Variation of Carbonic Acid pK'_{1g} in Blood and Urine during NaHCO₃ Infusion and NH₄Cl Loading: A Study of Two Renal Acidotic Patients

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In the Henderson–Hasselbalch equation, $pH = pK'_{1g} + \log\left(\frac{[HCO₃^-]}{[CO₂]}\right)$, the millimolar partition coefficient, per mmHg pressure (S), and $pK'_{1g}$ are considered to be constants. Combining these two constants gives $pH = pK'_{1g} + \log\left(\frac{[HCO₃^-]}{[CO₂]}\right)$. In this paper, we report the results of a study designed to test the constancy of $pK'_{1g}$. This was done by varying the blood and urine pH in two patients by administration of NaHCO₃ and NH₄Cl. The $pCCO₂$, pH, and total CO₂ were determined independently, and the values obtained were substituted in the equation, solving for $pK'_{1g}$. The $pK'_{1g}$ was variable in the blood and urine of both patients. In a 16-month-old child, suffering from a transient type of renal acidosis, a linear relationship between pH and $pK'_{1g}$ was noted. In blood, the relationship was $pK'_{1g} = 10.25 - 0.332 pH$, and for urine, $pK'_{1g} = 9.48 - 0.246 pH$. When this patient was restudied six months later, the results were confirmed. Similar observations were made in a 10-year-old patient suffering from distal tubular acidosis syndrome. A linear relationship between $pK'_{1g}$ and pH of both blood and urine was observed in this case also, but the equations were somewhat different. For blood, $pK'_{1g} = 8.55 - 0.129 pH$, and for urine, $pK'_{1g} = 7.78 - 0.028 pH$. The correlation coefficient with the line of best fit in these studies ranged from 0.81 to 0.95, indicating that these observations are statistically significant.

Additional Keyphrases: dissociation constant – CO₂ tension – blood gases – acid-base balance

Recently Natelson and Nobel (1, 2) drew attention to the question of the validity of the common use of a constant $pK'_{1g}$ for the calculation of plasma bicarbonate concentration from the blood pH and $pCCO₂$. In an earlier study, more than 10 years ago, the legitimacy of using the Henderson–Hasselbalch equation for clinical purposes was questioned by Trenchard et al. (3), who observed significant changes of $pK'_{1g}$ values in patients suffering from acute changes in their clinical condition. They concluded that “nomograms based on the constancy of $pK'_{1g}$ to assess acid–base disturbances may in certain circumstances be invalid.” Unfortunately, this report, although of prime importance, was not fully convincing because it described either single or a few incidental observations on patients of various types, subjected to different treatments.

For practical reasons we use $pK'_{1g}$ in this study (4, 5) instead of $pK'_{1g}$; $pK'_{1g}$ is defined as the exponent of the practical (“apparent”) coefficient $K'_{1g}$ of the overall first ionization equilibrium of carbonic acid, where g stands for carbon dioxide in the gas phase. The value of $pK'_{1g}$ equals the sum of $pK'_{1g}$ and the logarithm of the solubility (S) of carbon dioxide (see formulas under Materials and Methods). Generally, a constant value for $pK'_{1g}$ is assumed, and is based on in vitro observations in buffer solutions and blood serum or plasma from normal individuals.

The admission of a patient suffering from a transient type of renal-tubular acidosis, described in the early 1960’s by Lightwood et al. (6), prompted us to investigate this general assumption of a constant value of $pK'_{1g}$ in whole blood, during diagnostic NaHCO₃ infusion and acidifying NH₄Cl loading tests. Under these conditions, $pK'_{1g}$ values can be calculated from in vitro observations on whole blood. By the use of infusion pumps, the shift of the acid–base balance in the course of the experiments can be followed continuously. Recent measurements of the pH, $pCCO₂$, and total carbon dioxide in urine samples also enable us to calculate the $pK'_{1g}$ in this matrix. The experimental results can be interpreted with greater certainty because of the relatively large number of successive samples that can be assayed in these types of tests.

Materials and Methods

Patient S.L. (age 16 months, weight 8.9 kg) was admitted to our hospital for a hyperchloremic metabolic acidosis. Investigating the nature of this disturbance, we found a defect of acid–base regulation, both in proximal and distal tubules. Also, the serum potassium concentration was above normal (5.1–6.0 mmol/L). The patient was treated with a daily dose of oral NaHCO₃. Re-evaluation of the patient six months later demonstrated a spontaneous normalization of the renal acidification defect. During an oral NH₄Cl loading test, the patient produced an acid urine containing normal amounts of acids. Intravenous NaHCO₃ loading revealed that the reabsorption of bicarbonate was now normal, although small amounts were leaking into the urine below the normal renal bicarbonate threshold (24 mmol/L). In that period the serum potassium was about 5.0 mmol/L. Further details about the physiopathologic characteristics of this transient type of tubular acidosis have been published in detail elsewhere (7).

The patient was subjected to a “NaHCO₃ infusion test,” as described by Edelmann et al. (8). Every 20 min during more than 4 h, a venous blood sample was drawn and heparinized. Urine specimens were also collected every 20 min via a catheter. Air was introduced into the bladder, and subsequent manual compression of the abdominal wall expressed urine, which was collected into a small vial. For measurements of the pH, $pCCO₂$, and total carbon dioxide in urine, a midstream portion (2 mL) was collected as anaerobically as possible by means of a disposable syringe.

Tests for NH₄Cl were performed as described by Edelmann et al. (8). In all specimens, pH and $pCCO₂$ were measured within 3 min, and the corresponding (plasma/urine) total carbon dioxide was measured within 10 min after collection.

A second patient (M.S., age 10 years, weight 28 kg) suspected to have a classic distal tubular acidosis was included in this study for comparison. An NaHCO₃-infusion test was performed, and $pK'_{1g}$ was calculated from the data obtained from venous blood and from urine.

pH and $pCCO₂$ Measurements

pH and $pCCO₂$ were measured by means of a pH-blood gas
Fig. 1. Variations of \( pK_{1g} \) in blood (A), related to blood pH (B), in patient S.L. O, first bicarbonate infusion test; *, second ammonium chloride loading/bicarbonate infusion test (observations 7–9 are excluded). The upper bar indicates the period of the first bicarbonate infusion. The lower bar refers to the second test. Arrows in B indicate the direction of change of \( pK_{1g} \) with pH in the course of the experiment.

**analyser (IL 413; Instrumentation Laboratory, Inc., Lexington, MA 02173) (9).**

Specimens were introduced manually in the micro mode setting of the instrument, and all determinations were performed at least in triplicate. For quality control we used three different ampouled, tonometered buffer solutions as described by Maas et al. (10) supplied by Wilten Diagnostica, the Netherlands; respective stated values for pH and \( pCO_2 \) (mmHg): 7.12 and 65.1, 7.37 and 44.4, and 7.60 and 22.2.

**Total CO\(_2\) Measurements**

The heparinized venous blood samples were centrifuged within 3 min of withdrawal by means of a microcentrifuge (Skalar, Delft, the Netherlands) at 12000 X g. Total \( CO_2 \) was measured by means of a micromethod (17) in which the increase of the \( pCO_2 \) is measured after the sample is acidified by the addition of dilute lactic acid. For analysis in urine, the volume injected into the measuring chamber (20–50 \( \mu L \)) depends on the actual total \( CO_2 \) content, so that the electrode signal is close to the observed signal obtained by calibration. All determinations were performed at least in triplicate.

**Calculations**

According to the Henderson–Hasselbalch equation,

\[
pH = pK_1 + \log \left( \frac{HCO_3^-}{S \cdot pCO_2} \right)
\]

(1)

or, rearranged in linear form,

\[
pK_1 = pH - \log HCO_3^- + \log pCO_2 + \log S
\]

(2)

where \( pK_1 \) signifies the first dissociation constant (about 6.1), \( S \) is the solubility of gaseous \( CO_2 \) expressed in mmol/L/mmHg (about 0.03), and \( HCO_3^- \) is the bicarbonate concentration in mmol/L of plasma. In the literature, some recommend that \( S \) be incorporated into the dissociation constant, using for this the symbol \( K_{1g} \). Equation 2 now becomes (5):

\[
pK_{1g} = pH - \log HCO_3^- + \log pCO_2
\]

(3)

\( pK_{1g} \) in plasma at pH 7.40 and at 37 °C can be derived from experimental data of Maas et al. (4), and is equal to 7.614. According to the same author, the corresponding value in whole blood is 0.01 higher because of a systematic difference in measured pH between plasma and whole blood.

For \( S \) a constant value of 0.0304 mmol/L-mmHg is assumed in plasma at 37 °C. For calculations of \( pK_{1g} \) in urine, we chose the value of 0.0326 mmol/L-mmHg for \( S \), which equals the value for physiological saline at 37 °C.

**Results**

The curves in Figure 1A demonstrate the in vivo changes of \( pK_{1g} \) in venous blood when the acid–base equilibrium is changed by relatively rapid NaHCO\(_3\) infusion or by an oral dose of NH\(_4\)Cl.

The upper curve data for blood, obtained during the first admission of the patient, show for the first 10 observations a relatively great variation of the calculated \( pK_{1g} \). However, the course of the \( pK_{1g} \) shows a more or less gradual decrease. Observations 9 and 10 in this curve may be questioned, because of sampling problems due to clogging of the in-dwelling cannula. Support for erroneous results of \( pK_{1g} \) of the observations 9 and 10 is found by the calculation of the differences between the actual HCO\(_3^-\) concentration and the calculated base excess. Because the shift of the acid–base balance in this patient is solely related to a renal loss of bicarbonate, this difference, taking the \( pCO_2 \) variations into consideration, must be constant: we calculated a mean value for all observations...
Fig. 2. Variations of \( pK'_{\text{ig}} \) in urine (A), related to pH (B), in patient S.L. six months after experiment shown in Fig. 1. 

A: ο, first bicarbonate infusion test (freshly voided specimens); x, data of the same samples stored at \(-20^\circ\text{C}\) for two months. B: ο, first bicarbonate infusion test; △, same data obtained during the second admission; Δ and ○, data after ammonium chloride loading during the first admission on two different days.

(except 9 and 10) of 23.0 ± 1.0 (SD) mmol/L, while for the observations 9 and 10 the differences were 19.7 and 18.7 mmol/L, respectively.

From this curve it can also be seen that the initially high \( pK'_{\text{ig}} \) value decreases to a value close to the plasma value at 37 °C reported in the literature (4), namely 7.614. In the course of this NaHCO₃ infusion the initial blood pH changed from 7.23 to 7.49.

The lower curve of Figure 1A shows the results from a combination of an oral NH₄Cl loading test and a subsequent NaHCO₃ infusion test. This experiment was performed during the second admission of the patient to our hospital six months later. Acidifying the patient’s blood (the pH changed from 7.37 to 7.28) by means of NH₄Cl increases the \( pK'_{\text{ig}} \) (observations 1–5), whereas the opposite occurs during the subsequent infusion of NaHCO₃ after the blood pH has returned to its original starting value (observations 10–17). In the period represented by observations 6–9, \( pK'_{\text{ig}} \) remains about constant.

In Figure 1B the \( pK'_{\text{ig}} \) values are related to their respective pH values. Observation 9 and 10 of the first bicarbonate infusion test (open circles) have been excluded for reasons mentioned earlier in this section. From this figure one can see that in blood, during relatively rapid pH shifts caused by NaHCO₃ or by NH₄Cl, \( pK'_{\text{ig}} \) changes inversely with pH. Although the pH dependency of \( pK'_{\text{ig}} \) in all experiments is about the same, the absolute \( pK'_{\text{ig}} \) values differ for the various experiments.

Figure 2A demonstrates the \( pK'_{\text{ig}} \) values in urine specimens. The sample numbers, indicating 20-min periods of collection, coincide with those for blood samples in Figure 1A. Figure 2A shows that the \( pK'_{\text{ig}} \) values in urine vary gradually but significantly in the course of the NH₄Cl infusion test. Also shown (far left) is the mean ± standard deviation, calculated from observations made by Mainzer and Bruhn in 1931 (12).

The broken line in Figure 2A demonstrates the results calculated from the original urine specimens after storage for two months at \(-20^\circ\text{C}\). The \( pCO_{2} \) decreased to values between 5 and 30 mmHg; pH increased, ranging between 7.39 and 8.54; and bicarbonate concentration was between 3 and 104 mmol/L. Aside from observation 6 ([HCO₃⁻] = 3 mmol/L), which may be doubtful, the changes of \( pK'_{\text{ig}} \) in this case generally confirm the observations in the fresh urine samples. The urine samples had been thawed a few times for additional investigation during this period of storage.

Six months later, the patient was capable of producing acid urine. Accordingly, only a few (alkaline) urine samples could be obtained during the second set of NH₄Cl and NaHCO₃ tests, from which data could be used for the calculation of \( pK'_{\text{ig}} \) (not shown in Figure 2A, but included in Figure 2B as closed circles).

In Figure 2B all urine \( pK'_{\text{ig}} \) values are presented in relation to their respective pH values obtained from the various NH₄Cl loading and NaHCO₃ infusion tests. Results obtained on different days (including a six-month delay) fit into one statistical population when related to the pH (inverse relationship).

The second patient (M.S.) was also investigated by means of a NaHCO₃ infusion test. In this case a change of \( pK'_{\text{ig}} \) in blood was found in the course of the experiment (not shown). This case also showed an inverse relationship between \( pK'_{\text{ig}} \) and the blood pH (Table I). In the urine specimens the variation of \( pK'_{\text{ig}} \) was small.

The results obtained in both patients S.L. and M.S. are presented as orthogonal regression equations, in which \( pK'_{\text{ig}} \) and pH are considered to be variables. From Table I we conclude that in blood: (a) independently of the type of test (NH₄Cl loading or NaHCO₃ infusion), for patient S.L. the change of \( pK'_{\text{ig}} \) in relation to pH, expressed as \( \Delta pK'_{\text{ig}}/\Delta \text{pH} \), has a constant value (about \(-0.36\), experiments 1–3); (b) for patient M.S. the calculated value for \( \Delta pK'_{\text{ig}}/\Delta \text{pH} \) is significantly less (\(-0.126\), experiment 4), but is still about threefold the value reported for human and canine serum at 37.5 °C \((-0.044\) (13)); and (c) the differences between the values of \( \Delta pK'_{\text{ig}}/\Delta \text{pH} \) mentioned under (a) and (b) cannot be attrib-
Table 1. Relation between $pK'_{1g}$ and pH in Blood and Urine of Two Patients

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Test and pH range</th>
<th>No. observations</th>
<th>Regression equation: $pK'_{1g} = b_0 + b_1 \cdot pH$</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous blood, S.L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>bicarb.</td>
<td>7.23–7.49</td>
<td>13</td>
<td>10.25</td>
<td>-0.352</td>
<td>-0.862</td>
</tr>
<tr>
<td>2</td>
<td>NH$_4$Cl</td>
<td>7.34–7.29</td>
<td>5</td>
<td>10.47</td>
<td>-0.392</td>
<td>-0.861</td>
</tr>
<tr>
<td>3</td>
<td>bicarb.</td>
<td>7.37–7.29</td>
<td>6</td>
<td>12.85</td>
<td>-0.718</td>
<td>-0.940</td>
</tr>
<tr>
<td>Venous blood, M.S.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>bicarb.</td>
<td>7.37–7.45</td>
<td>8</td>
<td>10.26</td>
<td>-0.357</td>
<td>-0.866</td>
</tr>
</tbody>
</table>

Urine, S.L.

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Test and pH range</th>
<th>No. observations</th>
<th>Regression equation: $pK'_{1g} = b_0 + b_1 \cdot pH$</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>bicarb.</td>
<td>7.10–7.78</td>
<td>12</td>
<td>9.46</td>
<td>-0.248</td>
<td>-0.919</td>
</tr>
<tr>
<td>6</td>
<td>bicarb.</td>
<td>7.10–7.86</td>
<td>14</td>
<td>8.99</td>
<td>-0.185</td>
<td>-0.824</td>
</tr>
<tr>
<td>7</td>
<td>bicarb.</td>
<td>7.39–8.54</td>
<td>12</td>
<td>9.07</td>
<td>-0.178</td>
<td>-0.799</td>
</tr>
</tbody>
</table>

Urine, M.S.

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Test and pH range</th>
<th>No. observations</th>
<th>Regression equation: $pK'_{1g} = b_0 + b_1 \cdot pH$</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>bicarb.</td>
<td>6.80–7.50</td>
<td>9</td>
<td>7.78</td>
<td>-0.028</td>
<td>-0.820</td>
</tr>
</tbody>
</table>

* First admission.
* Second admission.
* Urine stored two months at -20°C.
* Freshly voided urine.

See text for discussion of meaning of different numbers of observations.

In the absence of marked influences on renal function and markedly alters urinary composition. Therefore, we calculated two regression equations in experiment 5: with and without observations 15 and 16 (Figure 2A), that is, with and without acetazolamide. A comparison of the corresponding values of $\Delta pK'_{1g}/\Delta pH$ (Table 1, experiment 5) in this case shows a significant difference. On the other hand, there is remarkable agreement if the observed $\Delta pK'_{1g}/\Delta pH$ of experiment 5 (all observations), -0.185, is compared with the calculated $\Delta pK'_{1g}/\Delta pH$ of experiment 6, where the specimens were stored for two months at -20°C (including the $pK'_{1g}$ values after acetazolamide administration), -0.178.

Apparently, during storage at -20°C, the actual values of $pK'_{1g}$ do not change in the presence of the drug; the differences in $pK'_{1g}$ before and after storing samples 15 and 16 are 0.002 and 0.008, respectively. This is in contrast with the findings in other urine samples, for which we observed a mean increase in $pK'_{1g}$ of about 0.05 ± 0.02 (SD). After storage, the observed $pCO_2$ values were between 5 and 30 mmHg.

The reliability of our observations, and hence the precision of the analytical methods involved, may be derived from the following considerations: (a) $pK'_{1g}$ in the urine of patient M.S. (experiment 8) is almost constant, and about equals the value reported in literature for plasma, and (b) at the same time, the blood specimens of patient M.S., which were monitored in one analytical series (experiment 4), show variable, and pH-dependent, $pK'_{1g}$ values.

Moreover, a systematic error in our findings is not likely because calculation of the $pK'_{1g}$ value for pH 7.40, according to the regression equations in Table 1, gives a mean value for blood of about 7.61 (experiments 1–3) and for urine, about 7.62 (experiment 7; i.e., all observations). The accuracy of the results, expressed in the standard deviation (SD) of $pK'_{1g}$, can be obtained from the variance of the orthogonal regression statistics by calculating the pH dependency of $pK'_{1g}$ in each experiment. We calculated an SD of between 0.006 and 0.013 (excluding experiments 6 and 7, Table 1).

Table 2 presents the calculated linear regression between $pK'_{1g}$ and pHCO$_3$$. The experiment numbers refer to the experiments in Table 1. In contrast to the relationship between $pK'_{1g}$ and pH (see Table 1), the regression coefficients ($b_1$) vary greatly for the various experiments in blood of patient S.L. (experiments 1–3) but not in urine (experiments 5–7). Moreover, this relationship is significant for patient S.L. (see $r$ values) but is virtually absent for patient M.S. during bicarbonate infusion.

Surprisingly, if these regression equations of $pK'_{1g}$ with pHCO$_3$ are substituted into their respective equations of
pK′ values with pH (Table 1) the relationships between pH and pHCO$_3$ are almost identical for experiments 1 and 4 in venous blood and experiments 5 and 7 in urine. For these cases ΔpH/ΔpHCO$_3$ varied between 0.60 and 0.65.

We observed no significant correlation between pK′$_{14}$ and the negative logarithm of pCO$_2$ (r = 0.30).

**Discussion**

Several conclusions can be drawn from these results: (a) fast, in vivo shifts of the acid–base balance may affect the value of pK′$_{14}$, as calculated by the formula pK′$_{14}$ = pH – log HCO$_3$ + log pCO$_2$; (b) variation of pK′$_{14}$ is linearly related to variations of pH and of the negative logarithm of the bicarbonate concentration; (c) variations of pK′$_{14}$ can be observed both in blood and urine; (d) the change of pK′$_{14}$ with pH (ΔpK′$_{14}$/ΔpH) may be constant for one patient but will differ, under identical conditions, from patient to patient; (e) pH-related variations of pK′$_{14}$ in freshly voided, alkaline urine specimens can be observed even after two months of storage at −20 °C; and (f) except for two urine specimens containing a carbonic anhydrase inhibitor (acetazolamine), pK′$_{14}$ in the stored urine samples deviate from the original pK′$_{14}$ values in the freshly voided specimens.

Howorth (14) stresses the importance of variation of solubility for carbon dioxide in plasma, the result of changes in molarity, plasma proteins, and plasma lipid, as the major cause of variations in calculated pK′$_{14}$.

In an early study of urine samples, Mainzer et al. (12) noted a wide variation of urine pH (5.81–6.30). Their calculations involved a variable solubility coefficient, determined separately for each urine specimen. From their data we calculate a mean pK′$_{14}$ of 7.558 ± 0.119 (95% confidence limits: 7.32–7.80, n = 19); for the solubility coefficient we calculate a coefficient of variation of 4.5%.

It seems reasonable to assume that one common cause in blood and urine may account for the observed variation of pK′$_{14}$. If so, then we can exclude all explanations attributed on the one hand to components or variations present solely in blood, and on the other hand to those observed only in urine specimens. Hence the influence of (e.g.) plasma proteins, erythrocyte carbamino compounds, or variable ionic strength (in urine) can be ruled out as a possible cause of major variations of pK′$_{14}$. In our view, two possibilities for explaining the variable pK′$_{14}$ remain: (a) there is a “pseudo” equilibrium, and the Henderson–Hasselbalch equation cannot be used; or (b) there is a chemical equilibrium in blood and urine, but the present form of the Henderson–Hasselbalch equation is invalid for this case.

Possibility (a) can almost be excluded if the results of the various experiments are compared. For instance, variation of the bicarbonate infusion rate in patient S.L. (experiments 1 and 3, Table 1) does not lead to variation of ΔpK′$_{14}$/ΔpH, nor is there a relation between the infusion rate and the variation of pK′$_{14}$ with the pHCO$_3$ (same experiments, Table 2). Also the observation that, after NH$_4$Cl-loading, patient S.L. retains an almost identical ΔpK′$_{14}$/ΔpH (experiment 2, n = 5, Table 1) does not fit into the theory of the absence of chemical equilibrium. The fact that all values of pK′$_{14}$ calculated from urine experiments (experiment 7, Table 1) fit into one regression equation and the fact that stored urine samples still demonstrate variable, pH-dependent pK′$_{14}$ values (experiment 6, Table 1) also support this thesis.

Hill et al. (15) observed slow pH changes in blood plasma after carbon dioxide exchange. These observations, however, cannot explain our findings, and do not support the theory about possible absence of chemical equilibrium, because the rate of the change of pH (observed in dogs), expressed as t$_{1/2}$, was less than 10 s. In our case the first measurements were performed about 3 min after obtaining the specimens from the patient.

The only theory left is that the practical form of the Henderson–Hasselbalch equation used for calculation is not always valid. When the Henderson–Hasselbalch equation is used, one of the assumptions made is that the bicarbonate concentration equals the total carbon dioxide content in plasma minus the dissolved carbon dioxide, which ignores the possible influence of erythrocyte carbamino carbon dioxide in blood. This influence is not very likely because, as has been discussed earlier, it cannot have an effect on both matrices, blood and urine. Another reason is that assuming the presence of bound carbon dioxide would lead to calculation of pK′$_{14}$ values that are too low, whereas in our studies, pK′$_{14}$ values are higher than those reported in literature (between 7.61 and 7.62; see ref. 4).

Other assumptions in the literature (3, 13), such as the variation of the solubility coefficient and possible role of hypoxic (in our case, venous) blood, can explain neither the relatively high variations of pK′$_{14}$ nor the presence of those variations both in blood and in urine.

An alternative explanation would be the influence of pH on the equilibrium H$_2$CO$_3$ ⇌ (CO$_2$)$_2$ + H$_2$O. Normally, this equilibrium is far to the right and the concentration of dissolved carbon dioxide [(CO$_2$)$_2$] in plasma is about 1000 times greater than the concentration of carbonic acid. Thus if, through this reaction mechanism, pH influences pK′$_{14}$, the only possibility left is that in our experiments pH inhibits somewhat the activity of the enzyme carbonic anhydrase. Our data, however, contradict this hypothesis by the same arguments mentioned earlier against the absence of equilibrium.

When all this is taken into consideration, the only explanation left is that yet another chemical component or components may be involved in the chemical equilibrium. Although the second dissociation constant of carbonic acid is very small (pK = 10.32), we may tentatively consider introduction of the divalent carbonate ion for further study to explain the pH- and pHCO$_3$-dependent pK′$_{14}$ variations during fast, in vivo shifts in the acid–base balance.

The theoretical importance of our findings for physiology as such can hardly be evaluated because we studied only two patients. However, we believe that variation of pK′$_{14}$ can play a role in the explanation of certain observations—for instance, in renal physiology. Perez et al. (16), analyzing the variables influencing urinary carbon dioxide tensions during bicarbonate and water loading in normal men, established that the cause of the observed high urinary carbon dioxide tension is due to an insufficiently rapid rate of dehydration of the intraluminal carbonic acid in the distal nephron to permit carbon dioxide equilibration.

Considering our observations, this theory of a limited rate of dehydration of carbonic acid can be questioned. Our findings suggest that the pH-dependent differences of the (apparent) chemical equilibrium of carbonic acid among blood, urine, and, eventually, the intracellular space are the cause of final urinary pCO$_2$ values higher than that of blood.

This study gives evidence that, at least during acute shifts, investigations of the acid–base balance that are based only upon pH and pCO$_2$ determinations in blood and urine may lead to erroneous conclusions. Values of calculated bicarbonate in blood differed from the concentrations obtained by actual measurement by +23% to −13%.

**References**

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Letter.