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Effect of Acetazolamide on Renal Tubular Bicarbonate Reabsorption in Newborn Dogs (40685)[1]

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In a previous report (1) we demonstrated that under conditions where isovolemia was maintained, there was no renal tubular maximum (T_M) for bicarbonate reabsorption in newborn dogs, and that as much as 50 μeq of bicarbonate per milliliter glomerular filtrate could be reabsorbed. Moreover, when carbonic anhydrase was inhibited by 50 mg/kg acetazolamide, only 4.5 μeq/ml g.f.r. of bicarbonate reabsorption was inhibited, an amount less than 20% of the total bicarbonate reabsorption, indicating that the major portion of renal bicarbonate reabsorption occurs independent of the effects of carbonic anhydrase. A recent report by Mathisen *et al.* (2), however, suggests that the dose of 50 mg/kg of acetazolamide may have been insufficient to completely inhibit carbonic anhydrase. The present study was carried out, therefore, to establish a dose–response relationship for acetazolamide in the newborn dog, and to determine whether the conclusions reached in our earlier paper were valid, or instead, may have been inaccurate due to incomplete inhibition of carbonic anhydrase.

Methods. Studies were carried out on a total of 15 mongrel puppies, of either sex, 5–30 days of age. The animals were anesthetized by an i.v. injection of pentabarbitol, 25 mg/kg, and placed on a temperature-controlled heating board regulated to maintain rectal temperature at 37°. The trachea was exposed and cannulated to clear the airway and to apply positive-pressure ventilation. Polyethylene catheters of appropriate size (PE50 or PE60) were placed in the right external jugular vein for infusion of ^{3}H-labeled inulin and maintenance fluids, in the right femoral vein for infusion of bicarbonate-enriched blood, in the ascending aorta via the right carotid artery for recording of blood pressure, in the descending aorta via the left femoral artery for withdrawal of blood, and in both ureters for collection of urine.

All animals were infused with a maintenance solution containing glucose (50 g/liter), sodium (20 meq/liter), potassium (10 meq/liter), and chloride (30 meq/liter) at a rate of 0.06 ml/min·kg. A priming injection of ^{3}H-labeled inulin, 3–5 μCi/kg, was followed by a constant infusion of labeled inulin at a rate of 0.06 μCi/min·kg. After a 1-hr equilibration period, a 30-min control clearance was taken.

Plasma bicarbonate levels were changed by the exchange transfusion technique described previously (1). This technique permitted large changes in plasma bicarbonate levels without changing body water and sodium since body weight, hematocrit, and plasma sodium concentrations remained constant throughout the experiment. Blood was withdrawn from the femoral artery at a rate of 1 ml/min·kg while bicarbonate-enriched blood was infused simultaneously at exactly the same rate into the femoral vein. Bicarbonate-enriched blood was prepared by separating plasma from the blood of an adult donor dog, dialyzing the plasma against a high-bicarbonate, multielectrolyte solution (Table I), and then recombining the high-bicarbonate plasma and the red cells to a hematocrit identical to that of the experimental puppy.

Every 30 min during the exchange transfusion, renal clearance studies were performed. Calculations of the renal clearances were made from the blood withdrawn during the exchange transfusion and the urine that was collected over that 30-min clearance period.

Following the collection of two to three clearance studies, acetazolamide was administered at various doses to three different groups of animals. During the acetazolamide infusion, the exchange transfusion with high-bicarbonate blood was continued to maintain high plasma bicarbonate levels.

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TABLE I. COMPOSITION OF MULTIELECTROLYTE SOLUTION FOR EXCHANGE TRANSFUSION

	mM
Na^+	140
K^+	5
HCO_3	140
Mg^{2+}	0.85
Ca^{2+}	2.5
Cl^-	10
SO_4^{2-}	0.85

In five puppies acetazolamide was infused intravenously at a priming dose of 5 mg/kg followed by a maintenance dose of 5 mg/kg/hr for the duration of the experiment (usually another 2 hr). Clearance studies were performed every half hour beginning immediately after the administration of the priming dose.

Five puppies received a priming dose of 50 mg/kg acetazolamide followed by a maintenance infusion of 50 mg/kg/hr for 1 to 2 hr. Then these puppies received another acute dose of acetazolamide at 100 mg/kg followed by a sustaining dose of 100 mg/kg/hr for another 2hr. Clearance studies were performed every half hour beginning immediately after the administration of the first dose.

Five puppies received a dose of 500 mg/kg acetazolamide followed by a maintenance infusion of 500 mg/kg/hr for 2 hr and as in the other experiments, half-hour clearance studies were begun immediately after the administration of the priming dose.

Blood and urine pH and pCO_2 were measured with a Radiometer PHM71MK2 acid–base analyzer. Plasma and urine bicarbonate were calculated from the Henderson–Hasselbalch equation using a pK' of 6.1 for blood (3) and of $6.33 - 0.5([Na+] + [K+])^{1/2}$ for urine (4) estimating the ionic strength of urine as the sum of the concentrations of sodium and potassium.

Sodium concentration of plasma and urine samples were determined by atomic absorption spectrometry (Perkin–Elmer). Plasma and urine inulin were determined from samples placed in Instagel solution for scintillation counting (Packard) of tritium. Hematocrit was measured on all blood samples. Blood pressure was measured directly from the ascending aorta with a transducer (Statham) and recorder (Hewlett–Packard).

The g.f.r. was equated with inulin clearance. The amount of bicarbonate filtered was calculated as the product of g.f.r. and plasma bicarbonate concentration. The amount of bicarbonate reabsorbed was calculated as the difference between the amount of bicarbonate filtered and that excreted and expressed as microequivalents per ml g.f.r. Similar calculations were made for sodium. All results were analyzed with standard statistical computer programs available at the University of Cincinnati.

Results. A bicarbonate titration curve of values taken from all of the animals prior to administration of acetazolamide is presented in Fig. 1. As is readily apparent from the figure, no T_M for bicarbonate was found, and as much as 50 μeq bicarbonate could be reabsorbed per ml g.f.r. Except for a few cases, all of the filtered bicarbonate was reabsorbed even when the plasma bicarbonate exceeded 30 meq/liter. On the average, 0.19 μeq/ml g.f.r. of bicarbonate was excreted, amounting to less than 1% of the filtered load (Table II). When acetazolamide was administered, bicarbonate reabsorption fell and excretion increased (Figs. 2 and 3). As can be seen from the bicarbonate titration curve following the various doses of acetazolamide (Fig. 2), there was still no T_M for bicarbonate even with plasma values exceeding 50 meq/liter. At 5 mg/kg of acetazolamide, reabsorption was inhibited less than at the higher doses ($P < 0.01$), but there was no difference among the three higher doses ($P > 0.05$, using analysis of variance). The line of regression

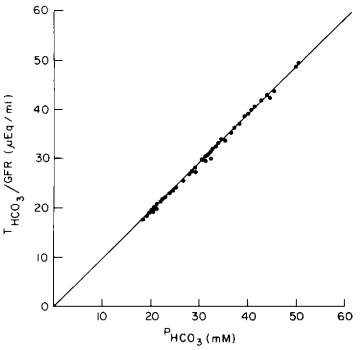

FIG. 1. Bicarbonate reabsorption ($T_{HCO_3/g.f.r.}$) as a function of plasma bicarbonate (P_{HCO_3}) before administration of acetazolamide. The straight line represents complete reabsorption of filtered bicarbonate.

TABLE II. BICARBONATE EXCRETION ($E_{HCO_3/g.f.r.}$), FRACTIONAL BICARBONATE EXCRETION ($F.E._{HCO_3}$), AND FRACTIONAL SODIUM EXCRETION ($F.E._{Na}$) IN NEWBORN DOGS UNDER VARIOUS DOSES OF ACETAZOLAMIDE

Dose (mg/kg)	$E_{HCO_3/g.f.r.}$ (μeq/ml)	$F.E._{HCO_3}$ (%)	$F.E._{Na}$ (%)
0	0.19 ± 0.07[a]	0.72 ± 0.25	0.43 ± 0.09
5	1.99 ± 0.20	6.12 ± 0.65	2.64 ± 0.27
50	4.84 ± 0.35	15.80 ± 2.20	4.55 ± 0.30
100	4.90 ± 0.36	16.30 ± 1.80	5.02 ± 0.35
500	6.13 ± 0.78	15.25 ± 1.84	5.57 ± 0.84

[a] Values are mean ± SE.

drawn in the figure is based on the combined data from the three higher dose groups and its slope, 0.96, is not significantly different from 1. In other words, the same amount of bicarbonate was inhibited by acetazolamide independent of the plasma bicarbonate concentration or filtered bicarbonate load. This is shown further in Fig. 3 which reveals the lack of correlation ($P > 0.10$ for all analyses) of bicarbonate excretion (representing that portion of bicarbonate reabsorption inhibited by acetazolamide) and plasma bicarbonate for each group ($r = 0.17$, 0.24, 0.06, and 0.45 for the 5, 50, 100, and 500 mg/kg dose groups, respectively) as well as for all the groups combined ($r = 0.18$). Also apparent from Fig. 3 is that there is no difference in bicarbonate excretion among the three higher dose groups of acetazolamide although bicarbonate excretion is lower at the 5 mg/kg dose.

Since there was no correlation between excreted bicarbonate and plasma bicarbonate, it is possible to analyze the data from the means of the various dosage groups (Table II). Using analysis of variance, no difference in bicarbonate excretion could be found among the three higher dosage groups ($P > 0.05$) but bicarbonate excretion was lower at the 5 mg/kg dose ($P < 0.01$). Bicarbonate excretion at the higher acetazolamide dose levels averaged 5.3 μeq/ml g.f.r. Fractional bicarbonate excretion (excreted divided by filtered) and fractional sodium excretion followed similar dose–response patterns. At the three higher acetazolamide dose levels, the puppies excreted only 15–16% of the filtered bicarbonate and 5% of the filtered sodium. It should be noted, however, that fractional bicarbonate excretion was slightly greater at lower plasma bicarbonate levels than at higher levels since filtered load was changing with changing plasma bicarbonate levels while excreted bicarbonate was not. At plasma bicarbonate levels of 25 m*M* fractional bicarbonate excretion averaged 21% while at levels of 40 m*M*, it was 13%.

Discussion. Results from the present study confirm results from our previous studies performed on different animals (1). When a bicarbonate titration curve is performed in the neonatal dog and body fluid volume is maintained constant by exchange transfusion, there is no renal T_M for bicarbonate reabsorption and this reabsorption is essentially complete, at least up to values of 50 μeq/ml g.f.r.

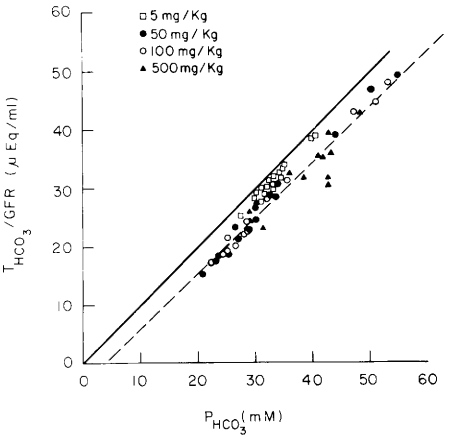

FIG. 2. Bicarbonate reabsorption ($T_{HCO_3/g.f.r.}$) as a function of plasma bicarbonate (P_{HCO_3}) after administration of the various doses of acetazolamide. The straight line represents complete reabsorption of filtered bicarbonate. The dashed line represents the regression equation based on the data from the three highest dosage groups, $y = 0.96x - 3.72$.

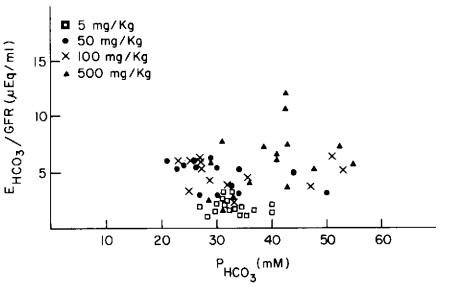

FIG. 3. Bicarbonate excretion ($E_{HCO_3/g.f.r.}$) as a function of plasma bicarbonate (P_{HCO_3}) after administration of the various doses of acetazolamide.

In the earlier study, we wished to investigate the role of carbonic anhydrase in renal bicarbonate reabsorption in the neonatal dog and chose a dose of 50 mg/kg of acetazolamide to inhibit the enzyme. At that time, based on published reports (5, 6), we believed that this dose was sufficient to completely inhibit carbonic anhydrase. However, recent reports have suggested that carbonic anhydrase inhibition may have been incomplete at the acetazolamide dosage used. Mathisen, *et al.* (2) found that in adult dogs under conditions of volume expansion and ethacrynic acid administration, at a dose of acetazolamide of 30 mg/kg, renal bicarbonate reabsorption was 49% of the filtered load but at a dose of 500 mg/kg, it fell to 19% of the filtered load. In the present study at a dose of 50 mg/kg, bicarbonate reabsorption averaged 84–85% of the filtered load and did not change when the dose was raised to 100 or to 500 mg/kg. There are a number of factors that may account for the discrepancies between the two studies. One explanation for the difference may be that mechanisms of renal bicarbonate reabsorption could be different in the neonatal than in the adult dog. Although small differences in bicarbonate reabsorptive mechanisms between adult and newborn dogs cannot be ruled out, it is unlikely that large differences exist for the following reasons. The bicarbonate excretion response of puppies at acetazolamide doses of 5 and 50 mg/kg in the present study could be predicted from the acetazolamide dose–response curve done in adult dogs by Maren (7). Also, the values for the carbonic anhydrase dependent and independent bicarbonate reabsorption in puppies from these and previous experiments, are similar to those obtained by Garg (8) in nonexpanded adult dogs. In those studies benzolamide, when administered at doses calculated to completely inhibit carbonic anhydrase, inhibited the reabsorption of from 4 to 5.7 μeq bicarbonate/ml g.f.r. whereas acetazolamide at the higher dosage levels, inhibited an average of 5.3 μeq bicarbonate/ml g.f.r. in puppies of the present study. Moreover, other studies from our laboratory (9) investigating the interrelationships among bicarbonate, sodium, and chloride reabsorption in the proximal tubule have revealed similarities between newborn and adult dogs.

Another explanation for the differences of results may be related to the different fluid states of the animal between the two studies. For example, animals in the present study were not volume explanded whereas those of Mathisen *et al.* were. In addition, animals in the present study maintained constant fluid balance throughout the experimental procedure. Volume expansion is known to inhibit bicarbonate and sodium reabsorption, (10) which probably accounted for the greater degree of inhibition of bicarbonate reabsorption, even at the lower acetazolamide dosage, in the animals of Mathisen *et al.* (2).

The reason for the large difference in the response at the very high bicarbonate dose between the present study and that of Mathisen *et al.* may be found in the results of earlier studies by Relman *et al.* (11) and Maren (12). Relman *et al.* (11) found that a dose of 500 mg/kg of acetazolamide administered to adult dogs did indeed produce a large increase in bicarbonate and sodium excretion compared to that from an acetazolamide dose of 10 mg/kg. However, when an N^5-methyl analog of acetazolamide was administered at the same high dose of 500 mg/kg, there were also a large increase in bicarbonate excretion. This large bicarbonaturia occurred even though there were only negligible amounts of carbonic anhydrase-inhibiting drug found in the blood or urine since the analog had negligible carbonic anhydrase-inhibiting effect and less than 0.1% of the drug was converted to the active carbonic anhydrase inhibitor, acetazolamide (12). These results with the acetazolamide analog suggest that the increase in bicarbonate excretion from the large dose of acetazolamide was due not to carbonic anhydrase inhibtion but to some other factor. Since the large dose of acetazolamide, as well as its analog, produced a large increase in blood pH (due to the buffer base properties of the drugs), the authors suggested that the change in bicarbonate excretion could have been due to changes in body pH. Unfortunately, in the report of Mathisen *et al.*, there is no data relating to pH changes due to infusion of the large dose of acetazolamide. Since there was no control experiment with a noncarbonic anhydrase-inhibiting analog, there is no certainty that

their results could not be explained by a noncarbonic anhydrase inhibitory action of the large dose of acetazolamide, perhaps related to body pH changes. In the present experiment, the exchange transfusion technique permitted stabilization of pH homeostasis to the extent that following infusion of acetazolamide at the 500 mg/kg dose, there was a statistically insignificant ($P > 0.05$) change of blood pH by only 0.01 units. This stabilization of pH may have prevented the noncarbonic anhydrase inhibitory effect of the large dose of acetazolamide, permitting a true dose–response effect on carbonic anhydrase inhibition. The present study, therefore, supports the conclusions of the earlier experiment from this laboratory (1) demonstrating that at high plasma bicarbonate levels in nonexpanded puppies, the carbonic anhydrase dependent portion of bicarbonate reabsorption accounts for less than 20% of the total renal bicarbonate reabsorption. In addition, in the neonatal dog the dose of acetazolamide of 50 mg/kg is sufficient to completely inhibit carbonic anhydrase activity since higher doses caused no increase in bicarbonate or sodium excretion.

Summary. Bicarbonate titration curves were determined in 15 neonatal dogs, 5 to 30 days of age, under conditions of isovolemia and with the administration of various doses of acetazolamide to inhibit renal carbonic anhydrase. Under each experimental condition used, no T_M for bicarbonate reabsorption could be found. With no acetazolamide, essentially all of the bicarbonate filtered was reabsorbed even at filtered loads of 50 μeq/ml g.f.r. Acetazolamide at a dose of 5 mg/kg inhibited less renal bicarbonate reabsorption than at doses of 50, 100, and 500 mg/kg but there was no difference among the latter three doses. At the higher acetazolamide dose levels, 5.3 μeq of bicarbonate was inhibited per ml g.f.r., amounting to only 12–24% of the total bicarbonate reabsorption (depending upon plasma bicarbonate levels) indicating that the major portion of the renal bicarbonate reabsorption in the neonatal dog is independent of the effects of carbonic anhydrase.

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