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Effects of Euvolemic and Hypervolemic Hyperbicarbonemia on Segmental Nephron HCO_3 Reabsorption in the Newborn Dog[1]

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ABSTRACT. Studies were undertaken to determine the effect of elevated plasma bicarbonate concentration ($PHCO_3$) with and without volume expansion on segmental nephron HCO_3 reabsorption in newborn and adult dogs using the technique of distal blockade. Reabsorption of bicarbonate per mL glomerular filtrate ($RHCO_3$/GFR) in the proximal nephron was more suppressed in euvolemic newborns than euvolemic adults as $PHCO_3$ was increased from baseline to 50–70 mM. Inasmuch as total nephron reabsorption has been shown to be essentially complete under these conditions in both newborns and adults, distal nephron HCO_3 delivery and the fraction of the distal HCO_3 load reabsorbed must have been greater in euvolemic newborns than adults when $PHCO_3$ was elevated. Total nephron $RHCO_3$/GFR was less suppressed by $NaHCO_3$ volume expansion in the newborn than it was in the adult. However, proximal nephron $RHCO_3$/GFR was similarly suppressed by $NaHCO_3$ volume expansion in newborns and adults. Thus, the $NaHCO_3$-expanded newborn must have reabsorbed a greater proportion of the increased distal HCO_3 load than did the $NaHCO_3$-expanded adult. Proximal nephron HCO_3 reabsorption is a balance between reabsorption effected by active proton secretion and passive HCO_3 back leak. Euvolemic increase in peritubular HCO_3 concentration has been shown to suppress proximal tubular proton secretion; volume expansion increases proximal tubule HCO_3 permeability and back leak. Thus, we interpret these results to indicate that: *1)* the limited capacity of the immature proximal tubule for proton secretion is most likely responsible for the greater suppression of proximal $RHCO_3$/GFR by increased $PHCO_3$ in euvolemic newborn dogs compared with adults; *2)* the increase in HCO_3 permeability of the immature proximal nephron with volume expansion is no greater than that which occurs in the mature proximal nephron with volume expansion; and *3)* the immature distal nephron displays a greater propensity for HCO_3 reabsorption as $PHCO_3$ is increased than does the mature distal nephron, with or without volume expansion. (*Pediatr Res* 27: 604–611, 1990)

Abbreviations

$FRHCO_3$, fractional reabsorption of the filtered bicarbonate load
GFR, glomerular filtration rate
$PHCO_3$, plasma bicarbonate concentration
$RHCO_3$/GFR, reabsorption of bicarbonate per mL glomerular filtrate
U:PNa, urine sodium to plasma sodium ratio
U:POsm, urine osmol to plasma osmol ratio
(U—P)P_{CO_2}, difference in P_{CO_2} between urine and plasma

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For many years it had been claimed that newborn infants had a lower plasma threshold for bicarbonate excretion than did adults (1–3). However, the infusion of bicarbonate in these studies probably expanded extracellular volume. When $PHCO_3$ was progressively elevated in newborn dogs without producing volume expansion by exchange transfusion with high $PHCO_3$ and low plasma chloride concentration whole blood, there was no tubular maximum for bicarbonate and renal tubular bicarbonate reabsorption was essentially complete over a range of $PHCO_3$ from 10 to 50 mM (4). Thus, it seems that suppression of bicarbonate reabsorption by extracellular volume expansion, rather than a limited resorptive capacity, may well have accounted for the lower plasma threshold observed in newborn infants. On the other hand, volume expansion natriuresis is attenuated in the newborn compared with the adult (5–8). This attenuated natriuresis is due to enhanced fractional reabsorption of sodium by the immature distal nephron (5, 7, 9, 10). Volume expansion actually suppresses fractional sodium reabsorption by the proximal nephron more in the newborn than in the adult (7).

In our study, the effect of elevated $PHCO_3$ without volume expansion on proximal nephron bicarbonate reabsorption and the effect of elevated $PHCO_3$ with volume expansion on segmental bicarbonate reabsorption were investigated in the newborn and adult dog using the technique of distal nephron blockade. The purpose was to compare how volume expansion modifies the segmental response of the immature and mature nephrons to an increased filtered load of bicarbonate.

MATERIALS AND METHODS

Studies were performed on newborn (4–22 d) and adult mongrel dogs of either sex. The protocol conformed to the *NIH Guide for the Care and Use of Laboratory Animals* (11) and was approved by the Laboratory Animal Users Committee of the State University of New York at Stony Brook.

Animals were anesthetized with 20–30 mg/kg of i.v. pentobarbital. Additional pentobarbital was given as necessary during surgery to maintain an adequate level of anesthesia. Rectal temperature was maintained at 36.9°C in the newborn dogs (12) on a warmed operating table with a servo-controlled heat lamp. Rectal temperature was maintained between 38 and 40°C in adult dogs (13) with an overhead radiant warmer. After cannu-

lation of the trachea, all animals were routinely placed on appropriate size volume-controlled ventilators (Harvard Apparatus Co., Inc., S. Natick, MA) with supplemental oxygen. Polyethylene catheters were placed in a femoral vein for infusion of ^{3}H-inulin in a maintenance solution with or without diuretics; in an external jugular vein for infusion of the urine replacement solution; in one or both femoral arteries for blood sampling and heart rate and blood pressure monitoring; and in each ureter for timed urine collection. In addition, the second femoral vein was catheterized in animals undergoing volume expansion for infusion of isotonic sodium bicarbonate solution. All incisions were surgically closed. After surgery, a priming injection of ^{3}H-inulin was given followed by constant infusion of labeled inulin in a glucose, multielectrolyte maintenance solution at a rate of 0.067 $mL \cdot min^{-1} \cdot kg^{-1}$. The respirator was adjusted so that the partial pressure of carbon dioxide in arterial blood was 35–45 mm Hg. After a 1-h recovery and equilization period, the experimental protocol was begun.

Protocol I. Euvolemic hyperbicarbonemia with distal blockade. The purpose of this protocol was to compare the effect of elevated $PHCO_3$ on proximal nephron bicarbonate reabsorption in the newborn and the adult in the absence of volume expansion. After the 1-h recovery and equilibration period, a priming injection of 2.5 mg/kg of ethacrynic acid and 2.0 mg/kg amiloride was given, followed by a constant infusion of these diuretics in the inulin/maintenance solution at rates of 1 $mg \cdot h^{-1} \cdot kg^{-1}$ and 2.4 $mg \cdot h^{-1} \cdot kg^{-1}$, respectively. As diuresis began, urine output was replaced volume for volume with a low bicarbonate solution (140 mM NaCl + 5 mM $KHCO_3$). When U:POsm was 0.95 to 1.05, a control renal clearance of 30 to 120 s was obtained. During this and all subsequent clearance periods, arterial blood was sampled for total plasma protein concentration, plasma sodium concentration, plasma potassium concentration, arterial pH, and P_{CO_2}. All blood sampled was replaced volume for volume with whole blood. Immediately after obtaining this control clearance, a high bicarbonate replacement solution (150 mM $NaHCO_3$ + 5 mM $KHCO_3$) was substituted for the low bicarbonate replacement solution. The respirator was subsequently adjusted to prevent a rapid rate of rise in P_{CO_2}. As $PHCO_3$ rose, four to seven additional clearances were obtained until $PHCO_3$ reached 60 to 70 mM, at which point the experiment was ended. Data were included in the analysis only from clearance periods during which the urine to plasma sodium concentration ratio (U:PNa), as well as the U:POsm, was confirmed to be 0.95 to 1.05.

Protocol II. $NaHCO_3$ volume expansion followed by distal blockade. The purpose of this protocol was to compare the effects of volume expansion on the relationships between $PHCO_3$ and segmental bicarbonate reabsorption in the newborn and the adult. After surgery, protocol II animals were paralyzed with pancuronium bromide and the respirator adjusted so that arterial P_{CO_2} was 35 to 45 mm Hg. Paralysis was maintained throughout the experiment and no subsequent respirator adjustments were made. No further surgery was performed after the administration of pancuronium bromide. After a 1-h period for recovery and equilibration, a control clearance of 30 to 60 min duration was obtained. An arterial blood sample was obtained at the midpoint of this and subsequent clearance periods for determination of total plasma protein concentration, plasma sodium concentration, plasma potassium concentration, pH, and P_{CO_2}. All blood sampled was replaced volume for volume with whole blood. After this initial control clearance, isotonic $NaHCO_3$ (150 mM) was infused at 2.0 $mL \cdot min^{-1} \cdot kg^{-1}$ for 15 min and then at 0.5 $mL \cdot min^{-1} \cdot kg^{-1}$ for the remainder of the experiment. After 45 min at the slower infusion rate, two consecutive clearances (of 30 min duration in the newborn and 15 min duration in the adult) were obtained. After these clearance collections, priming injections of 1.25 mg/kg of ethacrynic acid and 1.0 mg/kg amiloride were given, followed by the constant infusion of the diuretics in the inulin/maintenance solution at 1 $mg \cdot h^{-1} \cdot kg^{-1}$ and 2.4 $mg \cdot h^{-1} \cdot kg^{-1}$, respectively. Urine output during the diuretic infusion was replaced isovolumetrically with a solution containing 140 mM sodium, 5 mM potassium, 140 mM chloride, and 5 mM bicarbonate. After 1 h, three 5- to 15-min consecutive clearances were obtained. Only clearance periods with U:POsm and U:PNa ratios of 0.95 to 1.05 during distal blockade were used.

Sample Analysis. The tritium activity of plasma and urine samples was determined by liquid scintillation counting. Bicarbonate concentrations were calculated from the pH and P_{CO_2} of arterial blood and urine samples obtained with a commercial blood gas analyzer using the Henderson-Hasselbalch equation. A pK′ (negative logarithm of the apparent dissociation constant) of 6.1 was used for the calculation of $PHCO_3$. Urine bicarbonate concentration was calculated using a pK′ estimated from the equation 6.33 − 0.5b, where b is the sum of sodium and potassium concentrations in the urine in mol/L, to account for ionic strength (14). Plasma and urine sodium concentrations were determined by flame emission spectrophotometry. Plasma and urine potassium concentrations were determined by atomic absorption spectrophotometry. Osmolarity was determined by vapor pressure osmometry. Plasma protein concentration was measured by refractometry.

Calculations. GFR was equated with inulin clearance. Bicarbonate reabsorption was calculated as the difference between filtered bicarbonate load (GFR × $PHCO_3$) and bicarbonate excretion (urine flow rate × urine bicarbonate concentration). $FRHCO_3$ was calculated as 1 − (bicarbonate clearance/GFR × 100) and it thus expressed as a percent.

Renal tubular bicarbonate reabsorption is expressed as μmol of bicarbonate reabsorbed per mL of glomerular filtrate to correct for differences in filtered bicarbonate load due to differences in GFR between the newborn and the adult. $RHCO_3$/GFR before infusion of ethacrynic acid and amiloride represents total nephron $RHCO_3$/GFR. Bicarbonate reabsorption during diuretic infusion (all clearances in protocol I and the last set of clearances in protocol II) represents proximal nephron reabsorption. In protocol II, bicarbonate delivery to and reabsorption in the distal segment of the nephron (loop of Henle, distal convoluted tubule, and collecting duct) during $NaHCO_3$ expansion could be calculated. Distal bicarbonate load was calculated as the difference between filtered bicarbonate load during $NaHCO_3$ expansion without diuretics and proximal nephron $RHCO_3$. Distal $RHCO_3$/GFR was calculated as the difference between $RHCO_3$/GFR during $NaHCO_3$ expansion without diuretics and proximal $RHCO_3$/GFR. In both of these calculations, proximal $RHCO_3$/GFR was calculated as the product of the filtered bicarbonate load during $NaHCO_3$ expansion without diuretics and $FRHCO_3$ during $NaHCO_3$ expansion with diuretics. The fraction of the distal bicarbonate load that was reabsorbed in the distal nephron segments was calculated as the quotient of distal nephron bicarbonate reabsorption and distal bicarbonate load, multiplied by 100.

The percentage increase in extracellular volume during $NaHCO_3$ expansion in protocol II was calculated as [(plasma protein concentration during the control clearance/plasma protein concentration during volume expansion) − 1] × 100. This calculation assumes that total intravascular protein content remains constant during the experiment.

Data Analysis. All data are presented as mean ± SEM.

Comparisons of single measure data between newborn and adults within each protocol were made using the unpaired t test if the data were normally distributed or the Wilcoxon rank sum test if they were not. When single-measure data was normally distributed, but the F test for equality of variances indicated that group variances were significantly different, the unequal variance t test (15) was used to make statistical comparisons between newborns and adults.

In protocol I, both the observed $PHCO_3$ values and the number of clearance periods varied within and between the age groups as

a result of the nature of the experimental design. Therefore, linear regression analysis was used to obtain an equation that best related the $PHCO_3$ and the corresponding $RHCO_3$/GFR actually observed in each animal. The unpaired t test was then used to test whether the slopes of these regression equations were different between age groups. Composite regression equations were obtained for each group by calculating the mean ± SEM of $RHCO_3$/GFR values predicted by the individual regression equation at each of several $PHCO_3$ ranging from 20 to 60 mM. A composite regression line and 95% confidence interval was then generated for each age group describing the effect of raising $PHCO_3$ without volume expansion on $RHCO_3$/GFR. In addition, between- and within-age groups comparisons of the observed data under control conditions and at the highest $PHCO_3$ were made using two-factor analysis of variance with repeated measures of one factor.

Between- and within-age groups comparisons under control and $NaHCO_3$ volume-expanded conditions were made using two-factor analysis of variance with repeated measures of one factor. When the F value for interaction effect exceeded the critical F at the 0.05 level of significance, further comparisons were made using the least significant difference test. Comparisons of proximal and distal nephron function between age groups were made using the unpaired t test.

RESULTS

Protocol I. Studies were performed in seven newborn (mean age 15 ± 2 d, range 4–21 d) and six adult dogs. Arterial blood gas and plasma and urine electrolyte data for the control clearance period and the clearance period with the highest $PHCO_3$ in protocol I are presented in Table 1. $PHCO_3$ was similar in newborns and adults under control conditions. $PHCO_3$ was raised to similar values in both groups by replacing urine output with the high bicarbonate solution. These increases in $PHCO_3$ were not accompanied by extracellular volume expansion in either age group.

Proximal nephron $RHCO_3$/GFR was similar in newborns and adults under control conditions. Proximal nephron $RHCO_3$/GFR increased with $PHCO_3$ in both groups ($p < 0.001$), but not as much in the newborn (from 22.2 ± 1.1 to 40 ± 3.9 μmol/mL filtered) as in the adult (19.4 ± 4.3 to 47.0 ± 4.5 μmol/mL filtered), $p = 0.04$ for interaction effect. $FRHCO_3$ by the immature proximal nephron tended to be lower than that reabsorbed by the mature proximal nephron under both control conditions (90.9 ± 0.04 *versus* 95.6 ± 0.9%) and at the highest $PHCO_3$ observed (73.4 ± 4.1 *versus* 85.1 ± 2.4%), $p = 0.07$ for main effect of age).

Proximal nephron $RHCO_3$/GFR was linearly related to $PHCO_3$ in six of seven newborn ($r = 0.73$–0.99, $p = 0.08$–0.0001) and six of six adult dogs ($r = 0.97$–1.0, $p = 0.0003$–0.0001). The individual regression lines for all animals are shown in Figure 1. There was no correlation of the regression line slopes with age in the newborn group ($r = 0.05$, $p = 0.92$). The composite regression line and 95% confidence intervals are shown in Figure 2 for the newborn group (*left panel*) and for the adult group (*right panel*). The equation for these composite regression lines are $RHCO_3$/GFR = 0.581 $PHCO_3$ + 7.11 in the newborn and $RHCO_3$/GFR = 0.802 $PHCO_3$ + 2.62 in the adults. The slope of the adult composite regression line is significantly steeper than that of the newborn, $p = 0.05$ by unpaired t test.

Protocol II. Studies were performed in seven newborn (mean age 8 ± 2 d, range 4–19 d) and six adult dogs. Arterial blood gas and plasma and urine electrolyte data for control and $NaHCO_3$ expansion clearance periods in protocol II are presented in Table 2. Control $PHCO_3$ were similar in newborn and adults and similar to those in the protocol I animals.

Renal clearance data under control- and $NaHCO_3$-expanded conditions in the absence of distal blockade is shown in Figure 3. As expected, reabsorption of the filtered load under control conditions was essentially complete at control $PHCO_3$ values in the absence of volume expansion in both groups. $FRHCO_3$ was 99.9 ± 0.0% in newborns and 99.8 ± 0.1% in adults.

Isotonic $NaHCO_3$ infusion increased $PHCO_3$ and the filtered load of bicarbonate per GFR (to 34.0 ± 2.5 and 31.8 ± 2.2 μmol/mL filtered) and extracellular volume (by 24 ± 2 and 21 ± 4%) similarly in the newborn and adult. Inhibition of bicarbonate reabsorption was significantly greater in the adult than in the newborn under these conditions. $FRHCO_3$ in the volume-expanded state was 94.4 ± 1.9% in newborns and only 77.0 ± 1.9% in adults ($p = 0.0001$).

Segmental renal function during $NaHCO_3$ volume expansion is shown in Figure 4. Bicarbonate reabsorption in the presence of distal nephron transport blockade with ethacrynic acid and

Table 1. *Arterial blood gas and plasma and urine electrolyte data for control clearance period and clearance period with highest $PHCO_3$ in protocol I* *

	Control		Highest $PHCO_3$	
	Newborn	Adult	Newborn	Adult
pH†	7.39 ± 0.03	7.29 ± 0.05	7.61 ± 0.04	7.57 ± 0.02
P_{CO_2}, mmHg†,‡	42 ± 2	41 ± 5	55 ± 4	64 ± 4
$PHCO_3$, mM†	24.6 ± 0.9	20.3 ± 4.1	52.5 ± 3.4	55.5 ± 4.5
POsm, mM†,§	290 ± 4	298 ± 2	289 ± 2	289 ± 2
UOsm, mM†,§	294 ± 4	306 ± 4	284 ± 3	300 ± 2
U:POsm	1.01 ± 0.01	1.02 ± 0.01	1.01 ± 0.01	1.04 ± 0.01
PNa, mM§	134 ± 1	142 ± 1	134 ± 1	141 ± 0
UNa, mM§	133 ± 2	145 ± 1	135 ± 2	146 ± 1
U:PNa†	0.99 ± 0.01	1.02 ± 0.00	1.01 ± 0.01	1.03 ± 0.01
PK, mM†,§	4.9 ± 0.2	4.1 ± 0.2	4.8 ± 0.2	3.3 ± 0
UK, mM	7.3 ± 0.4	5.8 ± 0.4	7.2 ± 0.9	7.0 ± 0.7
TP, g/dL§	4.2 ± 0.1	5.2 ± 0.2	4.0 ± 0.2	5.1 ± 0.2

* POsm, plasma osmolarity; UOsm, urine osmolarity; PNa, plasma sodium concentration; UNa, urine sodium concentration; PK, plasma potassium concentration; UK, urine potassium concentration; and TP, total plasma protein concentration.

† $p \leq 0.05$ for bicarbonate effect (control *vs* highest $PHCO_3$) by analysis of variance.

‡ $p \leq 0.05$ for interaction effect by analysis of variance.

§ $p \leq 0.05$ for age effect by analysis of variance.

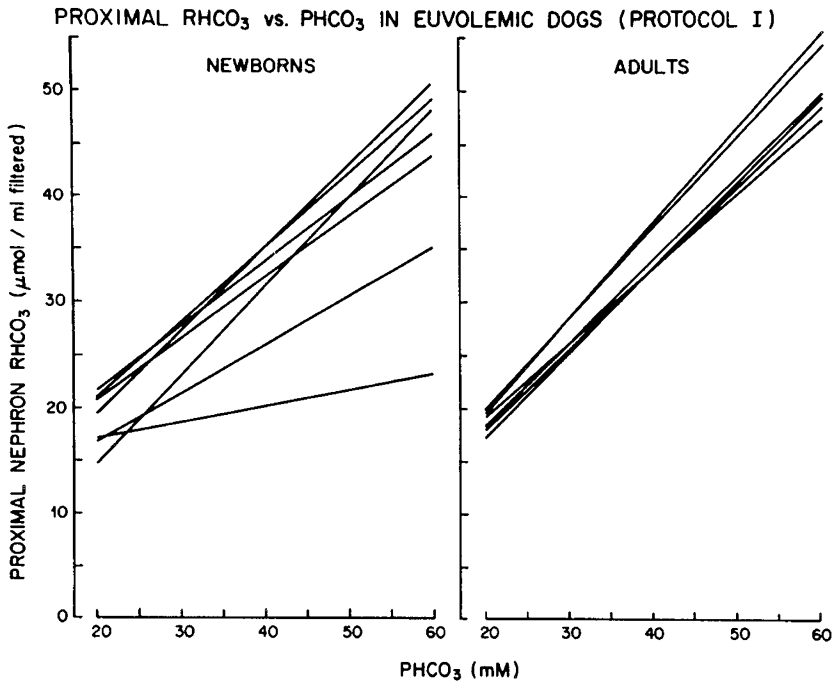


Fig. 1. Proximal $RHCO_3$/GFR *vs* $PHCO_3$ in euvolemic dogs (protocol I). Individual best-fit lines of regression of proximal nephron $RHCO_3$/GFR on $PHCO_3$ in seven euvolemic newborn dogs (*left panel*) and in six euvolemic adult dogs (*right panel*).

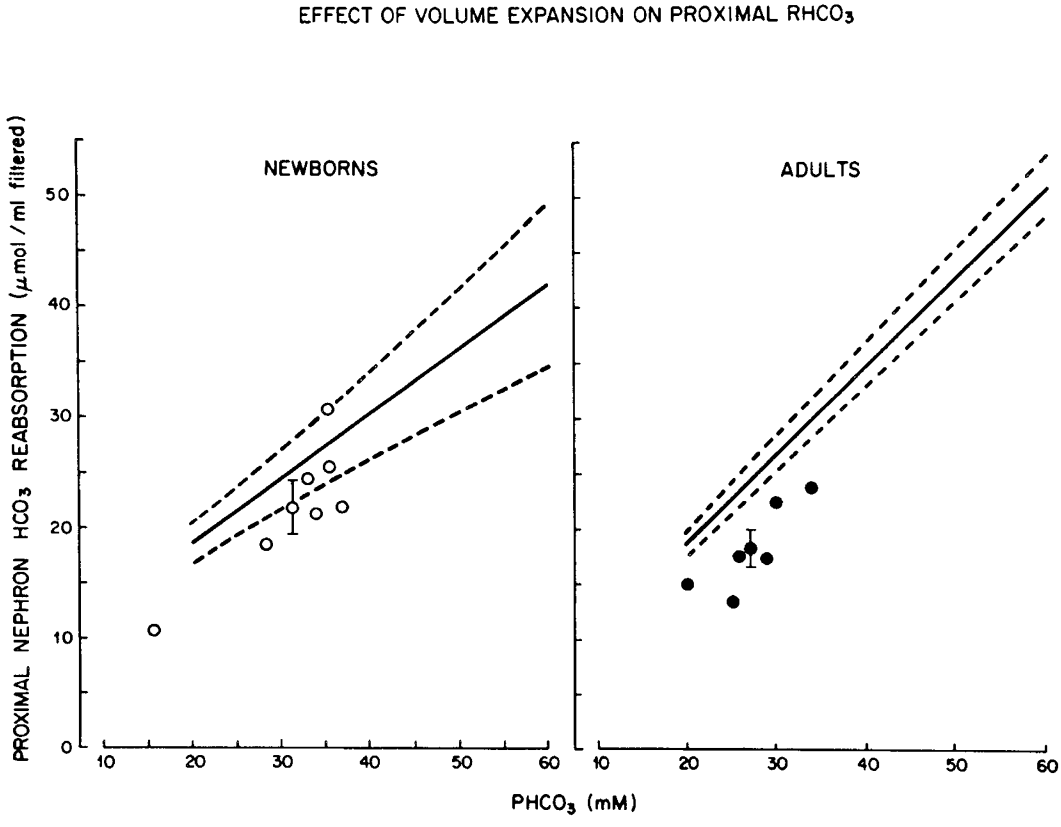


Fig. 2. Effect of volume expansion on proximal $RHCO_3$/GFR. *Left panel*: Proximal nephron $RHCO_3$/GFR *vs* $PHCO_3$ for each of the $NaHCO_3$ volume-expanded newborn dogs (protocol II) are depicted by *open circles*. The *open circle with SEM bars* depicts the mean for these seven animals. The composite regression line (*solid line*) and 95% confidence interval (*dashed lines*) relating $RHCO_3$/GFR to $PHCO_3$ in euvolemic (protocol I) newborn dogs is shown for comparison. *Right panel*: Proximal nephron $RHCO_3$/GFR *vs* $PHCO_3$ for each of the $NaHCO_3$ volume-expanded adult dogs (protocol II) are depicted by *filled circles*. The *filled circles with SEM bars* depicts the mean for these six animals. The composite regression line (*solid line*) and 95% confidence interval (*dashed line*) relating $RHCO_3$/GFR to $PHCO_3$ in euvolemic (protocol I) adults is shown for comparison.

Table 2. *Arterial blood gas and plasma and urine electrolyte data for control and $NaHCO_3$-expanded clearance periods in protocol II**

	Control		$NaHCO_3$-expanded	
	Newborn	Adult	Newborn	Adult
pH†	7.40 ± 0.03	7.38 ± 0.02	7.52 ± 0.04	7.53 ± 0.02
P_{CO_2}, mmHg	41 ± 2	40 ± 4	43 ± 2	39 ± 2
$PHCO_3$, mM†	24.7 ± 2.1	22.2 ± 14	34.0 ± 2.5	31.8 ± 2.2
POsm, mM†	294 ± 4	294 ± 4	283 ± 3	286 ± 2
UOsm, mM†,‡,§	856 ± 65	1579 ± 91	376 ± 87	494 ± 67
PNa, mM§	130 ± 2	142 ± 3	129 ± 2	143 ± 3
UNa, mM§	48 ± 15	135 ± 61	48 ± 19	212 ± 28
PK, mM†,§	5.0 ± 0.3	4.1 ± 0.1	3.7 ± 0.2	3.0 ± 0.1
UK, mM†	133 ± 24	150 ± 21	92 ± 39	30 ± 5
TP, g/dL†,§	4.4 ± 0.2	6.0 ± 0.4	3.5 ± 0.1	5.0 ± 0.4

* POsm, plasma osmolarity; UOsm, urine osmolarity; PNa, plasma sodium concentration; UNa, urine sodium concentration; PK, plasma potassium concentration; UK, urine potassium concentration; and TP, total plasma protein concentration.

† $p \leq 0.05$ for bicarbonate effect (control *vs* $NaHCO_3$-expanded) by analysis of variance.

‡ $p \leq 0.05$ for interaction effect by analysis of variance.

§ $p \leq 0.05$ for age effect by analysis of variance.

amiloride is shown on the left of each panel of bar graphs. This is proximal nephron bicarbonate reabsorption. Distal nephron bicarbonate reabsorption during volume expansion is shown on the right of each panel in Figure 4. U:POsm and U:PNa during distal blockade were 1.00 ± 0.02 and 1.00 ± 0.01, respectively, in the newborn and 0.99 ± 0.1 and 1.00 ± 0.01 in the adult. There was no significant difference in proximal nephron $RHCO_3$/GFR between the newborn and the adult at $PHCO_3$ values of 32.3 ± 2.8 and 27.4 ± 2.0. However, proximal nephron $RHCO_3$/GFR for three of the seven newborns was within the 95% confidence interval of the line describing the relationship of $PHCO_3$ and proximal $RHCO_3$/GFR without volume expansion and the group's mean proximal $RHCO_3$/GFR was at the lower limit of the 95% confidence interval (Fig. 2, *left panel*). On the other hand, none of the adults had a proximal nephron $RHCO_3$/GFR that fell within the 95% confidence interval of the line in adults without volume expansion and thus neither did mean proximal $RHCO_3$/GFR for the adult group (Fig. 2, *right panel*).

Because there was no difference in filtered bicarbonate load per GFR or proximal $RHCO_3$/GFR between the newborn and the adult, distal bicarbonate delivery per GFR was similar (*top right panel*, Fig. 4). However, distal $RHCO_3$/GFR was greater in the newborn than the adult (8.5 ± 1.3 *versus* 3.8 ± 1.8 μmol/mL filtered, $p = 0.06$) and, therefore, so was the percent of the distal load that was reabsorbed (83 ± 6% *versus* 28 ± 10%, $p < 0.001$).

DISCUSSION

The newborn kidney is frequently characterized as bicarbonate wasting. There was no evidence of this in a previous study from this laboratory in which $PHCO_3$ was progressively elevated in newborn dogs without accompanying volume expansion. Tubular reabsorption of filtered loads as high as 50 μmol/mL of glomerular filtrate was essentially complete (4). This study demonstrates that tubular bicarbonate reabsorption was suppressed in the newborn and the adult when the increase in filtered load was accompanied by extracellular volume expansion. However, the newborn reabsorbed a significantly greater fraction of the filtered load than did the adult. Thus, the immature kidney conserves bicarbonate when filtered bicarbonate is increased whether or not there is an accompanying increase in extracellular volume.

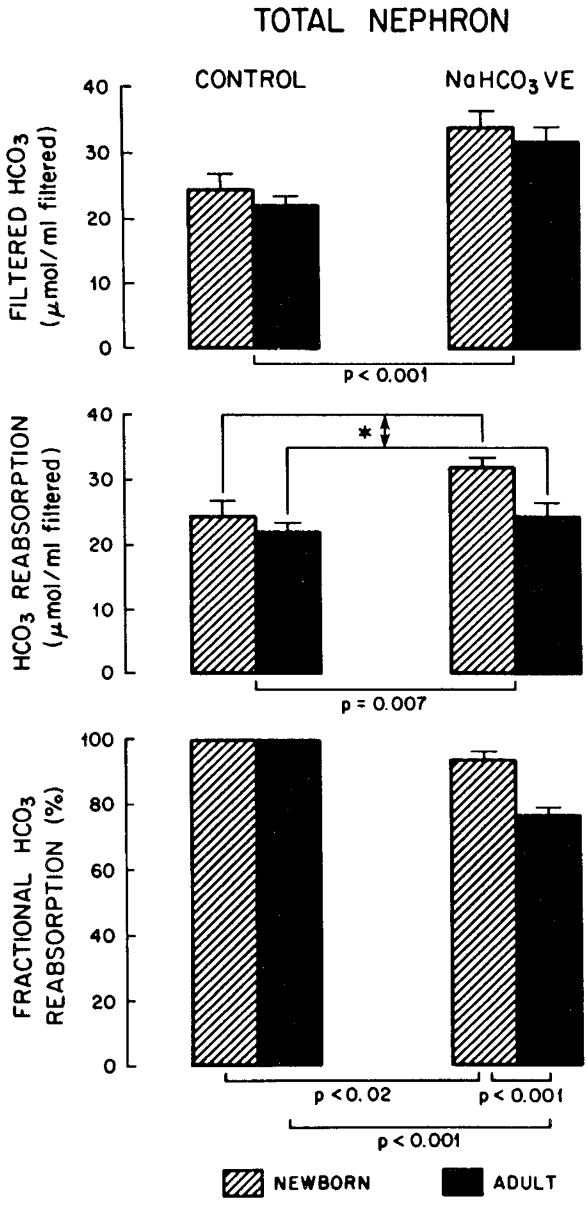
Discussion of protocol I results and of the segmental loci of bicarbonate reabsorption when extracellular volume is expanded and/or $PHCO_3$ elevated is dependent upon the validity of the distal blockade technique. This technique is based on the rationale that urine excreted during the administration of ethacrynic acid and amiloride at appropriate dose rates resembles tubular fluid at the end of the proximal straight tubules. Two assumptions must be met for this to be the case.

First, ethacrynic acid and amiloride must have no effect on the proximal tubule electrolyte reabsorption. In the doses used in this study and in the absence of secondary contraction of extracellular volume, this is likely the case (16–20). The high concentration of amiloride required to block Na^+-H^+ exchange in the proximal tubule makes it unlikely that this action is physiologically relevant (21).

Second, distal electrolyte reabsorption and secretion must be completely blocked. To assure this, clearances during distal blockade were included in the data only if urine to plasma sodium concentration and osmolarity ratios were 0.95–1.05. The mean urine to plasma chloride concentration ratio decreased from 1.26 ± 1.01 to 1.04 ± 0.05 in adults in protocol I as $PHCO_3$ rose and proximal bicarbonate reabsorption decreased, and was 1.20 during $NaHCO_3$ volume expansion in protocol II. These are the ratios expected if distal blockade is complete and urine resembles fluid at the end of the proximal tubule (22–25). However, continued isotonic reabsorption during distal blockade is not excluded by these ratios. The dose of amiloride used in this study abolishes nearly all potassium secretion (17, 26–28) and inhibits bicarbonate reabsorption in the collecting tubule (29, 30). Moreover, proximal fractional bicarbonate reabsorption in protocol I under control conditions in the adult dog (95.6%) was very close to the $FRHCO_3$ of 94% before the distal tubule in hydropenic dogs by micropuncture (31). Thus, urine flow rate and composition during ethacrynic acid and amiloride administration reasonably approximates tubular fluid flow rate and composition at the end of the proximal straight tubule.

The calculation of distal nephron transport from sequential clearances without and with diuretics in protocol II requires a third assumption: that the change in transport after diuretic administration and reequilibration can be principally attributed to inhibition of distal nephron function and not due to an effect of time. This assumption is supported by previously reported results of $NaHCO_3$ expansion in time control experiments (32).

$RHCO_3$/GFR by the proximal nephron was linearly sup-



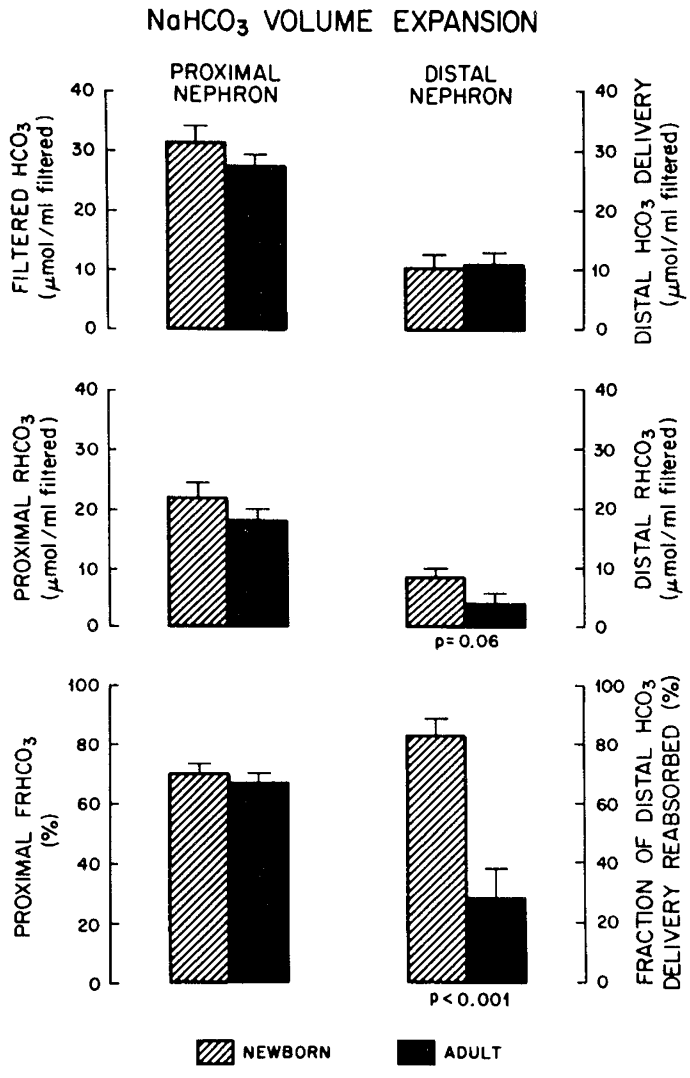


Fig. 3. Total nephron bicarbonate handling under control conditions and during $NaHCO_3$ expansion (protocol II). *Right panel*: Filtered HCO_3 load per GFR, total nephron $RHCO_3$/GFR and $FRHCO_3$ under control conditions (*left*) and during $NaHCO_3$ volume expansion (*right*) in newborn (*open bars*) and adult (*solid bars*) dogs. *Bar heights* depict age group means and *error bars* depict SEM.

Fig. 4. Segmental nephron $RHCO_3$/GFR during $NaHCO_3$ expansion (protocol II). Proximal (*left*) and distal (*right*) nephron bicarbonate loads per GFR, $RHCO_3$/GFR, and $FRHCO_3$ in $NaHCO_3$ volume-expanded newborn and adult dogs. Proximal values are those obtained during volume expansion with ethacrynic acid and amiloride blockade of distal nephron function. Distal values are calculated from values obtained during volume expansion with and without distal blockade. There was a main effect of age on proximal nephron $RHCO_3$ ($F = 4.57$, $p = 0.05$).

pressed in both the newborn and adult as $PHCO_3$ was progressively elevated from control values to 60–70 mM when extracellular volume was maintained constant. Although $RHCO_3$/GFR was similar at $PHCO_3$ near control values, suppression of proximal nephron $RHCO_3$/GFR was more pronounced in the newborn than in the adult as $PHCO_3$ rose. Volume expansion with isotonic $NaHCO_3$ significantly suppressed proximal $RHCO_3$/GFR more than elevation of $PHCO_3$ alone in both age groups at comparable $PHCO_3$. In contrast to hyperbicarbonemia alone, however, there was no difference between the age groups in the degree of suppression produced by hyperbicarbonemic volume expansion. If anything, the decrement in proximal nephron $RHCO_3$/GFR with volume expansion over that with comparable elevation of $PHCO_3$ alone was less in the newborn than the adult (Figs. 2 and 3). We have observed the same tendency with NaCl loading (32). Furthermore, this is opposite of the effect that volume expansion has on proximal nephron sodium reabsorption (5, 7, 10, 32). The former suggests that the decrement in proximal $FRHCO_3$/GFR seen in protocol II over that in protocol I is the result of volume expansion per se; the latter suggests that suppression of proximal nephron $RHCO_3$ by volume expansion is not directly linked to an effect on proximal sodium reabsorption.

Alpern *et al.* (33) have demonstrated that bicarbonate absorption in the proximal nephron can be described by two parallel components, both sensitive to luminal bicarbonate concentration: *1*) saturable proton secretion, and *2*) a passive bicarbonate leak. Active proton secretion is suppressed by increases in $PHCO_3$ but is unaffected by volume expansion. Passive bicarbonate leak is determined by the proximal tubule bicarbonate permeability, which increases with volume expansion, and the transtubular concentration gradient (34). The more pronounced suppression of proximal $RHCO_3$/GFR observed in the newborn by euvolemic hyperbicarbonemia, then, could be the result of a lesser capacity for proton secretion or a greater back leak of bicarbonate in the late proximal tubule as luminal bicarbonate concentration falls along the tubule. However, the similar (or perhaps less pronounced) suppression of proximal $RHCO_3$/GFR by volume expansion in newborns at comparable $PHCO_3$ suggests that the

increase in HCO_3 permeability with volume expansion is similar (or perhaps even less) in the newborn than the adult. Therefore, the limited capacity of the immature proximal nephron for proton secretion seems to be the most likely explanation for the differences in $RHCO_3$/GFR observed between the age groups in protocol I.

Calculation of distal nephron (which by this method includes the loop of Henle, the distal convoluted tubule, and the collecting tubule) load and reabsorption in protocol II revealed greater $RHCO_3$/GFR of a similar HCO_3 load/GFR in the newborn *versus* the adult. The design of protocol I did not allow total nephron reabsorption to be determined in these animals. However, previous studies performed in our laboratory have shown that bicarbonate reabsorption is essentially complete in newborn and adult dogs up to $PHCO_3$ of 50 mM in the euvolemic state (4). Thus, reabsorption of bicarbonate in the distal nephron must increase commensurately with the delivered load, so that it is essentially complete over the range of $PHCO_3$ observed in protocol I. Moreover, the fraction of the filtered load that must be reabsorbed distally increases more in the euvolemic newborn than in the euvolemic adult as $PHCO_3$ rises. As $PHCO_3$ rises from control values to the values observed during $NaHCO_3$ expansion to 60 mM, the fraction of the filtered load reabsorbed distally increases from 9 to 19 to 30% in the euvolemic newborn and 7 to 10 to 15% in the euvolemic adult. Thus, the immature distal nephron displays a greater propensity for bicarbonate reabsorption than does the mature distal nephron with elevation of $PHCO_3$ whether or not there is concomitant volume expansion.

As discussed above, these conclusions require the assumption that distal reabsorption of bicarbonate is completely (or at least similarly) inhibited in the newborn and adult kidney by ethacrynic acid and amiloride. If diuretic administration inhibited distal nephron bicarbonate reabsorption less completely in the adult than it did in the newborn, then distal bicarbonate delivery and reabsorption would actually be greater in the adult than what we concluded them to be. If this were the case, then delivery of bicarbonate per GFR to the distal nephron and distal nephron $RHCO_3$/GFR could actually be similar in the newborn and the adult as $PHCO_3$ is elevated. To investigate this possibility, we examined the $(U—P)P_{CO_2}$ (35). The $(U—P)P_{CO_2}$ is due largely to proton secretion in the presence of tubular bicarbonate in the distal nephron. Increased delivery of bicarbonate to the distal nephron also increases $(U—P)P_{CO_2}$. The $(U—P)P_{CO_2}$ was greater in the newborn than in the adult during diuretic administration in both protocol I (31 ± 6 *versus* 8 ± 7 mm Hg, $p = 0.03$) and protocol II (13 ± 3 *versus* 2 ± 4 mm Hg, $p = 0.03$). Thus, these data are inconsistent both with greater distal bicarbonate delivery per GFR and with greater distal $RHCO_3$/GFR in the adult than the newborn. Although $(U—P)P_{CO_2}$ also varies with medullary countercurrent trapping of CO_2 (35), it is unlikely that this factor accounts for these differences in $(U—P)P_{CO_2}$. Shorter loops of Henle (36) and relatively greater medullary blood flow (37) in the immature kidney would tend to decrease medullary CO_2 trapping and the $(U—P)P_{CO_2}$ in the newborn. If anything, then, these $(U—P)P_{CO_2}$ data suggest that inhibition of distal nephron bicarbonate reabsorption may have been more, not less, complete in the adult than in the newborn. In this case, segmental nephron differences in renal tubular bicarbonate reabsorption per GFR between the newborn and the adult would be even greater than we have estimated them to be.

This greater avidity of the distal nephron when distal delivery is increased is also observed when distal sodium and chloride delivery are increased (7, 32) and thus is probably common to more than one segment of the distal nephron. The factors responsible for this greater propensity for electrolyte reabsorption when delivery is increased cannot be determined from these studies. It is difficult to identify a single factor that could effect the reabsorption of these electrolytes in different segments. It should be noted that reabsorption of sodium, chloride, and bicarbonate per g kidney are much lower in the newborn than in the adult under all of these experimental conditions, even though the fractions of the distal loads reabsorbed are higher, because the distal loads are much lower as a result of a lower GFR per g kidney in the newborn (7, 32). Thus, even if the reabsorptive capacities per g kidney of these segments of the immature distal nephron were less than those of the mature distal nephron, it is possible that these capacities might be exceeded in the adult but not in the newborn. Enhanced electrolyte reabsorption by the immature distal nephron when delivery is increased could, then, be the result of the lower GFR and the resultingly lower distal electrolyte delivery per g kidney in the newborn.

In summary, as $PHCO_3$ and filtered HCO_3 load increase there is less suppression of $RHCO_3$/GFR in the newborn than in the adult dog whether or not these increases are accompanied by extracellular volume expansion. Suppression of proximal nephron $RHCO_3$/GFR is more pronounced in the euvolemic newborn than in the euvolemic adult. Volume expansion, on the other hand, produces no greater inhibition of proximal $RHCO_3$ in the newborn than it does in the adult. We conclude that the immature proximal tubule has a lower capacity for active proton secretion, but similar increase in bicarbonate permeability in response to volume expansion, than the mature proximal tubule. The greater $RHCO_3$/GFR by the whole nephron observed in the newborn compared with the adult as $PHCO_3$ rises with or without volume expansion, then, is the result of greater $RHCO_3$/GFR by the immature than mature distal nephron as distal bicarbonate delivery is increased. The same phenomenon is observed when distal delivery of sodium and chloride are increased, suggesting that the avidity of the immature distal nephron for electrolytes when delivery is increased is not localized to one segment, but probably shared at least by the thick ascending limb of the loop of Henle and the collecting duct.

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REFERENCES

1. Tudvad F, McNamara H, Barnet HD 1954 Renal response of premature infants to administration of bicarbonate and potassium. Pediatrics 13:4–16
2. Edelmann Jr CM, Rodriguez-Soriano J, Boichis H, Gruskin AB, Acosta MI 1967 Renal bicarbonate reabsorption and hydrogen ion excretion in normal infants. J Clin Invest 46:1309–1317
3. Svenningsen NW 1974 Renal acid-base titration studies in infants with and without metabolic acidosis in the postnatal period. Pediatr Res 8:659–672
4. Kleinman LI 1978 Renal bicarbonate reabsorption in the new-born dog. J Physiol (Lond) 281:487–498
5. Aperia A, Elinder G 1981 Distal tubular sodium reabsorption in the developing rat kidney. Am J Physiol 240:F487–F491
6. Goldsmith DI, Drukker A, Blaufox MD, Edelmann Jr CM, Spitzer A 1979 Hemodynamic and excretory response of the neonatal canine kidney to acute volume expansion. Am J Physiol 237:F392–F397
7. Kleinman LI, Banks RO 1983 Segmental nephron sodium and potassium reabsorption in newborn and adult dogs during saline expansion. Proc Soc Exp Biol Med 173:231–237
8. Merlet-Benichou C, de Rouffignac C 1977 Renal clearance studies in fetal and young guinea pigs: effect of salt loading. Am J Physiol 232:F178–F185
9. Kleinman LI 1975 Renal sodium reabsorption during saline loading and distal blockade in newborn dogs. Am J Physiol 228:1403–1408
10. Schoeneman MJ, Spitzer A 1980 The effect of intravascular volume expansion on proximal tubular reabsorption during development. Proc Soc Exp Biol Med 165:319–322
11. NIH Guide for the Care and Use of Laboratory Animals. Office for Protection from Research Risks, National Institutes of Health, Bethesda, MD
12. Fox MW 1963 The clinical behavior of the neonatal dog. J Am Vet Med Assoc 143:1331
13. Fuller JL 1951 Genetic variability in some physiological constants of dogs. Am J Physiol 166:20–24
14. Hastings AB, Sendroy Jr J 1925 The effect of variation in ionic strength on the apparent first and second dissociation constants of carbonic acid. J Biol Chem 65:445–455
15. Snedecor GW, Cochran WG (eds) 1977 Statistical Methods, 6th ed. Idaho State University Press, pp 114–116

16. Dirks JH, Cirksena WJ, Berliner RW 1965 The effect of saline infusion on sodium reabsorption by the proximal tubule of the dog. J Clin Invest 44:1160–1170
17. Duarte CG, Chomety F, Giebisch G 1971 Effect of amiloride, ouabain, and furosemide on distal tubular function in the rat. Am J Physiol 22:632–639
18. Wilczewski TW, Olson AK, Carrasquer G 1974 Effect of amiloride, furosemide, and ethacrynic acid on Na transport in the rat kidney. Proc Soc Exp Biol Med 145:1301–1305
19. Meng K 1975 Comparison of the local effects of amiloride hydrochloride on the isotonic fluid absorption in the distal and proximal convoluted tubule. Pflugers Arch 357:91–99
20. Steen PA, Hartmann A, Kiil F 1981 Ethacrynic acid inhibits transcellular NaCl reabsorption in dog kidneys in doses of 1 to 10 $mg \cdot kg^{-1}$ and proximal bicarbonate-dependent reabsorption at higher doses. J Pharmacol Exp Ther 219:505–509
21. Benos DJ 1982 Amiloride: a molecular probe of sodium transport in tissues and cells. Am J Physiol 242:C131–C145
22. Walker AM, Bott PA, Oliver J, MacDowell MC 1941 The collection and analysis of fluid from single nephrons in the mammalian kidney. Am J Physiol 134:580–595
23. Bennet CM, Clapp JR, Berliner RW 1967 Micropuncture study of the proximal and distal fluid in the dog. Am J Physiol 213:1254–1262
24. Neumann KH, Rector Jr FC 1976 Mechanism of NaCl and water reabsorption in the proximal convoluted tubule of rat kidney. J Clin Invest 58:1110–1118
25. Liu FY, Cogan MG 1984 Axial heterogeneity in the rat proximal convoluted tubule. I. Bicarbonate, chloride, and water transport. Am J Physiol 247:F816–F821
26. Stoner LC, Burg MB, Orloff J 1974 Ion transport in cortical collecting tubule; effect of amiloride. Am J Physiol 227:453–459
27. Banks RO, Kleinman LI 1978 Effect of amiloride on the renal response to saline expansion in new-born dogs. J Physiol (Lond) 275:521–534
28. Lorenz JM, Kleinman LI, Disney TA 1986 Renal response of newborn dog to potassium loading. Am J Physiol 251:F513–F519
29. McKinney TD, Burg MB 1978 Bicarbonate absorption by rabbit cortical collecting tubules *in vitro*. Am J Physiol 234:F141–F145
30. Tam SC, Goldstein MB, Stinebaugh BJ, Chen C-B, Gougoux A, Halperin ML 1981 Studies on the regulation of hydrogen ion secretion in the collecting duct *in vivo*: Evaluation of factors that influence the urine minus blood P_{CO_2} difference. Kidney Int 20:636–642
31. Wong NLM, Quamme GA 1981 Tubular handling of bicarbonate and chloride in the dog. Am J Physiol 241:F219–F223
32. Lorenz JM, Kleinman LI, Disney TA 1986 Lack of anion effect on volume expansion natriuresis in the developing canine kidney. J Dev Physiol 8:395–410
33. Alpern RJ, Cogan MG, Rector Jr FC 1982 Effect of luminal bicarbonate concentration on proximal acidification in the rat. Am J Physiol 243:F53–F59
34. Alpern RJ, Cogan MG, Rector Jr FC 1983 Effects of extracellular fluid volume and plasma bicarbonate concentration on proximal acidification in the rat. J Clin Invest 71:736–746
35. Alpern RJ, Warnock DG, Rector Jr FC 1986 Renal acidification mechanisms. In: Brenner BM, Rector Jr FC (eds) The Kidney. WB Saunders, Philadelphia, pp 206–249
36. Boss JM, Dlonha H, Kraus M, Krecek J 1963 The structure of the kidney in relation to age and diet in white rats during the weaning period. J Physiol (Lond) 168:196–204
37. Kleinman LI, Reuter JH 1973 Maturation of glomerular blood flow distribution in the newborn dog. J Physiol (Lond) 228:91–103