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Thermodynamical Aspects on the Determination of Bicarbonate in Urine

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ABSTRACT

Despite the fact that the composition of urine varies a lot during the day, this has essentially been neglected as a factor of importance in determinations of urine bicarbonate. The investigations reviewed in combination with an own study shows that qualitative and quantitative factors in urine composition impacts the solubility of carbon dioxide as well as the dissociation constant for the bicarbonate buffer system. These two "constants" are of outstanding importance in the determination of bicarbonate, using the total carbonic acid method as well as the carbon dioxide equilibration method. Nomograms are presented to quantify the influence of different urine compositions on the determinations of bicarbonate in final urine and tubular fluid.

INTRODUCTION

Since many decades it has been a well-known fact that the ligands used in the chemical description of biological acid-base equilibria are influenced by different physical and chemical properties of the solution under study. For many biological fluids this fact has been made, for example, for blood (27), for cerebrospinal fluid (24, 25), for amniotic fluid (17) and also for artificial fluids (9, 32).

A common denominator for these biological fluids is the relatively restricted variation in composition from time to time and also between different individuals.

As a sharp contrast, final urine varies in composition considerably even from hour to hour in the same individual. The circadian pH-variation (15) is a well-known fact as well as the postprandial "alkalaine tide". But the wide range in electrolyte composition and osmolality, makes a considerable impact on the chemical analysis of components in the bicarbonate buffer system.

Consider two final urine samples from the same individual , the same day; one of them 150 mOsm/kg and the other 600 mOsm/kg. Provided the osmolality reflects the NaCl content the bicarbonate activities can be calculated using the Henderson-Hasselbalch equation (see Table 1). Thus, despite the fact that the pH and Pco_2 are identical in the same samples, the bicarbonate activity measured might vary around 50% depending upon chemical factors other than the bicarbonate activity itself.

TABLE 1

	150 mOsm/kg	600 mOsm/kg	
pKa	6.117	5.919	Fitzsimons and Sendroy, (9)
S	0.03145	0.02915	Van Slyke et al., (32)
HCO_3^-	6	9	

Table 1.The influence of osmolarity on the bicarbonate activity in urine. The calculations are based on a Pco_2 of 40 mm Hg (5.3 kPa) and a pH of 6.8 for both urines.

In biological fluids other than urines, the bicarbonate variations as a function of thermodynamical ligands is smaller and usually well-known (for a recent review see Siggaard Andersen, (27)).

The aim of the present paper is to present ligands for the bicarbonate determination in urine as a function of different compositions of this fluid. A review of the literature in this field is presented as well as a study of primary urine (ultrafiltrate). An evaluation of different methods for the bicarbonate determination in urine is made.

METHODS

The bicarbonate concentration can be analyzed in different ways among which the determination of total carbonic acid, Tco_2 is the most widely used. This technique cannot, however, differ between CO_2, HCO_3^- and CO_3^{2-}, which is a considerable drawback when dealing with fluids of unknown pH and CO_2 concentrations. This is most times the case in compartment studies in or adjacent to single cells. Pco_2 electrodes are now made for in vivo studies in micropuncture research (8,30), but will probably wait a couple of years for a more wide-spread use.

The bicarbonate activities in biological fluids are determined mainly by using the equilibration technique as thoroughly used and described for blood and other fluids

(27). This technique is also suitable for samples in nano-liter size as described by Karlmark and Sohtell (18). For carbonic anhydrase rich fluids (eg blood), chemical equilibrium is assumed and the calculation of bicarbonate activity is based upon single measurements of pH and Pco_2.

If chemical non-equilibrium (disequilibrium) is prevailing, the Henderson-Hasselbalch equation cannot be used. In such a case an immediate responding and highly specific bicarbonate electrode is the only method. Such an electrode is described (19) but is not yet developed enough to permit reliable and valid measurements.

As long as the non-bicarbonate buffer concentration is low as compared to the bicarbonate activity the equilibration technique is rather insensitive to differences in actual pH and Pco_2 and is preferred mainly because its simplicity and also the fact that it results in a measure of the activity and not barely by the concentration. The total-CO_2 technique for bicarbonate determinations gives a more unspecific total concentration of bicarbonate buffer constituents and gives a considerable error in acid urines.

Irrespective of which of the mentioned techniques that is to be used for bicarbonate determinations in urine, the CO_2 concentration must be measured. This is easily performed with a Pco_2 electrode as long as the sample volume is 100 µl or more. For the calculation of the bicarbonate activity the solubility factor (S) and pKa are then of vital importance. In addition, knowledge of the actual pH is also vital in very alkaline urines (where the amount of CO_3^{2-} gives a substantial contribution (5 - 10 %) to total-CO_2) and also in highly buffered urine.

So far experimental investigations of the S and pKa for urines are not published. We used here one method described by Siesjö (24) to study S and another by Siesjö (25) to study pKa. Primary urine was artificially made as an ultrafiltrate of rat plasma, filtered through a Diaflo membrane (PM 10, Amicon Corp., Lexington, Mass., USA).

a) The solubility coefficient (S): The solution investigated was acidified with HCl to a pH of about 2.5 and then equilibrated with the humidified 5 % CO_2 in oxygen gas mixture for 1 hour at 38°C. The carbonic acid content of an equilibrated sample was made volatile by depositing it in a HCl solution in a Conway unit, which was modified as shown in Fig.1. The CO_2 formed was trapped in a $Ba(OH)_2$ solution. The sealing between the plugs and the lid was made by the use of acidified carboxymethylcellulose to avoid leakage of CO_2. The diffusion time was 90 min and the change of the $Ba(OH)_2$ solution due to the reaction with CO_2 was immediately analyzed by titration with HCl standard. S was measured as a relation between the total carbonic acid content (Tco_2) in acidified solution and the partial pressure of carbon dioxide. The validity of the method was tested by determining the S factor for distilled water and a 160 mmol/l NaCl solution.

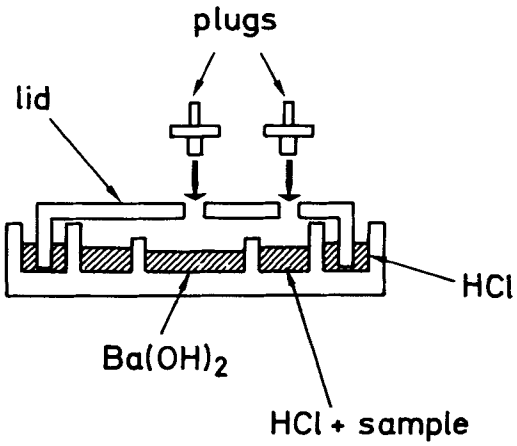

Fig.1. The circular Conway unit (made of glass), here seen from the side. The plugs are sealed to the lid with acidified carboxymethylcellulose.

b) The first apparent dissociation constant (pKa): The solution was first equlilibrated in a humidified gas of 5% CO_2 in oxygen for one hour at 38°C. The pH at this equilibration was measured with a glass electrode and the Pco_2 was measured as described above. This value was inserted in the Henderson-Hasselbalch equation.

RESULTS AND DISCUSSION

a) The solubility coefficient: The results are presented at the bottom of Table 2. Van Slyke et al (32) presented data, indicating that not only the ionic strength was of importance for the CO_2 solubility. Of considerable importance was also the influence of the different ionic species. The solubility of CO_2 is depressed by ions in proportion to their concentrations (Table 3). The table shows the depression of the solubility of CO_2 for different ions extrapolated to a concentration of 1 mol/l. In lower concentrations the depression in solubility is proportionally reduced (for phosphate this linearity is not strict, however). It must be pointed out that the table is not useable for concentrations above 300 mmol/l of individual ions. For biological pure salt solutions, Siggaard Andersen (27), summarized the different data of table 3 in a formula, which describes an estimation of the ionic influence but in terms of a weighed ionic strength. But his approximation is not valid for urines, due to the wide range of ionic composition during different physiological diuretic conditions.

Table 2

S	°C	Solution	
0.03304	38	H_2O	Bohr,C., (4)
0.03222	38	H_2O	Van Slyke et al., (32)
0.03015	38	Plasma (human)	-"-
0.03215	38	H_2O	Bartels and Wrbitzky, (2)
0.03007	38	Plasma (ox)	-"-
0.03262	37.5	H_2O	Siesjö, (24)
0.03229	38	H_2O *	-"-
0.03136	37.5	160 mmol/l NaCl	-"-
0.03105	38	160 mmol/l NaCl *	-"-
0.03223	38	H_2O	Austin et al., (1)
0.03013	38	Sera (human)	-"-
0.0311	37	Amnion fluid (human)	Johnell, (17)
0.03074	38	Ultrafiltrate	This study (SE= 0.0018,n= 24)
0.03065	38	160 mmol/l NaCl	-"- (SE= 0.00004,n=30)
0.03233	38	H_2O-"-	-"- (SE= 0.00009,n=47)

Table 2. Summary of data concerning the solubility of CO_2 in different kinds of solutions. In case the solubility was presented as the Bunsen coefficient (liter gas dissolved /liter/ unit atmosphere pressure), we have normalized it into the solubility coefficients (mmol/l/mm Hg). Those values presented with a * are a temperature correction to 38° C from the preceding value (see text).

Table 3

H^+	0.00000
$HC_2O_4^-$	0.00117
$Lactate^-$	0.00296
Cl^-	0.00130
K^+	0.00238
Na^+	0.00382
$H_2PO_4^-$	0.00615
HCO_3^-*	0.00130

Table 3. The molar depression of carbon dioxide solubility (Van Slyke et al., (32). The * denotes that the value is taken from Harned and Davies (12) as the same as that for chloride. It lacks direct experimental support, however.

Normally final urine does not contain significant amounts of neither proteins nor lipids. During pathological conditions, however, this could be the case, and would then make a serious impact on bicarbonate determinations. The solubility of CO_2 increases with a higher lipid concentration but decreases with a high protein concentration. Renal experimental techniques nowadays permits sampling from afferent and efferent arterioles as well as from the Bowman's capsule. Primary urine contains protein which thus will decrease the bicarbonate concentration. On the other hand one can expect an increased bicarbonate concentration in primary urine as the concentration of the plasma proteins increases during the ultrafiltration . This is due to an augmentation of the Donnan effect as well as of a reduced CO_2 solubility in plasma (Siggaard Andersen, pp41,(27); Sohtell (29)).

b) The first apparent dissociation constant: The results from rat ultrafiltrate are shown at the bottom of Table 4. It must be born in mind that older literature in this field describes acid-base chemistry with another definition of pH, than that of to-day (3). Furthermore, the analytical methods are considerably improved.

Table 4

pKa	°C	Solution	
6.3222	38	μ = 0	Hastings and Sendroy, (13)
6.105	38	Serum (human)	Hastings et al.,(14)
6.330	38	μ = 0	Stadie and Hawes, (31)
6.092	38	Serum (human)	Robinson et al., (22)
6.089	38	Serum (dog)	-"-
6.3089	38	μ = 0	MacInnes and Belcher, (20)
6.09	38	μ = 160 mmol/l	Danielson et al., (6)
6.112	37	Serum (ox+dog+human)	Dill et al., (7)
6.3002	38	μ = 0	Harned and Davies, (12)
6.086	37.5	Serum (dog+human)	Severinghaus et al., (23)
6.316	38	μ = 0	Fitzsimons and Sendroy, (9)
6.103	37.5	Serum (human)	Gambino, (10)
6.13	37.5	Serum (human)	-"- (11)
6.127	37.5	μ = 160 mmol/l	Siesjö, (25)
6.328	38	μ = 0	Siggaard Andersen, (26)
6.120	37	Amnion fluid	Johnell, (17)
6.101	38	Ultrafiltrate	This study (SE= 0.002, n = 42)

Table 4. Summary of data, describing the pKa in different solutions at body temperature. μ= 0 mol/l means an extrapolation of data from salt solutions.

As shown in Table 3, the pKa is strongly influenced by the ionic strength. A spectrum of formulas in literature are described to substantiate this relation. Many formulas, based on the Debye-Huckel equation, are restricted to too diluted solutions, however, to be of importance in analysis of biological fluids. Fig. 2 visualizes two of these formulas, describing the pKa as a function of ionic strength (other relations are described by Manov et al., (21), Hägg, (16), Slatopolsky et al. (28) and Siggaard Andersen, (27). The figure also indicates reasonable values of the ionic strength in tubular fluids. It is clearly seen that the wide range in ionic strength in these urines makes a considerable impact on the pKa and thus finally on the bicarbonate determination.

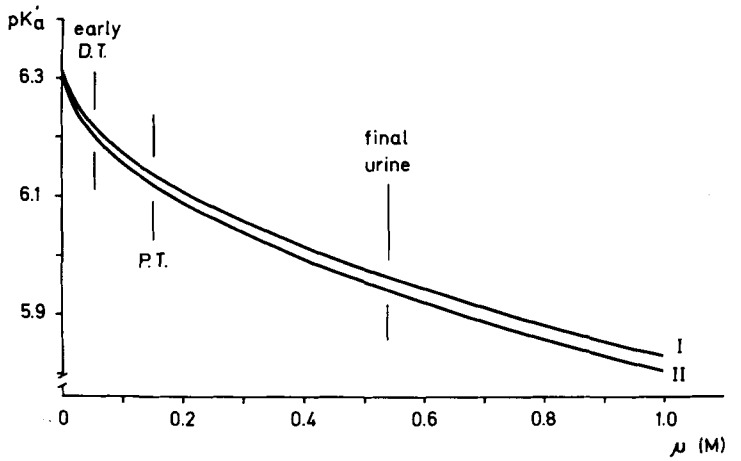

Fig. 2. pKa as a function of the ionic strength (μ). I: $pKa' = 6.322 - 0.5 \sqrt{\mu}$ (Hastings and Sendroy, (13) II: $pKa' = 6.316 - 0.512 \sqrt{\mu}$ (Fitzsimons and Sendroy, (9)). Reasonable mean ionic strengths for proximal tubule (PT), early distal tubule (DT) and final urine are indicated.

The influence of pH itself on the first apparent dissociation constant is sparsely studied in biological solutions. In cerebrospinal fluid (Siesjö, (25)) and human amniotic fluid (Johnell, (17)) no influence of pH on pKa was found. In serum, however, as well as in 150 mmol/l NaCl solution Siggard Andersen (26) found a decrease in pKa with increasing pH but only above pH 7.0 - 7.5. Similar dependence for serum was found by Severinghaus et al. (23). In alkaline solutions containing proteins, the carbamino-CO_2 concentration might influence on the bicarbonate activity as is further discussed by Siesjö (25).

c) Urinary bicarbonate: Fig. 3 shows a hypothetical urine of pH 6.8 and Pco_2 of 40 mm Hg (5.3 kPa). Reasonable S and pKa values are furthermore inserted in the Henderson-Hasselbalch equation and the bicarbonate activity increases with increasing S for a certain pKa.

The influence of different temperatures on the bicarbonate value is formulated by Siggaard Andersen (27) and shows that 1°C increase, also increases the bicarbonate activity with about 1.6 %. Thus an analysis of the bicarbonate concentration in a solution from a 38°C animal which is analyzed at room temperature results in significantly too low a value (more than 25%).

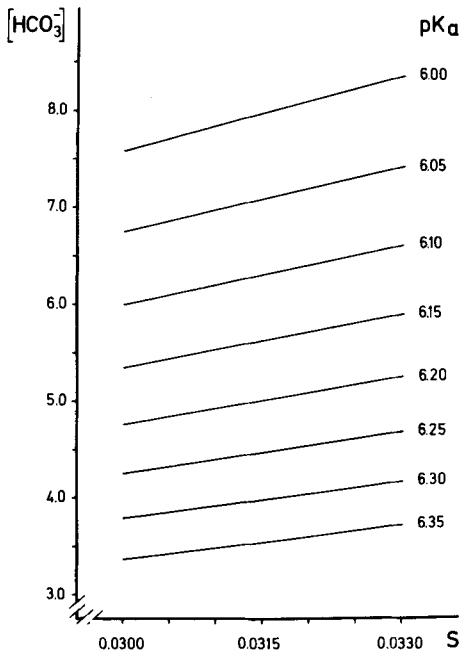

Fig. 3 The bicarbonate activity as a function of pKa and S in the Henderson-Hasselbalch equation. The example is based on equilibrium condition with a pH of 6.8 and a Pco_2 of 40 mm Hg.

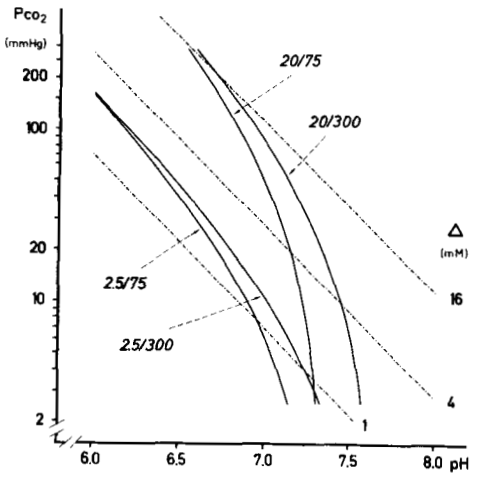

Fig 4 CO_2-equilibration curves of non-bicarbonate buffers. The reaction evaluated is the following:

$$HPO_4^{2-} + CO_2 + H_2O \rightleftharpoons H_2PO_4^- + HCO_3^-$$

The change of $HPO_4^{2-} \longrightarrow H_2PO_4^-$ is expressed as Δ which also will illustrate the equimolar increase in HCO_3^-. The curves are calculated according to Siggaard Andersen (27). The solutions used in the calculations are: phosphate buffers with equal concentrations for $H_2PO_4^-$ and HPO_4^{2-} of 2.5 mmol/l (left) and 20 mmol/l (right) at ionic strengths of 75 mmol/l and 300 mmol/l respectively. In the figure the dashed lines indicate iso-bicarbonate lines. For conclusions, see text.

The equilibration technique for the bicarbonate determination in solutions containing non-bicarbonate buffers yields equilibration lines which are curved as shown in Fig. 4. It is well visualized in the figure that two different Pco_2 values are not enough for the construction of the equilibration line.

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