean | 2.28 | 9.38 | 7.59 | 145.9 | 253.2 | 7.97 | 20.0 | 7.1 | 3.4 | 0.8 | 12.1 | 9.0 | 3.73 | 0.12 | 0.02 |
| SE | ±0.07 | ±0.84 | ±0.69 | ±40.3 | ±25.0 | ±1.20 | ±1.6 | ±1.3 | ±1.0 | ±0.2 | ±2.8 | ±0.9 | ±0.89 | ±0.06 | ±0.01 |
| *n* | (7) | (10) | (6) | (8) | (16) | (7) | (10) | (6) | (8) | (16) | (7) | (10) | (6) | (8) | (16) |
| Group II, acetazolamide, 20 mg/kg per h | | | | | | | | | | | | | | | |
| Mean | 1.75 | 3.89 | 3.73 | 31.8 | 29.0 | 31.1 | 50.3 | 39.5 | 178.3 | 209.4 | 80.0 | 51.4 | 47.3 | 26.6 | 31.8 |
| SE | ±0.12 | ±0.22 | ±0.82 | ±5.9 | ±2.5 | ±0.9 | ±2.3 | ±1.6 | ±23.8 | ±13.1 | ±3.6 | ±3.5 | ±10.5 | ±4.3 | ±2.4 |
| *n* | (12) | (16) | (4) | (6) | (9) | (12) | (16) | (4) | (6) | (9) | (12) | (16) | (4) | (6) | (9) |
| *P* I vs. II | <0.005 | <0.001 | <0.001 | <0.025 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.005 | <0.001 | <0.001 |
| Group III, acetazolamide, 50 mg/kg per h | | | | | | | | | | | | | | | |
| Mean | 1.48 | 5.03 | 3.74 | 22.5 | 30.8 | 28.1 | 63.9 | 40.1 | 134.6 | 216.0 | 79.5 | 53.1 | 50.8 | 23.6 | 28.3 |
| SE | ±0.11 | ±0.49 | ±0.63 | ±1.7 | ±2.7 | ±1.0 | ±3.7 | ±3.9 | ±9.0 | ±10.1 | ±4.3 | ±3.8 | ±6.1 | ±1.8 | ±3.4 |
| *n* | (9) | (14) | (11) | (10) | (9) | (9) | (14) | (11) | (10) | (10) | (9) | (14) | (11) | (10) | (9) |
| *P* I vs. III | <0.001 | <0.001 | <0.001 | <0.01 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| *P* II vs. III | NS | <.05 | NS | NS | NS | <.05 | <.005 | NS | NS | NS | NS | NS | NS | NS | NS |
| Group IV, mannitol, 12.5% | | | | | | | | | | | | | | | |
| Mean | 2.36 | 3.50 | 6.08 | 29.25 | 32.40 | 10.4 | 16.8 | 5.5 | 3.9 | 3.9 | 17.0 | 17.5 | 3.6 | 0.6 | 0.6 |
| SE | ±0.36 | ±0.66 | ±1.40 | ±2.94 | ±3.96 | ±2.9 | ±0.42 | ±1.2 | ±0.9 | ±0.9 | ±4.2 | ±2.0 | ±1.8 | ±0.2 | ±0.2 |
| *n* | (5) | (7) | (6) | (9) | (8) | (5) | (7) | (6) | (9) | (8) | (5) | (7) | (6) | (9) | (8) |
| *P* I vs. IV | NS | <0.001 | NS | <0.025 | <0.001 | NS | NS | NS | NS | NS | NS | <0.005 | NS | <0.02 | <0.005 |
| Group V, mannitol (12.5%) + acetazolamide, 50 mg/kg per h | | | | | | | | | | | | | | | |
| Mean | 1.53 | 2.86 | 3.02 | 9.45 | 16.92 | 26.4 | 42.2 | 34.1 | 87.9 | 118.5 | 70.3 | 64.6 | 50.2 | 39.6 | 30.8 |
| SE | ±0.11 | ±0.16 | ±0.61 | ±0.51 | ±0.81 | ±1.6 | ±1.9 | ±3.6 | ±5.3 | ±5.7 | ±4.8 | ±4.4 | ±4.4 | ±3.8 | ±1.5 |
| *n* | (9) | (15) | (8) | (10) | (13) | (9) | (15) | (8) | (10) | (13) | (9) | (15) | (8) | (10) | (12) |
| *P* III vs. V | NS | <0.001 | NS | <0.001 | <0.001 | NS | <0.02 | NS | <0.001 | <0.001 | NS | <0.02 | NS | <0.005 | NS |
| *P* IV vs. V | <0.025 | NS | <0.02 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

*Abbreviations used in this table*: BCD, base of the papillary collecting duct; DT, distal tubule; LOH, loop of Henle; LP, late proximal; TCD, tip of the papillary collecting tubule.

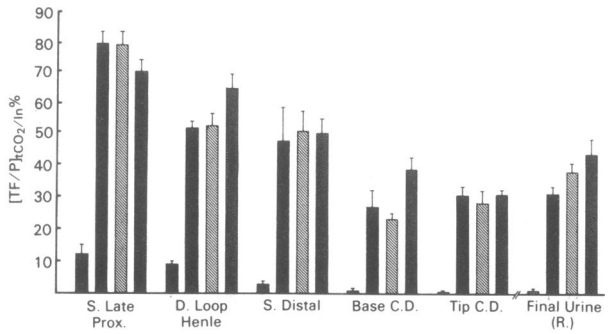


FIGURE 1 Summary of micropuncture data. The fraction of filtered bicarbonate ($[TF/P]_{tCO_2/In}$ %) delivered to each nephron segment is displayed for each experimental group in the following order (left to right): controls, acetazolamide 20 mg/kg, acetazolamide 50 mg/kg, and mannitol plus acetazolamide (50 mg/kg). The fractional excretion from the right kidney (final urine, extreme right) is included for comparison. Bars represent mean values±SE. Acetaz., acetazolamide; S. Late Prox., superficial late proximal; D. Loop Henle, descending loop of Henle; S. Distal, superficial distal; Base and Tip C.D., base and tip of the papillary collecting duct, respectively; (R.), right kidney.

differ significantly when descending or ascending vasa recta were analyzed. These results were combined. Six pairs of ascending or descending vasa recta and loop of Henle fluid were collected in group III rats and five pairs were obtained in group V rats. These results demonstrate that during acetazolamide administration alone, the $tCO_2$ concentration in tubule fluid (57.4±1.6 mM) exceeded the concentration in vasa recta (36.7±2.5) by 20.9±3.3 mM ($P < 0.001$). After combined acetazolamide and mannitol administration the concentration gradient favoring outward movement of bicarbonate was abolished (3.5±0.9, $P > 0.05$). These results were not altered when vasa recta values were corrected for plasma water and Gibbs-Donnan equilibrium ($\Delta tCO_2$ = 18.3±3.4 to 1.2±0.9 mM, $P < 0.0025$).

## DISCUSSION

Recent studies using new microelectrode techniques have demonstrated an acid disequilibrium pH in the proximal tubule during carbonic anhydrase inhibition and have supported proton secretion as the primary mechanism for acidification (3, 19). Controvery has persisted, however, regarding the mechanism by which the whole kidney carbonic anhydrase-independent component (~60–70%) of bicarbonate reabsorption is accomplished during the intravenous administration of a standard dose of an inhibitor of the enzyme (2, 6, 9, 20). In part, the debate is based on the seeming contradiction of the maintenance of such a large residual reabsorption of bicarbonate by the kidney despite the demonstration of inhibition of essentially 100% of the enzyme by in vitro techniques (8). Therefore, bicarbonate reabsorption per se (8), or recycling of carbonic acid (7) to provide a continuous source of protons for secretion, have been proposed to explain these observations. The salient features of these two hypotheses have been reviewed recently (6). That the uncatalyzed reaction would not be adequate to provide sufficient protons from the hydration of $CO_2$ to account for the remaining fraction of bicarbonate reabsorbed in either the proximal tubule or the whole kidney has been widely accepted (2, 3, 6, 9, 20).

There has been little consideration of the effect of carbonic anhydrase inhibition on nephron segments

TABLE III
*Total $CO_2$ Concentration in Deep Loop of Henle's Fluid vs. Vasa Recta Plasma*

| Condition | TF | VR | Uncorrected $\Delta[tCO_2]$‡ | Corrected§ $\Delta[tCO_2]$ |
|---|---|---|---|---|
| | *meq/liter* | *meq/liter* | *meq/liter* | *meq/liter* |
| Acetazolamide, 50 mg/kg per h | 57.4* ±1.6 | 36.7 ±2.5 | 20.9 ±3.3 (6) $P < 0.001$ | 18.3 ±3.4 (6) $P < 0.0025$ |
| Mannitol plus Acetazolamide, 50 mg/kg per h | 37.5 ±1.5 | 34.0 ±1.3 | 3.5 ±0.9 (5) NS | 1.2 ±3.4 (5) NS |

* Data presented as mean values±SE. ($n$) = number of pairs. $P$ value from paired $t$ test.
‡ $\Delta tCO_2$ = TF − VR [$tCO_2$], where TF is tubule fluid and VR, vasa recta.
§ Corrected for plasma water and Gibbs-Donnan equilibrium.

beyond the proximal tubule with regard to the segments responsible for, or the mechanism governing, this process. A preliminary report suggested significant reabsorption of bicarbonate in the superficial loop of Henle and distal tubule, the latter contribution of which was reduced by furosemide administration (21). In that study, however, juxtamedullary nephron function was not examined.

The present study was designed to compare: (*a*) the effect of two doses of acetazolamide (20 and 50 mg/kg per h) on both superficial and juxtamedullary nephron fractional bicarbonate reabsorption, (*b*) the effect of combined mannitol and acetazolamide administration on this function in these segments, and (*c*) to evaluate the transepithelial concentration gradient for bicarbonate between the loop of Henle and the vasa recta.

Several findings emerge. First, inhibition of carbonic anhydrase resulted in a significant increase in the fraction of bicarbonate delivered to each nephron segment evaluated. A significantly greater fraction was delivered out of the superficial proximal tubule than to the bend of Henle's loop of the deeper nephrons. Second, this difference in delivery was obliterated during combined mannitol and acetazolamide administration. Third, a significant portion of the filtered bicarbonate was reabsorbed between the superficial late proximal and late distal tubule as well as between the deep loop of Henle and the base collecting duct during acetazolamide administration. This observation suggests that either the proximal convoluted tubule, proximal straight tubule, the distal tubule, or the loop of Henle per se of either or both populations of nephrons was responsible for reabsorption of 30–40% of the acetazolamide-insensitive fraction. Fourth, a significant portion of the remaining fraction reabsorbed appeared to be in structures between the superficial distal tubule and the base of the collecting duct. Fifth, the favorable transepithelial concentration gradient between the deep loop of Henle and the vasa recta was obliterated during combined mannitol and acetazolamide administration. Finally, the observation that the segmental reabsorptive patterns were similar for both doses of acetazolamide suggests that incomplete inhibition of the enzyme is an unlikely explanation for the observations in this study.

The effect of acetazolamide and acetazolamide plus mannitol on fractional bicarbonate reabsorption is outlined in Fig. 2. The effect of acetazolamide on bicarbonate reabsorption in the superficial proximal tubule (80% inhibition) has been noted by other investigators using similar techniques in vivo (2, 3, 20) and in vitro (1, 5, 22). This study represents the first report of a significantly different fractional delivery when the superficial late proximal tubule and the deep loop of

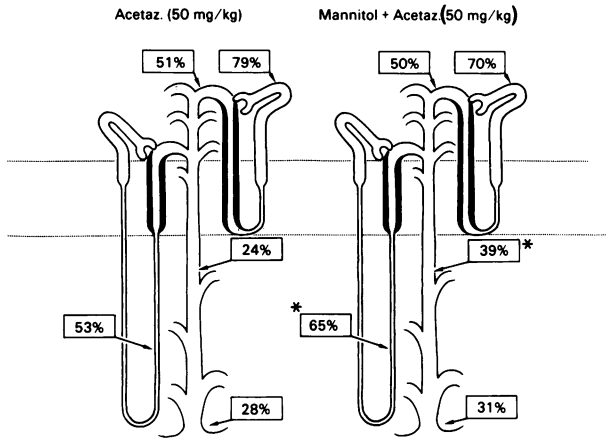


FIGURE 2 Schematic representation of segmental $tCO_2$ delivery. Numbers represent delivery to each segment noted for acetazolamide (Acetaz.) 50 mg/kg alone, left panel, and for mannitol plus acetazolamide, right panel. $^*P < 0.001$ vs. acetazolamide alone.

Henle are compared. Although these segments originate from different nephron populations, several conclusions regarding bicarbonate handling in these segments can be derived from such comparisons. The specific nephron segment responsible for the smaller fraction delivered to the deep loop of Henle cannot be stated with certainty when using the technique of in vivo micropuncture. Anatomical considerations suggest that either the juxtamedullary proximal convoluted tubule, pars recta, or the descending limb of Henle could contribute to this finding. Previous in vitro studies have noted an equal degree of inhibition by acetazolamide in the superficial and juxtamedullary proximal convoluted tubules (78 and 72%, respectively) (22). More importantly, the juxtamedullary proximal convoluted tubule demonstrated a higher basal reabsorptive rate so that a higher residual reabsorptive rate was present after acetazolamide (22). Moreover, Warnock and Burg (23) have reported that the uninhibited juxtamedullary proximal straight tubule reabsorbed significantly more bicarbonate than the superficial pars recta. One would conclude from these two studies that both the juxtamedullary proximal convoluted tubule and straight portion are likely candidates for the results noted in the present study. The descending limb of Henle would be an unlikely candidate since the permeability coefficient of all ions examined thus far has been extremely low (24). Furthermore, the observation that the delivery to the deep loop of Henle was similar to that delivered to the superficial distal tubule is further indirect evidence for this view (Fig. 2). In this respect, our findings suggest that a significant fraction of the acetazolamide-insensitive component of bicarbonate reabsorption is a re-

sult of reabsorption in the proximal straight tubule of the superficial population and the proximal straight and proximal convoluted portion of the juxtamedullary segment.

The specific nephron segment responsible for the reabsorption of ~20% of the filtered bicarbonate between the superficial distal tubule and base of the papillary collecting duct cannot be stated with certainty. Recent evidence from our laboratory supports a low capacity for bicarbonate reabsorption in the distal tubule (25). Similar conclusions have been reached from examination of the cortical collecting tubule in vitro (26). The capacity for bicarbonate reabsorption in the medullary collecting duct was shown to exceed the capacity of the cortical portion by these same investigators, however (26). We have recently demonstrated a significant acid disequilibrium pH at the base collecting duct during bicarbonate loading (11) but not in the late distal tubule under similar conditions (19). Taken together, these findings suggest that the predominant portion of the 20% reabsorbed between these two micropuncture sites (superficial distal tubule and base papillary collecting duct) during acetazolamide administration occurred in the medullary collecting duct.

Bicarbonate reabsorption along the papillary collecting duct was not observed in this study during administration of a carbonic anhydrase inhibitor (groups II, III, and V). This observation is compatible with the absence of carbonic anhydrase in this segment as noted in recent histochemical studies (27). Our finding of no significant bicarbonate in this segment during acetazolamide administration is similar to the recently reported finding of others (28).

In evaluating the nephron segments responsible for reabsorption of the acetazolamide-insensitive portion of bicarbonate reabsorption and the disparate effect on the superficial and juxtamedullary nephron population, we were intrigued by the relationship between bicarbonate reabsorption and water abstraction in these segments. Fractional bicarbonate reabsorption in segments beyond the proximal tubule occurred in association with a significant increase in the $TF/P_{In}$ and $tCO_2$ concentration (group III, Table II). During combined mannitol and acetazolamide administration, the effect of water abstraction in the deeper nephron segments was diminished. Note that a significant reduction in fractional reabsorption up to the loop of Henle and base collecting duct was observed. Such findings, although indirect, offer insight into the mechanism of bicarbonate reabsorption in the deeper nephron segments during carbonic anhydrase inhibition. Because acetazolamide administration dramatically increases the concentration of bicarbonate in tubule fluid in each nephron segment (Table II), a concentration gradient favorable for diffusive bicarbonate flux would be anticipated. The concentration gradient in the deeper nephrons can be estimated from the total $CO_2$ concentration in vasa recta plasma and loop of Henle fluid (Table III). In this case an outwardly directed concentration gradient between the loop of Henle and vasa recta was present during acetazolamide administration (18.3 meq/liter) but was abolished during combined mannitol and acetazolamide administration (1.2 meq/liter). A very large gradient was present at the base of the papillary collecting duct (~98 meq/liter). This gradient, although reduced, remained quite large even in the presence of mannitol. The concentration gradient across the proximal tubule, as predicted from this and other studies (20), was small. Neither the concentration gradient nor fractional reabsorption was altered in this segment after mannitol and acetazolamide, however. The permeability coefficient for bicarbonate is not known precisely for all segments of the nephron. Recent estimates from micropuncture and microperfusion studies in vivo and in vitro suggest that the superficial and juxtamedullary proximal convoluted and straight tubules are permeable to bicarbonate (29). The permeability coefficients in the cortical and medullary collecting tubules are not known for bicarbonate, although a recent study demonstrates that these segments are quite capable of bicarbonate transport (26). If one assumes a permeability coefficient in the range of $10^{-5}$ to $10^{-6}$ cm $s^{-1}$, despite these relatively low values, passive movement could still be significant in the presence of large concentration gradients such as suggested in this study between the late distal tubule (40–23 mM) (Tables I and II) and the base of the papillary collecting tubule (135–36 mM) (Tables II and III). For example, the calculated passive flux rate in the late distal tubule ($JtCO_2 = PtCO_2 \cdot \Delta C$), where J is flux, and $\Delta C$ is concentration gradient, would be 6.1 pmol $mm^{-1}$ $min^{-1}$ or 0.61 pmol $mm^{-1}$ $min^{-1}$ for permeability coefficients of $10^{-5}$ and $10^{-6}$ cm $s^{-1}$, respectively. At the base collecting tubule, the $tCO_2$ flux would be 35.2 pmol $mm^{-1}$ $min^{-1}$ or 3.53 pmol $mm^{-1}$ $min^{-1}$. These estimated values for passive bicarbonate flux are within the range reported for these segments perfused in vitro with symmetrical solutions (25 mM $NaHCO_3$) (26). Our results suggest, but do not prove, that acetazolamide administration inhibits bicarbonate reabsorption to a major extent in the proximal tubule, but that a significant degree of bicarbonate reabsorption is accomplished in the deeper and more distal segments by passive reabsorption driven by the favorable bicarbonate concentration gradient generated by the drug. A portion of this remaining reabsorptive process could occur from protons supplied by the uncatalyzed hydration of $CO_2$ or by recycling of carbonic acid as previously proposed (7).

Although speculative, we would also conclude that the observation of significant bicarbonate reabsorption by the whole kidney during complete inhibition of the enzyme does not provide evidence for a non-proton-dependent reabsorptive process when the enzyme is intact. Outwardly directed concentration gradients of the magnitude demonstrated in this study are not achieved during control conditions, as the bulk of filtered bicarbonate is reabsorbed in the proximal tubule so that the delivery of bicarbonate to more distal segments is low. Therefore, passive reabsorption, as suggested by our study during acetazolamide administration, would be considered a special but predictable result of inhibition of the enzyme and cannot be used to support a significant degree of passive bicarbonate reabsorption when the enzyme is intact.

Other explanations for the reabsorption of bicarbonate in the deep loop of Henle after acetazolamide that is inhibited by mannitol should be considered. Mannitol administration resulted in an increase in urinary flow rate even in the presence of acetazolamide, as expected (group V, Table I). Furthermore, a more pronounced reduction in water abstraction in the deeper nephrons was observed. Thus, one would anticipate an increase in flow rate in the segments comprising the juxtamedullary loop of Henle. Cogan and Rector (20) have recently examined the determinants of superficial proximal bicarbonate, chloride, and water reabsorption during carbonic anhydrase inhibition. These workers observed a significant decrease in fractional bicarbonate reabsorption in this segment as a result of acute volume expansion with Ringer's bicarbonate when carbonic anhydrase was inhibited. Plasma expansion with concomitant acetazolamide administration had no effect on fractional absorption in the proximal tubule, however. It is possible that an increase in flow rate in the deep nephrons would also be associated with a decline in fractional bicarbonate and salt transport in our study as well. Because carbonic anhydrase-independent acidification may be sensitive to changes in luminal concentrations of bicarbonate, the addition of mannitol in the inhibited state could be associated with a reduction in proton secretion as a result of a decrease in cellular acidification independent of carbonic anhydrase. We have not examined the role of flow rate or luminal bicarbonate concentrations per se in the present study. In addition, several differences in protocol in the above study (20) and our study preclude such comparisons. For example, Cogan and Rector (20) examined bicarbonate transport only in the superficial proximal tubule where the transepithelial bicarbonate concentration gradient was minimal and a disequilibrium pH was present (19). In contrast, we have measured very large concentration gradients in the deeper and more distal segments where disequilibrium is not present. These investigators also noted quite different results with plasma expansion and Ringer's expansion as mentioned (20). Although only colloid-free Ringer expansion decreased proximal tubule bicarbonate reabsorption in their study (20), the addition of mannitol, in our study, slightly increased fractional reabsorption (Table II). In order to exclude an effect of flow rate in the deep nephrons, studies during acetazolamide administration while altering flow rate or luminal bicarbonate concentration would be necessary, therefore. Nevertheless, carbonic anhydrase-independent proton secretion driven by high luminal bicarbonate concentrations that are reduced by mannitol appears to be a possible explanation for our findings.

If water abstraction resulted in a significant increase in the concentration of nonbicarbonate buffer in these same segments, back titration of bicarbonate by these buffers could also contribute to the apparent reabsorption of bicarbonate. This possibility seems less likely when one considers the actual amount of nonbicarbonate buffer necessary to explain our observations. Consider the increase in $TF/P_{In}$ from late proximal tubule to the loop of Henle during acetazolamide administration (1.6 to 4.5 or a 2.8 times increase). By water abstraction alone the predicted increase in $tCO_2$ concentration would be 30 to 84 mM in these same segments ($2.8 \times 30$ mM = 84 mM). The observed increase in $tCO_2$ concentration was from 30 to 53 mM (less than predicted). Therefore, the amount of nonbicarbonate buffer present in the acid form necessary to back titrate this amount of bicarbonate would be ~30 mM (84–53 mM), a concentration exceeding the amount of nonbicarbonate buffer available. Even larger concentrations would be necessary in the more distal segments. Buerkert and associates (30) have recently reported that the concentration of ammonium in the deep loop of Henle in control rats is 11 mM, whereas values in the base and tip of the papillary collecting duct are 94 and 122 mM, respectively. Similar measurements are not available after acetazolamide, however. Although back titration by nonbicarbonate buffer may occur, the quantitative contribution appears less than necessary to explain the results observed in this study.

In summary, significant nephron heterogeneity for transport of bicarbonate during carbonic anhydrase inhibition is demonstrated by our findings. The relationship between bicarbonate reabsorption and water abstraction in segments beyond the proximal tubule, the favorable transepithelial concentration gradient for bicarbonate reabsorption generated by acetazolamide in these segments, and the alteration of both of these parameters by mannitol administration suggests that the reabsorption of bicarbonate during inhibition

of the enzyme is accomplished, in part, by passive bicarbonate transport. The contribution of carbonic acid recycling would be anticipated to be most significant in segments in which an acid disequilibrium pH would be present during carbonic anhydrase inhibition (proximal tubule, medullary, and papillary collecting tubule) (11, 19, 31, 32). The quantitative contribution of carbonic acid recycling cannot be stated with certainty in the absence of information regarding the permeability of the luminal membrane to carbonic acid, or the magnitude of the disequilibrium pH in the juxtamedullary nephron segments. The evidence that bicarbonate reabsorption is accomplished predominately by proton secretion when the enzyme is intact appears overwhelming (19, 31, 32). The present results may not pertain to the normal physiological acidification in the kidney in the uninhibited state but emphasize the important contribution of favorable concentration gradients generated by inhibition of the enzyme. A more rigorous examination of this hypothesis requires knowledge of the permeability to bicarbonate and the magnitude of bicarbonate transport when similar concentration gradients are imposed in the segments in question.

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