BICARBONATE AND pH MEASUREMENTS IN THE PROXIMAL TUBULE OF THE RAT KIDNEY

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

G.F.R.	:	Glomerular filtration rate
mM	:	Millimoles per liter
m.V.	:	Millivolts
μ	:	Micron
μL	:	Micro-liter
O.d	:	Outer diameter
P	:	Probability
pCO_2	:	Partial pressure of carbon dioxide gas
$P_{HCO_3^-}$	:	Plasma bicarbonate concentration
S.E.	:	Standard error
T.F.	:	Tubular fluid
$(TF/P)_{HCO_3^-}$	:	Tubular fluid to plasma bicarbonate concentration ratio.
$1-(TF/P)_{HCO_3^-}$	:	Fractional reabsorption of bicarbonate ion
$U_{HCO_3^-}$	:	Urinary bicarbonate ion concentration

INTRODUCTION

GENERAL REVIEW

The indisputable importance of the extracellular bicarbonate ion concentration in the regulation of acid-base balance derives from the fact that 95% of extracellular buffering is due to the H_2CO_3-HCO_3^- system. The operation of the kidney on an ultrafiltrate of plasma in which bicarbonate is the major labile ion, (one that is synthesized and degraded by metabolic reactions and has buffer properties), accounts for the renal control of the reaction of the body fluids.

Several investigators (Gottschalk *et al*, 1960, Rector *et al*, 1964) utilized quinhydrone electrodes, built according to the design of Montgomery and Pierce (1935) to measure the bicarbonate ion concentration. The principle underlying the measurement is that the bicarbonate ion, being the predominant buffer of the tubular fluid, is expected to change slightly in concentration with varying CO_2 tension; so it is possible to use the pH determined at a known CO_2 tension to estimate the *in situ* bicarbonate ion concentration with some confidence (Clapp *et al*, 1963).

The use of the above electrode has elucidated the nature of the bicarbonate ion reabsorption. Clapp *et al* (1963), reported that approximately three quarters of the filtered bicarbonate is reabsorbed in the proximal tubule. The chloride ion concentration was reported by Walker *et al* (1941) to increase in the mammalian proximal tubules, a finding

which was later confirmed by Malvin et al (1958), and implied that bicarbonate ion concentration decreased below the plasma level. This is in agreement with the work of Rector et al (1964) who has reported that in rats under normal conditions, the bicarbonate reabsorption is faster than that of water. This results in a bicarbonate average concentration of 7.5 mM in the middle third of the proximal tubule as compared to a plasma concentration of 27 mM. Similar results were obtained by Gottschalk et al (1960) who reported that the bicarbonate concentration decreased from 25 to 10 mM in the proximal tubule of the rat kidney.

Micropuncture studies on the rat by Rector et al (1964) reveals that reabsorption of bicarbonate is not a linear function of plasma concentration, but displays saturation characteristics. In the dog, Pitts & Lotspeich, (1946) observed that while bicarbonate reabsorption varied proportionately with glomerular filtration rate, it remained essentially constant at a value of 2.5 mM/100 ml of G.F.R. over a wide range of $P_{HCO_3^-}$ concentration.

The rate of bicarbonate reabsorption has been shown to vary directly with the plasma carbon dioxide tension. Various explanation have been advanced which explain this observation in the light of the forthcoming discussion.

A study of the bicarbonate reabsorption proceeds along the lines of the following reaction which also describes

the buffering action of the HCO_3^--H_2CO_3 system.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

the equation relating the pH with the 2 components of the system is the Henderson-Hasselbalch equation: $pH = pK_1 + \log \frac{HCO_3^-}{H_2CO_3}$ The enzyme carbonic anhydrase catalyzes step 1 of the reaction which is otherwise slow. Step 2 is fast and is not influenced by the enzyme.

The action of carbonic anhydrase in catalyzing the hydration - dehydration reaction renders the system an effective buffer.

An important action of the enzyme is that of facilitating hydrogen secretion. Inhibition of carbonic anhydrase in a hydrogen secreting cell impairs markedly its secreting ability, (Berliner and Orloff 1956).

Carbonic anhydrase is involved in the reabsorption of filtered bicarbonate ion. Inhibition of the enzyme depresses the reabsorption and leads to the excretion of 50% of the filtered bicarbonate, a result found by Berliner and Orloff (1956), and confirmed by Clapp et al (1963).

The close relation between pH and bicarbonate concentration given by the Henderson-Hasselbalch equation, hardly requires emphasis. The measurement of the proximal tubule pH is fundamental for the understanding of bicarbonate reabsorption. Conflicting evidence has been presented regarding

the presence of a hydrogen concentration gradient between the proximal tubular fluid and blood. The proximal tubular pH in the Necturus and the dog is reported to be isohydric (Pierce and Montgomery, 1935, Giebisch, 1956, Berliner, 1952 Pitts and Lotspeich, 1946); whereas data obtained from the non diuretic rat indicate proximal acidification of the fluid (Clapp et al, 1963, Bank, 1962, Gottschalk et al, 1960 and Rector et al, 1965). While the validity of these measurements may be contested, the question of the isohydricity of the proximal tubule remains to be settled.

The simplest mechanism mediating the bicarbonate reabsorption is the tubular ion exchange theory proposed by Pitts and Alexander (1945) to account for urine acidification produced by the addition of hydrogen ion. This ion enters the lumen in response to the electrochemical gradient established by sodium reabsorption. The mechanism is dependent on the activity of luminal carbonic anhydrase to catalyze the dehydration of carbonic acid, which would otherwise accumulate in the lumen. The enzyme in the cell maintains a state of steady hydrogen ion secretion into the lumen.

If the uncatalyzed dehydration of the acid is to account for the observed rate of bicarbonate reabsorption, the steady state concentration of carbonic acid in the lumen must be at least ten times greater than that concentration that would exist were H_2CO_3 in equilibrium with the carbon

dioxide tension of the luminal fluid and plasma (Walser and Mudge). The resultant luminal pH would be lower than the pH calculated from the Henderson-Hasselbalch equation using the luminal concentration of bicarbonate and the pCO_2 of plasma, assuming complete equilibration of luminal H_2CO_3 with plasma CO_2. The difference between these two pH values is termed the disequilibrium pH.

The hydrogen ion secretion hypothesis is compatible with an equilibrium pH which would obtain in the presence of carbonic anhydrase or if carbonic acid diffuses out of the lumen.

Rector et al (1955) have demonstrated a disequilibrium pH in the rat proximal tubule only when carbonic anhydrase is inhibited. This constitutes an evidence that bicarbonate reabsorption in the proximal tubule is mediated through hydrogen ion secretion. According to Berliner (1952), bicarbonate reabsorption throughout the tubule is a manifestation of hydrogen ion secretion in exchange for sodium. The absorption of the latter accounts for acidification. The adrenal steroid effect on urine acidification is cited as an evidence for that.

However, according to Menaker (1948), it is not necessary to invoke hydrogen ion secretion to explain urine acidification, which can be effected by direct bicarbonate ion reabsorption. Brodsky (1955) is an advocate of this

mechanism the elements of which he considers to be:(a) an active transport of bicarbonate ion from the lumen to the cell, (b) a low luminal CO_2 tension due to the removal of bicarbonate.

If this hypothesis holds, carbonic acid would not accumulate, and would equilibrate with the plasma carbon dioxide, consequently no disequilibrium pH would result. The work of Rector et al (1965) which demonstrates the absence of a disequilibrium pH in the rat proximal tubule, supports the hydrogen ion secretory mechanism, but is compatible with the direct bicarbonate reabsorptive mechanism.

The question posed at this stage is whether the bicarbonate reabsorption and the substitution of hydrogen ion for fixed cation are the results of a single cellular mechanism or not. In order to determine this it is necessary to go back to the equation:

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

Urinary CO_2 tension greater than that of blood results from H^+ secretion whereas the opposite holds true if bicarbonate is directly reabsorbed.

Schilb and Brodsky (1966) working on turtle bladders, postulated a carbonic anhydrase independent process that actively transports bicarbonate from the mucosal to the serosal fluid. This accounts for the acidification of the

mucosal fluid which occurs concomitantly with a decrease in the concentration of carbon dioxide to a level below that of the serosal fluid. The fact that urinary bladder of turtle and gastric mucosae (Heinz and Obunk, 1954), can support transepithelial gradients of free CO_2 is in conflict with the notion that free CO_2 is always in a state of diffusion equilibrium across biological membranes.

As yet, there is no conclusive evidence for any of the two bicarbonate reabsorptive mechanisms. Both may be operative, the magnitude of their contribution to the transport varying under different conditions.

2. Scope of the Present Investigation

The quinhydrone microelectrode determinations may be questioned on the grounds that the electrodes are sensitive to oxidation - reduction reactions, and have significant protein errors. They also give an alkaline error even in moderately alkaline solutions.

In the present investigation, a glass-to-glass seal microelectrode is utilized to measure bicarbonate ion concentration in proximal tubular fluid under different conditions of bicarbonate infusions. pH data are obtained using the single unit pH microelectrode. The coupled findings of a proximal tubular fluid having an isohydric pH and a low bicarbonate concentration necessitates the presence of a low T.F. pCO_2 compared with plasma tension in the proximal tubule.

These results are compatible with the two bicarbonate reabsorptive hypotheses: the hydrogen ion secretory mechanism and the direct bicarbonate reabsorptive mechanism.

MATERIALS AND METHODS

1. Animal Preparation

Rats of the Wistair strain, weighing around 200 gms. were anesthetized by an intraperitoneal injection of .35 ml. of inactin (80 mg/ml). The animals were next placed on a thermostatic animal board inside a Faraday cage and subsequently tracheotomized. The right external jugular vein was exposed and cannulated with a fine polyethylene tubing for the infusion of solutions.

The animal was placed on its right side, and the left kidney was exposed through an abdominal incision. The kidney was then freed from fat and embedded with cotton inside a lucite cup. The latter was then firmly screwed to the animal board, and covered with mineral oil to prevent dehydration of the kidney. When necessary, the oil was equilibrated with 6% CO_2. Using thin forceps, the surface of the kidney was carefully cleaned under microscopic control. At this stage the kidney was ready to be punctured.

At the end of the experiment, blood was collected from a thin polyethylene cannula through the femoral artery. Urine was collected by puncturing the bladder and withdrawing urine into a small syringe.

2. Construction of the Single Unit pH Microelectrode

a. Micropuncture Pipette:

By means of a pipette-puller, an ordinary glass capillary with an O.d (outer diameter) of 1.43 mm was pulled into micropipettes. The latter were then bevelled on a rotating grinding stone to a tip diameter of 5-10μ. The tip was then cleaned with distilled water and acetone.

b. pH Capillary:

A corning 015 pH capillary was drawn on a microflame into their segments with an O.d of about 20μ. A segment, 2-3 cm in length was selected and waxed in the stem of an ordinary glass pipette, leaving the enclosed end patent.

c. Agar-KCl Reference Capillary

An ordinary glass capillary was pulled on a micro-flame. The resulting fine segment was filled completely with 2% agar gel containing 3M KCl.

d. Combination into a Single Unit

The pH capillary and the agar-KCl reference electrode were waxed together at the shoulders such that the tip of the latter extends slightly beyond the former. The pair were then inserted into the micropuncture pipette. Under microscopic observation controlled heat was applied on the outside of the pipette causing the wax to melt and fill the shank. Care was taken not to block the tips of the two capillaries. The pipette was then filled with a pH 7 buffer

and an Ag-AgCl wire was inserted into it as the internal reference element of the indicator pH capillary. The open end of the pipette was sealed with wax allowing the silver wire and the stems of the capillaries to emerge out as shown in Fig. 1.

The reference capillary was connected to a calomel, while the stem of the pH capillary was fitted into a polyethylene tubing connected to a syringe. The pH microelectrode can measure the pH of samples of less than .05 μL in volume

3. Construction of the Glass-to-glass Seal pH Microelectrode

a. Micropuncture Pipette:

Corning 0120 glass capillaries, which fuse readily with pH Corning glass, were used to prepare pipettes as previously described.

b. pH Capillary:

Corning 0150 pH capillaries were drawn manually on a microflame. The resulting thin segments had O.d of $\simeq 40\mu$.

c. Combination into a Single Unit:

The micropuncture pipette was placed in the platinum wire loop of the pipette puller such that the tip extended slightly beyond the loop. Under microscopic observation a thin segment of the pulled pH capillary was threaded down the shank of the pipette as far as possible. Using controlled heat the tip of the pH capillary was fused to the micropipette.

The stem of the latter was filled with pH 7 buffer into which an Ag-AgCl wire was introduced. The open end of the pipette was sealed with wax leaving the wire and the pH capillary stem emerging out as shown in Fig. 2. The latter was connected to a polyethylene tubing.

4. Calibration of the pH Microelectrodes

The pH microelectrodes were calibrated using three buffer standards. The calibration was performed under the same conditions as the experiments.

The electrodes were selected on the basis of a potential response of 55 m.v. or more per pH unit.

5. In Vivo Measurements of Intratubular pH

Measurements were made in the Faraday cage where the prepared animal was previously placed. By means of a micromanipulator the pH microelectrode was introduced into the lumen of the proximal tubule through a preformed hole in order to avoid breakage of its tip. The tubular fluid was withdrawn into the pH capillary by a combination of capillarity and very light suction. The pH capillary was inspected microscopically for complete filling. A Cary Vibrating Reed electrometer was used for measuring the potential, which was displayed on a recorder, and read after reaching a steady value. All equipment were grounded to a common copper bar.

6. Measurement of Blood pH

The femoral artery was cannulated with an enlarged version of the same electrode. The artery was unclamped intermittently to allow arterial blood to flow into the pH capillary. Potential measurements were made as indicated previously.

7. Checks on pH Measurement

a. Infusion of Carbonic Anhydrase

The enzyme carbonic anhydrase was administered to compensate for a possible decrease in contact time of the luminal fluid proximal to the site of puncture. The enzyme dose was 10 mg/Kg animal weight, and was administered in the 150 mM $NaHCO_3$ infusion.

b. CO_2 Equilibration of Mineral Oil

To rule out the possibility of CO_2 loss from the superficial cortical nephrones, the mineral oil covering the kidney was equilibrated with 6% CO_2. The pCO_2 of the oil is approximately equal to that of arterial blood.

8. Measurement of Bicarbonate Concentration in the Luminal Fluid

a. Infusion of Solutions

Animals were divided into groups which received different infusions by means of a Braun Infusion pump. Solutions infused were saline, 25mM $NaHCO_3$ + 125 mM NaCl,

75 mM $NaHCO_3$ + 75 mM NaCl and 150 mM $NaHCO_3$. Carbonic anhydrase and carbonic anhydrase inhibitor were each infused in 150 mM $NaHCO_3$ solutions. Infusion rates were usually 4.5 ml/hr.

b. Collection of Luminal Samples:

Micropipettes prepared as indicated previously were filled with colored oil (Sudan black in castor oil). The pipette was then fitted into a micromanipulator, by means of which the exposed kidney was approached under microscopic control. The selected tubule, usually parallel to the pipette was then punctured. Small droplets of colored oil were injected and their rate of flow observed. Slight negative pressure was then applied such that the oil droplets (in the tubule) distal to the site of puncture were held stationary. When a good collection had been insured, usually when the shank was completely filled, the pipette was withdrawn and a little oil from the layer covering the kidney was aspirated.

c. Collection of Blood and Urine Samples:

Blood samples were collected from a cannula through the femoral artery and into heparinized capillaries. One of the latter was centrifuged and a small amount of plasma was aspirated into a micropipette.

Urine was collected by puncturing the bladder. Another micropipette was used to collect a urine sample.

d. Transfer of Samples;

Under microscopic observation the tubular samples in the pipette were gently injected into a siliconized depression slide filled with paraffin oil (equilibrated with 6% CO_2). The tubular sample formed stationary tiny droplets under the paraffin oil.

e. pH Measurements of the Samples:

Ample time was given for the tubular samples to equilibrate with a continuously circulating layer of CO_2.

The reference electrode consisted of a 3M KCl filled micropipette with a tip diameter of 1µ which was connected to a calomel electrode. The pH microelectrode was calibrated using standard pH buffers. Under microscopic control the two electrodes were brought in contact with the sample under oil. A portion of the latter was withdrawn into the pH electrode leaving the KCl micropipette in contact with the remainder. A reading was taken when a steady value was reached.

f. pCO_2 Determinations:

Simultaneous with the pH measurements, the pCO_2 determinations were made on oil samples using a Severinghaus CO_2 electrode with a reproducibility of $\pm$ 1 mmHg. Blood and urinary pCO_2 were also determined.

g. pH of Blood:

In some instances the pH of blood was determined using a pH meter.

h. Reproducibility Checks:

Bicarbonate standards were prepared with the following concentrations: 30mM and 50 mM. These were treated in the same manner as the samples under study, and their pH was determined under the same experimental conditions.

i. Calculation of Bicarbonate Concentration:

The pH of the sample was determined under a constant known CO_2 tension.

By substituting the measured values in the Henderson-Hasselbalch equation:

$$pH = 6.1 + \log \frac{[HCO_3^-]}{0.0301\ [pCO_2]}$$

The bicarbonate concentration could be evaluated.

RESULTS

1. Simultaneous measurement of pH in the proximal tubule and arterial blood in vivo

a) Non-diuretic rat

Thirty eight in vivo measurements of pH in the proximal tubules of thirteen non-diuretic rats were made using the single unit pH micro electrode (Table I). The mean pH was found to be 7.42 ± .03 (S.E.), a value not significantly different from the pH of blood (7.45 ± .01) measured simultaneously using an enlarged version of the same electrode.

b) Rats receiving carbonic anhydrase infusions

Ten measurements of pH in the proximal tubule yielded a mean value of 7.40 ± .04, which was not significantly different from the simultaneous blood pH of 7.37 ± .01 (Table II).

Since carbonic anhydrase may be present on the luminal surface of the proximal tubule, an excess of the enzyme was infused to compensate for any decrease in the contact time of the intraluminal fluid proximal to the site of the electrode and to maintain the hydration-dehydration of CO_2 in equilibrium once the tubular fluid is within the pH capillary.

Table III shows that in both instances: the untreated rats and those receiving carbonic anhydrase infusions, the pH of tubular fluid was practically identical to that of blood. In other words the proximal tubule was isohydric, a

Table I

I. SIMULTANEOUS MEASUREMENT OF pH IN THE PROXIMAL TUBULE AND ARTERIAL BLOOD IN VIVO (UNTREATED RATS)

	Proximal Tubule	Blood
Mean pH	7.42	7.45
S.E.	0.03	0.003
# of samples	37	14

$.5 < p < .6$

Table II

SIMULTANEOUS MEASUREMENT OF pH IN THE PROXIMAL TUBULE AND ARTERIAL BLOOD IN VIVO IN THE RAT. (CARBONIC ANHYDRASE INFUSION)

	Proximal Tubule	Blood
Mean pH	7.40	7.37
S.E.	0.04	0.003
# of samples	10	3

$p < .7$

state which was also maintained during the infusion of the enzyme. ($.1 < p < .2$).

CO_2 loss from the lumina of superficial cortical nephrons would result in a false lowering of the intraluminal CO_2 tension and elevation of the in situ pH. As a check for this possibility, the intraluminal pH was measured when the mineral oil covering the kidney was continuously equilibrated with a 6% CO_2 mixture. The CO_2 tension of the equilibrated oil was in close agreement with that of the rat's arterial blood. With the CO_2 tension of the oil maintained to prevent CO_2 loss from the intraluminal fluid, the measured intraluminal pH was in close agreement with the simultaneous arterial blood pH.

Thus there seems to be no hydrogen gradient in the proximal tubule of the rat kidney.

2. Measurement of Bicarbonate Concentration of proximal tubular fluid and arterial plasma of the rat

a) Saline infusion

With saline infusion (Table IV), the mean proximal tubular bicarbonate ion concentration was found to be 13.39 mM as compared to plasma average concentration of 19.59 mM. The average TF/p bicarbonate concentration ratio was .63.

Table III

THE BLOOD-TUBULAR HYDROGEN GRADIENT OF UNTREATED RATS COMPARED TO THAT UNDER CONDITIONS OF CARBONIC ANHYDRASE INFUSION

	Untreated Rats	Carbonic Anhydrase Infusion
Mean pH (Blood-T.F.)	+0.007	-0.095
S.E.	$\pm$0.044	$\pm$0.033
# of samples	20	10

$.1 < p < .2$

Table IV

SIMULTANEOUS MEASUREMENT OF BICARBONATE CONCENTRATION IN THE PROXIMAL TUBULE AND ARTERIAL BLOOD DURING THE INFUSION OF 150 mM NaCl

Rat #	Tubular HCO_3^-	Plasma HCO_3^-	$(TF/p)_{HCO_3}$	$1-(TF/p)_{HCO_3}$
1	10	18.20	.56	.45
	10.5	"	.58	.42
	"	"	.60	.40
2	14.8	-	-	-
	13.5	-	-	-
	15.5	-	-	-
3	15.1	19.05	.79	.21
	12.6	"	.63	.37
	13.2	"	.69	.31
	13.2	"	.69	.31
	13.8	"	.69	.31
4	16.6	22.91	.72	.28
	13.2	"	.58	.42
	14.5	"	.63	.37
5	7.1	18.20	.39	.61
	11.22	"	.62	.38
	10.72	"	.59	.41
AV	13.39	19.59	.63	.37
S.E.	$\pm$0.51		$\pm$0.03	

b) Different bicarbonate infusions

The infusion of 125 mM NaCl + 25mM $NaHCO_3$ resulted in a lower luminal concentration of bicarbonate but a higher plasma concentration (Table V). This caused the (TF/p) bicarbonate to decrease significantly below the value found under conditions of saline infusion ($.001 < p < .1$).

The infusion of 75mM NaCl + 75mM $NaHCO_3$ caused a marked elevation in both plasma and tubular bicarbonate concentration (Table VI). The net effect was also a significant increase in the (TF/p) bicarbonate concentration ratio.

When isotonic bicarbonate solution was infused, plasma and tubular bicarbonate concentrations increased proportionately keeping the (TF/p) bicarbonate concentration ratio equal to its value under infusion of 75 mM $NaHCO_3$ (Table VII).

The fractional reabsorption expressed as 1 - TF/p is plotted against bicarbonate infusion. Fig. 3 shows that the fractional reabsorption decreases with increasing concentration of bicarbonate in the infusate, reaching a steady value with the infusion of 75 mM $NaHCO_3$ and above. This seems to indicate some form of saturation in the bicarbonate reabsorptive capacity.

Fig. 4 shows that the elevation of plasma bicarbonate, accompanying increased bicarbonate infusion, depresses the fractional reabsorption of the ion.

Table V

SIMULTANEOUS MEASUREMENT OF BICARBONATE CONCENTRATION OF PROXIMAL TUBULE AND ARTERIAL BLOOD (INFUSION OF 125 mM NaCl & 25 mM $NaHCO_3$)

Rat #	Tubular HCO_3^-	Plasma HCO_3^-	$(TF/p)_{HCO_3^-}$	$1-(TF/p)_{HCO_3^-}$	$U_{HCO_3^-}$
1	5.13	14.79	.35	.65	
	7.41		.50	.50	
	5.75		.39	.61	
	3.02		.20	.80	
	7.41		.50	.50	
	7.08		.48	.52	
2	12.30	22.9.	.54	.46	
	10.47		.46	.54	
3	9.5	20.42	.47	.53	1.18
	14.13		.69	.31	
	12.59		.62	.38	
	13.18		.65	.35	
	11.48		.56	.44	
4	13.49	19.95	.68	.32	3.31
	12.02		.60	.40	
	10.47		.52	.48	
	11.48		.58	.42	
	12.88		.65	.35	
5	12.59	23.99	.52	.48	
	12.30		.51	.49	
	13.49		.56	.44	
	10.72		.45	.55	
6	11.75	25.70	.46	.54	
	11.75		.46	.54	
	11.22		.44	.56	
	11.75		.46	.54	
	12.02		.47	.53	
	13.49		.52	.48	
AV	10.75	21.29	.51	.49	
S.E.	±.53		±.0192		

Table VI

SIMULTANEOUS MEASUREMENT OF BICARBONATE CONCENTRATION IN THE PROXIMAL TUBULE AND ARTERIAL BLOOD (INFUSION OF 75 mM $NaHCO_3$ and 75 mM NaCl).

Rat #	$T(HCO_3^-)$	$P_{(HCO_3^-)}$	$(TF/_P)_{HCO_3^-}$	$(1-TF/_P)_{HCO_3^-}$
1	23.99	33.88	.71	.29
	22.91		.68	.32
	21.88		.65	.35
	19.95		.69	.41
	25.70		.76	.24
2	12.95	24.55	.81	.19
	18.20		.74	.26
	17.78		.72	.28
	21.88		.89	.11
	20.89		.85	.15
3	27.54	35.48	.78	.22
	26.30		.74	.26
	30.20		.85	.15
	25.70		.72	.28
	30.20		.85	.15
	26.30		.74	.26
AV	23.71	31.30	.76	.24
S.E.	± .98		± .02	

Table VII

SIMULTANEOUS MEASUREMENT OF BICARBONATE CONCENTRATION IN THE PROXIMAL TUBULE AND ARTERIAL BLOOD (INFUSION OF 150 mM $NaHCO_3$)

Rat #	Tubular HCO_3^-	Plasma HCO_3^-	$(TF/p)_{HCO_3^-}$	$1-(TF/p)_{HCO_3^-}$
1	21.88	26.3	.83	.17
	20.89		.79	.21
	20.89		.79	.21
	17.38		.66	.34
2	18.20	25.12	.72	.28
	16.60		.66	.34
	25.12		1.00	0
3	27.54	61.66	.45	.55
	38.90		.63	.37
	74.13		1.20	.20
	50.12		.81	.19
	61.66		1.00	0
	40.74		.66	.34
4	28.84	46.77	.62	.38
	30.20		.65	.35
	26.92		.58	.42
	32.36		.69	.31
	33.11		.71	.29
	30.90		.66	.34
5	28.84	38.02	.76	.24
	31.62		.83	.17
	35.48		.93	.07
	30.20		.79	.29
AV	32.28	39.57	.76	.24
S.E.	2.95		$\pm$.03	

c) Infusions of carbonic anhydrase and its inhibitor

Initial experiments were conducted using the carbonic anhydrase enzyme and its inhibitor. With the infusion of the enzyme, the luminal concentration of bicarbonate ion fell, the opposite was the case when the carbonic anhydrase inhibitor was infused. Consequently it is observed that carbonic anhydrase enhances the absorption of bicarbonate which is depressed in the presence of the enzyme inhibitor.

d) Effect of plasma pCO_2 on bicarbonate reabsorption

In all the experiments the pCO_2 of blood was elevated, ranging between 47 - 65 mm.Hg. Over this range, no correlation could be found between blood pCO_2 and fractional reabsorption of bicarbonate.

3. Urinary pCO_2

When the infusion contained 25 mM $NaHCO_3$, urinary pCO_2 was lower than that of blood. However, with the increased load of bicarbonate in the infusion, the urinary pCO_2 was greatly elevated above that of blood; the value of the latter being in some instances less than 1/3 of the former.

No urinary pCO_2 was determined for untreated rats. However, the fact that the urinary pCO_2 was lower than blood pCO_2 during the infusion of 25 mM $NaHCO_3$, suggests that under

normal conditions the pCO_2 of urine is lower than that of blood.

When carbonic anhydrase enzyme and its inhibitor were separately infused, the pCO_2 of urine was noted to decrease in both instances.

Table VIII shows the results of bicarbonate measurements on samples with known bicarbonate concentrations. This operation was performed to check the validity of the method used in determining the bicarbonate concentration of tubular and plasma samples.

Table VIII

REPRODUCIBILITY AND PRECISION OF BICARBONATE MEASUREMENT ON $NaHCO_3$ STANDARDS

Standard #	True Conc.	Measured Conc.	Reproducibility	Precision
1	30	26.92		
	30	26.92	4.5% ± 6.2	$.05 < p < .1$
	30	26.92		
2	50	53.70		
	50	41.69		
	50	48.98		

DISCUSSION

The glass-to-glass-seal microelectrode has been shown to be well suited for the measurement of bicarbonate ion concentration on small volumes of fluid. It is superior to the quinhydrone microelectrode, the employment of which involves several errors discussed previously.

Using the afore-mentioned electrode. The mean concentration of bicarbonate in the proximal tubule is found to be 13.4 mM under condition of saline infusion. This agrees fairly well with the quinhydrone-measured concentrations reported by other investigators. (Gottschalk et al, 1960 and Rector et al, 1964). It also demonstrates the existence of a proximal transepithelial bicarbonate gradient resulting from a bicarbonate reabsorption rate faster than that of water.

However, during alkalosis induced by $NaHCO_3$ infusion, the TF/p bicarbonate ratio increases attaining in some instances the value of unity. This is due to the fact that the high concentration of bicarbonate in the glomerular filtrate exceeds the reabsorptive capacity of the proximal tubule so as water is removed isosmotically, the unreabsorbed bicarbonate is concentrated. This shows that the rate of bicarbonate reabsorption is not a linear function of its plasma concentration, instead it displays saturation kinetics. These findings are in agreement with those reported by Rector et al (1964).

When an infusion composed 25 mM $NaHCO_3$ + 125 mM NaCl

is substituted for isotonic saline, the bicarbonate concentration in the proximal tubule decreases despite the concomitant increase in plasma bicarbonate. This is consistent with the observation of Pitts and Lotspeich (1946) that the capacity of renal tubules to reabsorb bicarbonate decreases when an excess of chloride is infused, that no further decrease in tubular bicarbonate concentration and in $(TF/p)HCO_3^-$ occurs with the increasing bicarbonate filtered load, may be due to the saturation of the bicarbonate reabsorptive capacity of the proximal tubule.

Before attempting to go any further with the discussion of the bicarbonate reabsorption, it is essential to obtain a correct measure of pH in the proximal tubule.

The previous in vitro measurements on ultramicro samples involve several potential sources of error among which are the proper thermal equilibration, evaporation and CO_2 loss. Most of these difficulties are overcome, however, if the pH measurement is performed in vivo, as in the case of the present study, where the single unit microelectrode is utilized. The use of the latter has several advantages. CO_2 loss from the test sample is prevented by aspirating it internally. Moreover, the electrode has a large area/volume ratio and a minimal asymmetry potential.

pH measurements of the proximal tubular fluid, using the above electrode have yielded values, in close agreement

with the arterial blood pH measured simultaneously. The validity of these results is checked by infusing the enzyme carbonic anhydrase and by equilibrating the oil covering the kidney with 6% CO_2. In both cases, the pH of the tubular fluid was in good agreement with that of arterial blood.

Whereas results from this study agree with previous findings in indicating a low bicarbonate concentration in the proximal tubule, the pH is found to be nearly the same as that of arterial blood. The latter finding is in conflict with results reported by Rector et al (1965).

Writing up the Hendersen-Hasselbalch equation for the H_2CO_3 buffer system:

$$pH = 6.1 + \log \frac{[HCO_3^-]}{.0301\,[pCO_2]}$$

it becomes evident that the pCO_2 of the proximal tubular fluid must be low. In other words, we have to reject the assumption, made by other investigators, that the tubular fluid CO_2 is in equilibrium with plasma CO_2. Consequently while the proximal tubular bicarbonate concentration is found to be approximately 10 mM, the pCO_2 of the fluid has to be around 17 mm.Hg instead of 40 if the pH is to remain at a value closely approaching 7.4. Therefore a transepithelial CO_2 gradient has to exist across the proximal tubule to explain the absence of a hydrogen gradient. This is contrary to the notion that CO_2 always exists in a state of diffusion equilibrium across membranes.

Evidence for the existence of a CO_2 gradient comes from the work of Brodsky et al (1958) on the dog kidney, and Schilb and Brodsky on the turtle bladder.

It is in the light of the above findings that the hypotheses postulated to account for bicarbonate reabsorption will be examined.

The H^+ secretory mechanism mediating bicarbonate transport does not require the pCO_2 of the proximal tubular fluid to be in equilibrium with that of plasma. If the diffusion of CO_2 out of the lumen proceeds more rapidly than the rate of formation (by the carbonic anhydrase catalyzed dehydration of carbonic acid), then a state of low CO_2 can be maintained in the lumen. When carbonic anhydrase is absent from the luminal border, the accumulation of H_2CO_3 results in a tubular fluid pH lower than that of plasma, unless H_2CO_3 diffuses out of the lumen. In brief the state of isohydricity of the proximal tubular fluid is compatible with H^+ secretion if carbonic anhydrase is present or if H_2CO_3 diffuses out of the lumen.

The direct reabsorption of bicarbonate can explain a low luminal pCO_2. However, we have to postulate a continuous generation of a hydrogen acceptor to bind the hydrogen ion and maintain an isohydric fluid. Perhaps a buffer, present in the tubular fluid, such as di-sodium phosphate may be effective in that respect. Ammonia, secreted actively into

the tubular fluid, may act as the hydrogen acceptor and form ammonium ion, a major urinary constituent.

The low luminal pCO_2 may be due to the intracellular trapping of CO_2 by the proximal tubular epithelium. This is possible on the grounds that the kidney is a very active metabolic organ.

The CO_2 trapping hypothesis could very well account for low bicarbonate and pCO_2, and thus would explain the isohydricity of the proximal tubular fluid.

However, this hypothesis requires the secretion of hydrogen ion into the tubule. H^+ will react with bicarbonate to form carbonic acid. The carbonic anhydrase catalyzed dehydration of carbonic acid yields CO_2 which will subsequently be trapped.

In conclusion, the study demonstrates isohydricity and low bicarbonate concentration in the proximal tubular fluid. A low carbon dioxide tension in the proximal tubule is proposed in the light of the above results. The study does not provide evidence favoring one of the two current bicarbonate reabsorptive hypotheses, but is compatible with both.

CONCLUSIONS

1. The in vivo pH of the proximal tubular fluid in the non-diuretic rat is isohydric (same as arterial blood).

2. Isohydricity of the proximal tubular fluid is maintained when excess carbonic anhydrase is infused.

3. The mean bicarbonate concentration in the proximal tubule is 13.40 mM saline infusion. The (TF/p) bicarbonate concentration ratio is 0.63.

4. The fractional reabsorption of bicarbonate decreases with increasing bicarbonate concentrations in the infusions i.e. The bicarbonate reabsorption does not vary linearly with plasma bicarbonate.

5. Bicarbonate reabsorption by the proximal tubule is enhanced by exogenous carbonic anhydrase and diminished by the enzyme inhibitor.

6. No correlation was found between arterial blood CO_2 tension and the fractional reabsorption of bicarbonate in the proximal tubule.

7. The measured isohydricity and low bicarbonate concentration of proximal tubular fluid can only be explained by a low CO_2 tension of that fluid.

8. The study does not provide evidence favoring one of the two current bicarbonate reabsorption hypotheses.

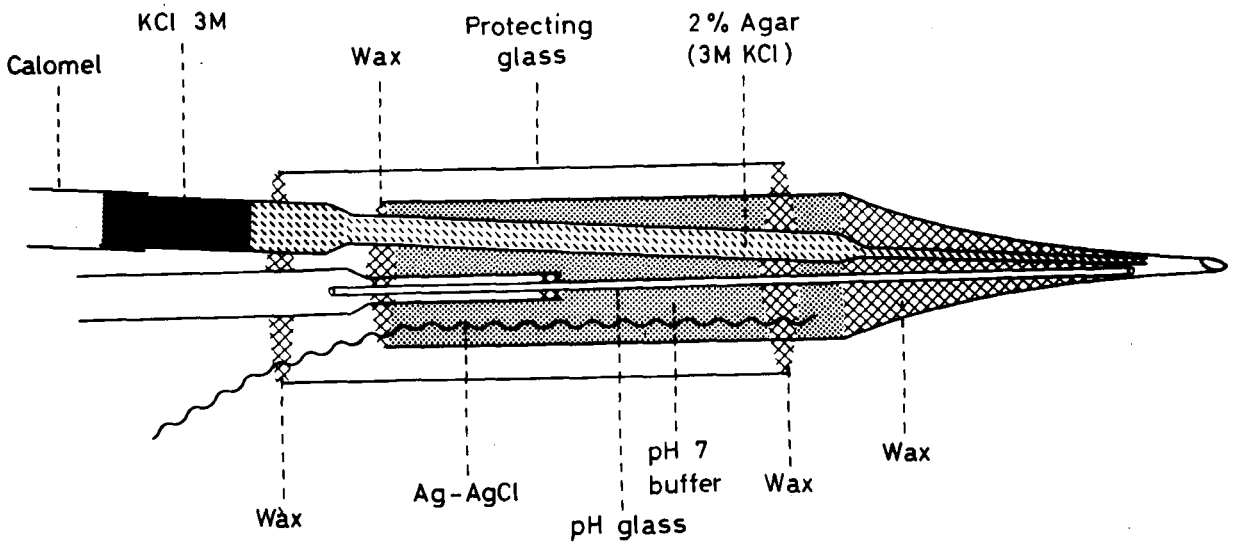

Fig. 1. Single unit pH glass ultra-microelectrode

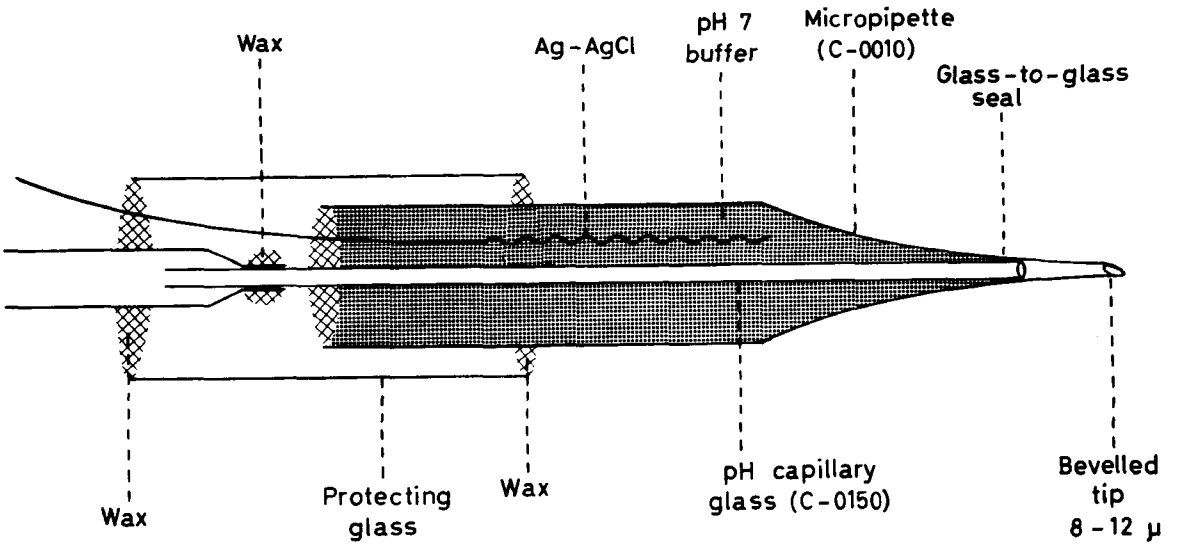

Fig. 2. Glass-to-glass seal pH microelectrode

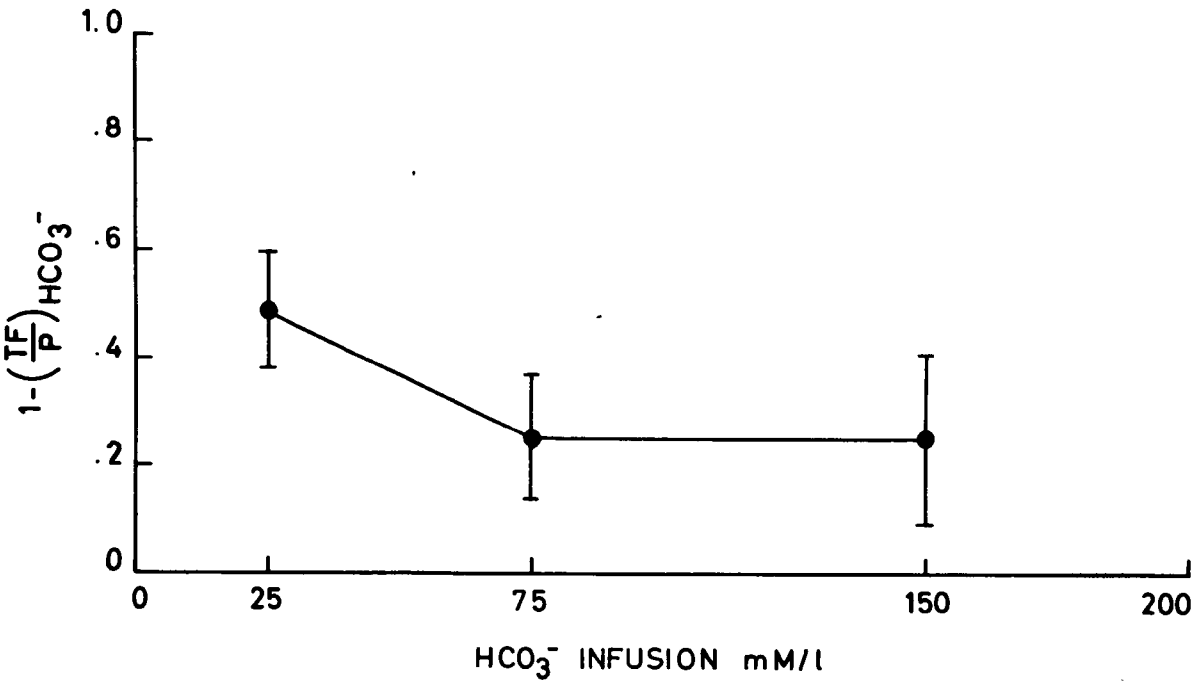

Fig. 3. Effect of increasing bicarbonate infusion on the fractional reabsorption of bicarbonate

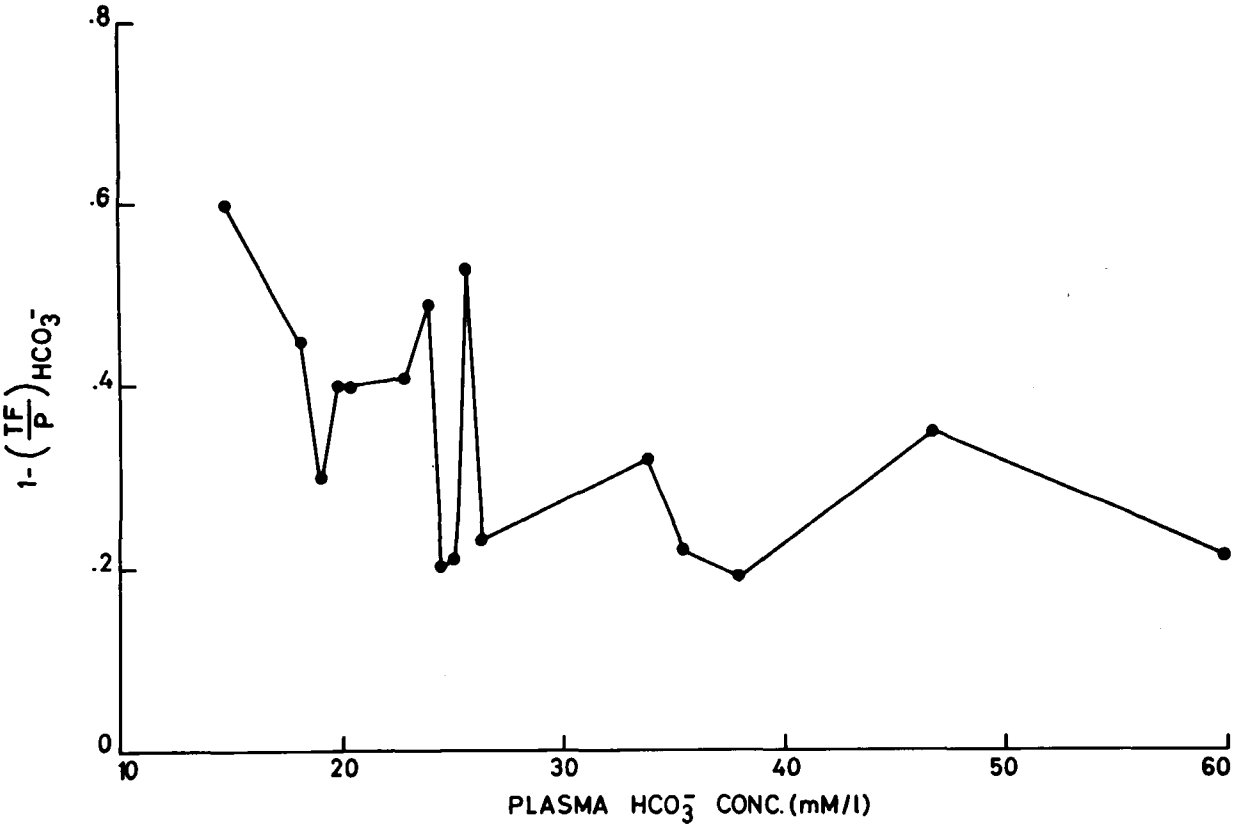

Fig. 4. Variation of fractional reabsorption of bicarbonate with changes in the plasma bicarbonate concentration

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