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Analysis of Variables Influencing Urinary pCO_2 during $NaHCO_3$ and Water Loading in Normal Man (39999)

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Introduction. During bicarbonate diuresis in normal subjects, urinary carbon dioxide tension (pCO_2) is considerably greater than that of the blood because in the distal nephron the rate of dehydration of the intraluminal carbonic acid generated as a result of distal hydrogen ion secretion is insufficiently rapid to permit CO_2 equilibration (1-9).

While it is recognized that, in addition to hydrogen ion secretion, several factors including urine pH, flow rate, and bicarbonate and phosphate levels influence urinary pCO_2 (2, 4-6, 9-18), there is uncertainty concerning their relative importance and interrelationships. The present paper reports the results of multiple regression analysis of the relationship between urinary pH, flow rate (V), bicarbonate and phosphorus concentration (U_{HCO_3} and U_P, respectively) and excretion rates ($U_{HCO_3}V$ and U_PV, respectively), and urinary pCO_2 in normal man, in an attempt to clarify the relative influence of these variables on urinary pCO_2.

Materials and methods. Thirteen studies were performed in 12 healthy out-patient volunteers, ages 21-51 years. Nine were male and three were female. None had clinical or laboratory evidence of renal, endocrine, or cardiovascular disease and all were ingesting a regular diet.

On the day of the study, 5% $NaHCO_3$ was administered orally in a dose of 2 mequiv/kg of body weight. This was given in less than 5 min with an amount of water sufficient to make the total fluid intake 500 ml. Urine was then collected under oil by spontaneous voiding at 20- to 30-min intervals. When urine pH exceeded 7.4, two or three further urine collections were made prior to determination of the maximal urine to arterial blood pCO_2 difference. This was done to ensure a consistently highly alkaline urine, thus avoiding misleading elevations of urine pCO_2 due to mixing of acid and alkaline urine in the urinary passages.

Immediately after measurement of the maximal difference between blood and urine CO_2 tension, seven of the subjects rapidly ingested 20 ml/kg body weight of water to obtain data extending over a wide range of urinary flow rates, pH, and urinary bicarbonate and phosphorus levels. Urine was collected serially every 20 to 30 min (approximately six observations per subject) for 2-3 hr. Arterial blood was not obtained after water-loading.

Written informed consent was obtained from each subject after explanation of the details of the procedures and any potential risks. The research was carried out according to the principles outlined in the Declaration of Helsinki and was approved by the Human Experimentation Committees of the University of Miami and the Miami Veterans Administration Hospital. No adverse effects occurred.

Determination of the pH and pCO_2 of blood and urine was completed within 5 min of collection on a radiometer Acid-Base Cart ABC-1. All urine and venous blood samples were measured for concentrations of sodium, potassium, phosphorus, and creatinine. In addition, the presence of glucose and protein was evaluated by reagent-impregnated plastic strips; neither was present in any of the samples tested. The analytical methods and calculations employed in our laboratory have been described previously (19, 20).

Statistical analysis. Statistical evaluation of the data was performed using both standard bivariate Pearson correlations and multiple regression analysis with a Univac 1106 at the computer center of the Univer-

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sity of Miami. The multiple regression linear model ($y = a_1x_1 + a_2x_2 + a_3x_3 + \cdots + B$) used was selected from a statistical package for the social sciences (SPSS) (21). Using the hierarchical method, R^2 change gives the component of variation attributable to the particular variable added in that step; the F ratio rates the independent influence of each variable when all variables have been introduced into the equation.

Results. The mean values of the variables influencing urinary $p\text{CO}_2$ (U$p\text{CO}_2$) following oral NaHCO$_3$ administration and prior to water-loading are given in Table I. The maximal difference between urinary and arterial blood $p\text{CO}_2$ was 47 ± 2 (SE) mm Hg, and arterial $p\text{CO}_2$ averaged 41 ± 2 mm Hg.

When the data of the pre- and post-water-loading periods were pooled (51 observations, 13 subjects), bivariate Pearson correlation analysis (Table II) showed a positive correlation between U$p\text{CO}_2$ and UpH, U_{HCO_3}, $\text{U}_{\text{HCO}_3}V$, and U_P, and a negative correlation with V ($P < 0.001$ for all). There was no correlation between U$p\text{CO}_2$ and $\text{U}_\text{P}V$ or creatinine clearance. Water-loading following bicarbonate-loading allowed analysis of the variables over a wide range of urine flow rate, pH, and bicarbonate and phosphate levels. Figure 1 shows the scattergrams illustrating these relationships.

TABLE I. RESPONSE TO NaHCO$_3$-LOADING.[a]

	Mean ± SE	Range
U$p\text{CO}_2$ (mm Hg)	88 ± 2	78–102
Arterial $p\text{CO}_2$ (mm Hg)	41 ± 2	35–46
U-A$p\text{CO}_2$ (max) (mm Hg)	47 ± 2	32–59
Urine pH	7.84 ± 0.03	7.63–7.98
Urine Flow (ml/min)	1.7 ± 0.2	0.8–2.6
U_{HCO_3} (μequiv/ml)	168 ± 12	76–249
$\text{U}_{\text{HCO}_3}V$ (μequiv/min)	274 ± 35	82–457
U_P (μg/ml)	367 ± 87	36–1179
$\text{U}_\text{P}V$ (μg/min)	566 ± 132	55–1650
C_{Cr}[b] (ml/min)	140 ± 12	100–216

[a] Values prior to water-loading at the time of the maximal urine to arterial blood $p\text{CO}_2$ difference (N = 13).

[b] Determined from the average of three to five collection periods.

TABLE II. BIVARIATE PEARSON CORRELATION ANALYSIS BETWEEN U$p\text{CO}_2$ AND VARIABLES ASSUMED TO INFLUENCE U$p\text{CO}_2$.[a]

	r	P value
U$p\text{CO}_2$ vs UpH	0.77	<0.001
U$p\text{CO}_2$ vs U_{HCO_3}	0.88	<0.001
U$p\text{CO}_2$ vs $\text{U}_{\text{HCO}_3}V$	0.45	<0.001
U$p\text{CO}_2$ vs U_P	0.53	<0.001
U$p\text{CO}_2$ vs V	−0.69	<0.001
U$p\text{CO}_2$ vs $\text{U}_\text{P}V$	−0.15	>0.2
U$p\text{CO}_2$ vs C_{Cr}	−0.12	>0.2

[a] Pooled data of pre (at the time of the maximal urine to arterial blood $p\text{CO}_2$ difference)- and post-water-loading periods.

The individual regression lines of U$p\text{CO}_2$ on V, UpH, U_{HCO_3}, and U_P for the seven subjects that underwent water-loading are shown in Fig. 2. The overall trend in the individual patients was in the direction of the changes indicated in Table II and Fig. 1. Analysis of covariance showed statistically significant differences between the individual slopes only in the case of U$p\text{CO}_2$ on U_P and U_{HCO_3}.

Multiple regression analysis (Table III) indicated that U_{HCO_3} (F ratio 11.6, $P < 0.005$) and U_P (F ratio 7.1, $P < 0.025$) were the only variables having a significant independent influence on U$p\text{CO}_2$. In this analysis, baseline and post-water-loading observations were pooled. The F ratio for the overall equation was 30.1 ($P < 0.001$).

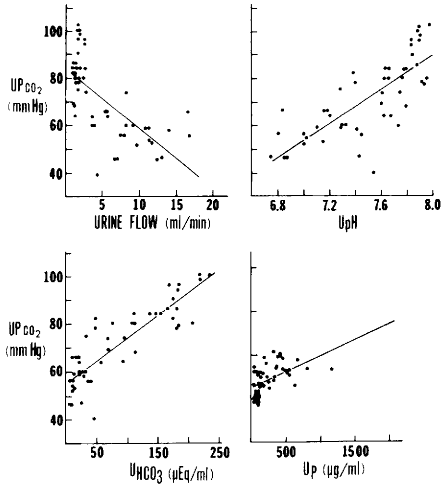

FIG. 1. Relationship between urinary carbon dioxide tension (U$p\text{CO}_2$) and urinary pH (UpH), urine flow rate, and urine bicarbonate (U_{HCO_3}) and phosphorus (U_P) concentrations. The data of the study periods following water-loading were pooled with the baseline values obtained at the time of determination of maximal U-A$p\text{CO}_2$ (51 observations, 13 subjects).

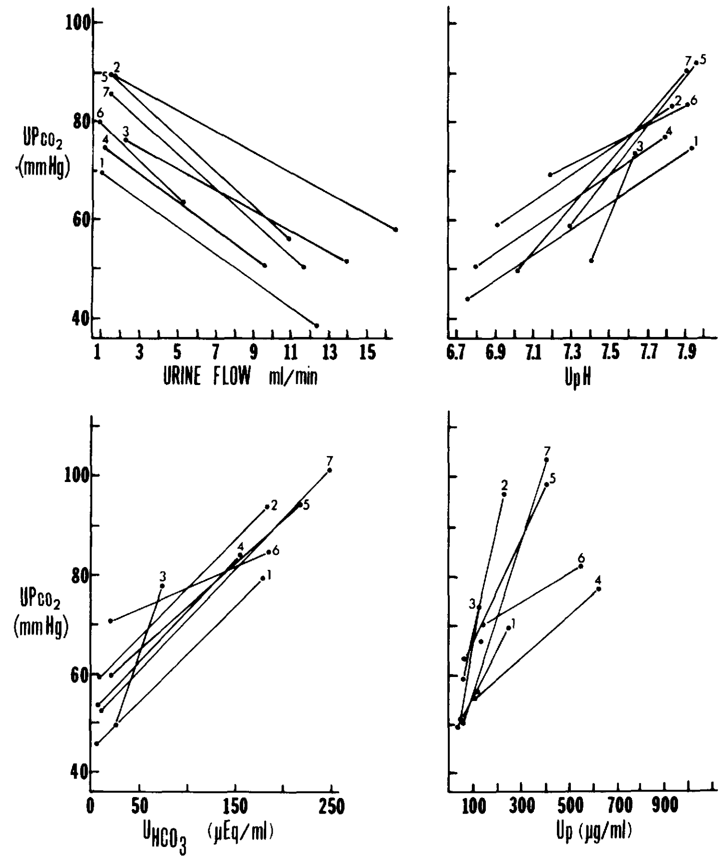

FIG. 2. Individual regression lines of $UpCO_2$ on V, UpH, U_{HCO_3}, and U_P for the seven subjects that underwent water-loading. Analysis of covariance showed statistically significant differences between the individual slopes only in the case of $UpCO_2$ on U_P and U_{HCO_3}.

TABLE III. MULTIPLE REGRESSION ANALYSIS OF FACTORS AFFECTING URINARY pCO_2.[a]

	U_{HCO_3} μequiv/ml	U_P μg/ml	$U_{HCO_3}V$ μequiv/min	C_{Cr} ml/min	UpH	V ml/min	U_PV μg/min
Range	4-249	36-1179	40-455	69-232	6.66-7.98	0.8-17	55-1650
R^2 change[b]	0.77	0.03	0.02	0	0	0	0
F ratio[c]	11.6	7.1	4.0	3.2	0.4	0.3	0
P[c]	<0.005	<0.025	NS[d]	NS	NS	NS	NS

[a] Pre- and post-water-loading data pooled (only 46 observations were included because C_{Cr} was not available in five H_2O loading periods in one patient).

[b] R^2 change indicates the component of variation attributable to the particular variable added in that step (hierarchical method).

[c] F ratio and corresponding P value rate the variables as to their independent influence when all variables have been introduced into the equation.

[d] Not statistically significant ($P \geq 0.05$).

The regression equation ($UpCO_2 = 0.13\ U_{HCO_3} + 0.02\ U_P - 0.06\ C_{Cr} + 0.04\ U_{HCO_3}V - 4.9\ \text{UpH} - 0.3\ V + 96$) derived from the data obtained in our patients predicts the values of urinary pCO_2.

Discussion. There are very few detailed data available in the literature concerning the maximal urine to blood pCO_2 difference in normal subjects (20, 22). In the present study, the response to oral $NaHCO_3$-loading was evaluated in 13 studies in 12 healthy subjects, and the findings confirm and amplify those of Halperin *et al.* (22) and are in agreement with our earlier observations (20).

Seven of the subjects underwent oral water-loading immediately following determination of maximal U-A$p\text{CO}_2$. Using standard bivariate analysis a positive correlation was noted between U$p\text{CO}_2$ and U_{HCO_3}, $U_{HCO_3}V$, UpH, and U_P, and an inverse correlation betwen U$p\text{CO}_2$ and V, over a wide range of urine flow rates, pH, and bicarbonate and phosphorus levels (Table II). Multiple regression analysis suggested that urinary bicarbonate and phosphorus concentrations were the predominant influences on U$p\text{CO}_2$ and that the independent influences of the other variables including U_PV, $H_{HCO_3}V$, and V were small. It is important to point out, however, that multiple regression analysis as utilized in this study, like other similar statistical techniques, can only indicate significant correlations between variables and cannot prove a cause and effect relationship.

Since the recent suggestion that U-A$p\text{CO}_2$ can be used as a sensitive qualitative index of distal nephron hydrogen ion secretion (22), there has been an upsurge of interest both in man (20, 23–25) and experimental animals (15–18, 26–28) in the determination of urinary $p\text{CO}_2$ during alkali-loading.

According to Halperin *et al.* (22), when the intraluminal pH is greater than that of the arterial blood neither back-diffusion nor gradient limitation of hydrogen ion secretion is operative when the urinary $p\text{CO}_2$ fails to increase appropriately after alkali administration. These investigators, therefore, have interpreted the finding of low U-A$p\text{CO}_2$ in patients with distal renal tubular acidosis (RTA) as indicative of decreased formation of H_2CO_3 (and defective cellular hydrogen ion secretion). In contrast, the supranormal bicarbonate T_m noted in some patients with classical distal RTA (29) and the finding that these patients tend to excrete less bicarbonate than alkali-loaded controls suggested to Sebastian *et al.* (23) that H_2CO_3 generation is not abnormal in this setting. Rather, these investigators suggested that rapid back-diffusion of H_2CO_3 accounts both for the low U-A$p\text{CO}_2$ and for obliteration of the disequilibrium pH which facilitates bicarbonate reabsorption (23). Sebastian *et al.* (23) believe, therefore, that the distal luminal membrane allows hydrogen ion back-diffusion when the pH of the luminal contents is low and H_2CO_3 back-diffusion when the pH is high. This explanation, however, has been criticized by Stinebaugh *et al.* since their studies demonstrated that the presumed abolition of the distal tubular disequilibrium pH by carbonic anhydrase infusion did not increase tubular reabsorption of bicarbonate (30).

The urinary $p\text{CO}_2$ is influenced by several variables in addition to distal tubular hydrogen ion secretion (2, 4–6, 9–18). It is known that elevated urine pH and bicarbonate and phosphate levels, and low urine flow tend to increase urinary $p\text{CO}_2$. Thus, when performing bicarbonate-loading studies for the detection of defects of distal tubular acidification it is desirable that these variables be comparable in the control and experimental groups. Bicarbonate serves as the substrate for H_2CO_3 generation (1–3), and dihydrogen phosphate ($H_2PO_4^-$) delays the dehydration of H_2CO_3 (13, 31) and provides hydrogen ion which back-titrates the bicarbonate in low-flow, bicarbonate-rich urine (6).

Several investigators have shown that the maximal elevation of U$p\text{CO}_2$ is associated with high urinary bicarbonate levels (1–5, 10, 11) and that urinary $p\text{CO}_2$ tends to be elevated when phosphate levels are high either spontaneously (11) or following infusions of phosphate (4, 5, 11). Antidiuresis tends to increase the U$p\text{CO}_2$ of alkaline urine (6, 10, 11), perhaps by increasing the concentration of buffer and bicarbonate, thus, facilitating the back-titration of the latter as the pH rises (6). The findings in the present study using bivariate analysis (Table II) are in good agreement with these concepts, since they show that low urine flow rate and high urinary levels of phosphate and/or bicarbonate are associated with higher urinary CO_2 tension. Since the Henderson–Hasselbalch equation dictates that under steady-state conditions when the urine pH is relatively fixed, the $p\text{CO}_2$ must be directly related to the bicarbonate concentration, it was expected that there would be a correlation between urinary pCO_2 and bicarbonate concentration in the present study. Multiple regression analysis was helpful, however, in assessing the influence on urinary pCO_2 of the various other variables over a very wide range of values produced by combined bicarbonate and water-loading.

Despite the above-mentioned studies, the relative influence of urine flow and urinary phosphate and bicarbonate levels on urinary pCO_2 is unclear. During bicarbonate-loading, an elevated urinary pCO_2 obtains with different levels of bicarbonate excretion (4, 32), and Thirakomen *et al.* (33) have stated that very low bicarbonate excretion in bicarbonate-loaded experimental animals with acute inferior vena cava constriction does not appear to limit the rise in urinary pCO_2.

Although Kennedy *et al.* (11) demonstrated a direct correlation between pCO_2 and buffer concentration in the urine, other studies are in conflict with this notion. Portwood *et al.* (4) demonstrated that urinary pCO_2 during $NaHCO_3$ diuresis can greatly exceed the pCO_2 of the arterial blood even when urinary phosphorus (buffer) is very low, and Dorman *et al.* (2) showed that a high urinary pCO_2 can occur in the presence of a very low phosphate buffer concentration or excretion rate. Portwood *et al.* (4) concluded that the excretion of buffer, although influencing urine CO_2 tension to some extent, has only a minor effect in the range of buffer excretion ordinarily encountered. Moreover, Thirakomen *et al.* (33) recently demonstrated in bicarbonate-loaded dogs with maximally alkaline urine that the formation of urinary pCO_2 was uninfluenced by urinary phosphate concentrations. These authors suggested that when urine is maximally alkaline almost all phosphate is in the HPO_4^{2-} form and that, as a consequence, its buffer effect (and thus its influence on urinary pCO_2) is minimal.

Although several investigators have shown an inverse relation between urine pCO_2 and flow rate (6, 10, 11), Rector *et al.* (5) and Portwood *et al.* (4) also demonstrated that $UpCO_2$ can remain elevated despite high urine flow rates as long as U_{HCO_3} (and presumably distal delivery of HCO_3) remains high.

The findings in the present paper are in agreement with other studies suggesting that urinary bicarbonate, and to a lesser extent, urinary phosphate concentrations are the important determinants of the CO_2 tension of alkaline urine. In the range of values noted, V, $U_{HCO_3}V$, U_PV, and creatinine clearance appeared to have no important independent influence on urinary pCO_2.

Summary. In the present study the relative influence of several variables, other than distal renal tubular hydrogen ion secretion, on urinary pCO_2 was evaluated. Thirteen studies were conducted in 12 healthy volunteers aged 21–51 years who received 2 mequiv/kg body weight of $NaHCO_3$ orally. When urine pH exceeded 7.4, urinary pCO_2 and arterial blood pCO_2 were measured. The maximal difference between urine and arterial blood pCO_2 was 47 ± 2 (SE) mm Hg. In order to obtain data extending over a wide range of urine flow rates, pH, and bicarbonate and phosphorus levels, seven subjects subsequently ingested 20 ml/kg body weight of water. Bivariate Pearson analysis (pre- and post-water-loading data pooled) showed a positive correlation between $UpCO_2$ and the following variables: UpH ($r = 0.77$), U_{HCO_3} ($r = 0.88$), $U_{HCO_3}V$ ($r = 0.45$), and U_P ($r = 0.53$) ($P < 0.001$ for all). A negative correlation was obtained between $UpCO_2$ and V ($r = -0.69$, $P < 0.001$). Multiple regression analysis suggested that U_{HCO_3} and U_P were the predominant influences on $UpCO_2$ and that the independent influences of the other variables were small and not statistically significant.

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