THE FIRST DISSOCIATION EXPONENT OF CARBONIC ACID AS A FUNCTION OF pH

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Ever since Henderson in 1908 introduced Guldberg & Waage's Law of Chemical Equilibrium (1867) in biology applied to the dissociation of carbonic acid, new studies on the first dissociation exponent of carbonic acid in plasma have appeared in the literature (Hasselbalch 1916, Warburg 1922, Hastings, Sendroy & Van Slyke 1928 and Severinghaus, Stupfel & Bradley 1956).

There is, however, still discussion about the correct value, the latest figures varying from 6.086 (Severinghaus et al. 1956) to 6.13 (Gambino 1961) at a temperature of 38° C.

Further, Severinghaus et al. (1956) found a considerable variation of pK with pH (ΔpK / ΔpH = -0.044 at 37.5° C) without explaining this variation in detail. A similar variation was found in cerebrospinal fluid by Alexander, Gelfand & Lambertsen (1961), and finally Van Heist, Visser & Maas (1961) briefly reported a variation in pure bicarbonate solutions.

The purpose of this study was to determine the pK once more, using the special technique developed in this laboratory (Siggaard Andersen, Engel, Jørgensen & Astrup, 1960) and to attempt to clarify the different reasons for this apparent variation in the pK.

Theory.

The equation defining the pK1' used in biology is the Henderson-Hasselbalch equation

\[ pH = pK_1' + \log \frac{[HCO_3^-]}{\alpha \cdot pCO_2} \] (1)

where \([HCO_3^-]\) expresses the millimolar concentration of bicarbonate, and \(\alpha \cdot pCO_2 = [CO_2]\) expresses the millimolar concentration of dissolved carbon dioxide, plus carbonic acid (Henry's law). The equation shows that simultaneous determination of pH, \([HCO_3^-]\), and pCO2, allows a calculation of pK1'.

pK1' can be expressed by means of the "thermodynamic" dissociation exponent (pK1) together with the activity coefficients for bicarbonate and carbon dioxide:

\[ pK_1' = pK_1 + \log f_{HCO_3^-} - \log f_{CO_2} \] (2)

According to Debye & Hückel the activity coefficient of ions depends on the ionic strength \(\mu = \frac{1}{2} \sum v^2 [x]\) (cf. Edsall & Wy-
man 1958, p. 273). The contribution of the protein ions to the ionic strength is uncertain. Most often a protein polyanion is taken to be equal to an equivalent amount of monovalent anions when calculating the ionic strength. As a first approximation, \( \log f_{HCO_3} = -A \sqrt[2]{\mu} \), where \( A \) is a constant which can be derived theoretically (approximately 0.5). At higher ionic strengths, \( (\mu > 0.05) \) the activity coefficient is a more complicated function of the ionic strength.

\( f_{CO_3} \) is equal to \( a_0/\alpha \) where \( a_0 \) is the solubility factor for pure water.

The function between \( pK_{1'} \) and \( \mu \) at 38°C for pure aqueous solutions was studied experimentally by Hastings & Sendroy (1925), and recalculation of their results (Fitzsimons & Sendroy, 1961) gives

\[
pK_{1'} = pK_1 - 0.512 \sqrt[2]{\mu}
\]

The function between \( pK \) and the temperature is given by the Van't Hoff equation:

\[
\frac{dpK}{dT} = \frac{-Q}{2.3 \cdot R \cdot T^3}
\]

where \( Q \) is the heat of dissociation (cf. Edsall & Wyman 1958, p. 203). In order to integrate this equation \( Q \) must be known as a function of temperature. According to studies by Shedlovsky & MacInnes (1935), and Harned & Davis (1943), the function between \( pK_1 \) and temperature is not linear.

\( \alpha \) is a modified Bunsen absorption coefficient expressing the concentration of dissolved \( CO_3 \) plus carbonic acid (mMol/l) per mm Hg \( pCO_2 \). It was determined experimentally for water and plasma by Van Slyke, Sendroy, Hastings & Neil (1928), and shown to vary with the concentration and type of salt, and also the concentration of lipoids and proteins. In 1960 Bartels & Wrbitzsky redetermined \( \alpha \) from 15°C to 38°C, and found good agreement with the value of Van Slyke et al. (1928). The values of Bartels & Wrbitzsky were used in the present work. At 38°C for pure water \( \alpha = 0.03215 \), for human plasma \( \alpha = 0.0300 \). At 25°C for pure water \( \alpha = 0.04392 \) and for plasma \( \alpha = 0.0402 \). Concerning the value \( \alpha \) in pure water, the reader is referred to the discussion by Siesjö (1962). In aqueous salt solutions, \( \alpha \) can be derived from the value for pure water by division with \( f_{CO_3} \), which can be found from the work of Van Slyke et al. (1928). For 25 mMol/l NaCl \( f_{CO_3} = 1.008 \) and for 150 mMol/l NaCl \( f_{CO_3} = 1.031 \). According to Harned & Davis (1943) the effect of \( HCO_3^- \) is equal to the effect of \( Cl^- \).

Summarizing, it is necessary to distinguish between \( pK, pK_{1'}, pK_1'' \) and \( pK_{1'}^', \). \( pK_1 \) is the “thermodynamic” dissociation exponent using activities in the equation (1). It varies with temperature but is independent of ionic strength and pH. \( pK_{1'} \) is the dissociation exponent using molar concentrations instead of activities in equation (1) (with the exception of pH). It varies with temperature and ionic strength but not with pH. \( pK_1'' \) is the value to be used when the apparent bicarbonate concentration, determined by a titration procedure, is used in equation (1). It varies with pH according to the present investigation. \( pK_{1'}' \) is the value to be used when the apparent bicarbonate concentration, determined gasometrically, is used in equation (1).

MATERIAL AND METHODS

The \( pK_1'' \) values were determined at 38°C and 25°C in pure aqueous solutions consisting of NaOH with, and without, the addition of NaCl of ionic strengths of 0.150 and 0.025. Some measurements...
were made using solutions containing potassium instead of sodium.

Plasma from 8 normal fasting subjects was investigated at the same temperatures. The blood was drawn after a stasis of 10 minutes. After dilution of the plasma, (factor 0.80) following the addition of acid or base, the protein concentration in all cases was found to be within the normal range (6.4 to 8.3 g/100 ml).

pH was measured by means of a pH-meter, PHM 4 (Radiometer), and a slightly modified capillary glass electrode (Radiometer) of the completely fused type, together with a thermostated KCl, Hg2Cl2-electrode (Siggaard Andersen, Engel, Jørgensen & Astrup, 1960). A small piece of glass capillary tube was attached to the posterior end of the electrode in order to prevent the tendency of the fluid to flow out of the electrode into the calomel cell. In order to diminish electrostatic effects from the suction tube, a suction device was attached which sprang back, disconnecting the electrode from the suction tube. With each pH measurement, the electrode was soaked with the sample for 1 minute before the final filling and measurement.

The National Bureau of Standard's (NBS) pH scale was used. By means of the phthalate and borate buffers, the electrode response was found to be 99.5 to 99.3 per cent of the theoretical. 100 per cent deflection of the meter was obtained by adjusting the temperature compensation potentiometer at a temperature 1°C lower than the temperature of measurement. The routine buffer was a 1/15 mol/l phosphate buffer (pH approx. 7.37), the pH of which was measured accurately by means of a precision phosphate buffer of pH = 7.381 (Baumwoll Spinner & Kokholm Petersen, 1961) obtained from Radiometer. Agreement was found between this precision buffer, and a NBS phosphate buffer, pH = 7.382 at 38°C, prepared from salts obtained from the NBS. The pH values of this buffer at different temperatures according to the NBS scale are given by Bower, Paabo & Bates (1961).

The pCO2 value was determined by equilibrating the solution with a gas mixture of known CO2 concentration (Siggaard Andersen, Engel, Jørgensen & Astrup 1960). The gas mixtures were obtained from AGA (Copenhagen), and analysed in connection with each experiment by means of a Haldane apparatus, or the previously described gasometer (Siggaard Andersen & Jørgensen 1961). The sulfuric acid in the levelling flask of the latter was replaced with mercury, leaving only a small meniscus of sulfuric acid. Four different gas mixtures were used: 2.0, 4.1, 9.7 and 20.4 per cent (v/v) CO2 in O2.

The bicarbonate concentration was determined by three different methods:

a) In the experiments using pure aqueous solutions, the concentration of base equivalents (OH-) was titrated with hydrochloric acid after adjustment of the acid with sodium borate. During equilibration with CO2, OH- is converted to HCO3-. At ionic strength 0.150, the following bicarbonate concentrations (meq/l) were used: 3.9, 13.9, 23.9, 33.9, 43.9 and 53.9. At ionic strength 0.025 the bicarbonate concentration 25 meq/l was used.

b) In the experiments with plasma, HCO3- was determined by a special titration procedure:

Principle:

Take for example a given plasma sample (A), and add a known amount of acid, e.g. 10.0 meq/l, without diluting or changing the ionic strength, thus giving the plasma sample (B). The pH-log pCO2 lines are determined for both samples (A) and (B) by equilibrating with known CO2-tensions, and measuring the equilibrated pH values (Fig. 1). At any arbitrary pH value, e.g. 7.400, we have

\[ [\text{HCO}_3^-]_B = [\text{HCO}_3^-]_A = 10.0 \]

This equation follows from the fact that the same pH value in A and B gives the same dissociation of non-bicarbonate buffers in A and B. Thus all the added acid must be buffered by the bicarbonate ions. The validity of this assumption will be discussed later. The pCO2 values of samples A and B at a pH value of 7.400, are read from the pH-log pCO2 graph (pCO2_A = 36.0 and pCO2_B = 19.3).

1 The molar concentration is temperature dependent due to the heat expansion of the solution. Concentrations determined at room temperature (23°C) were corrected to 38°C by division by 1.005.
From the Henderson–Hasselbach equation we have for A

$$7400 = pK_{1}\prime\prime + \log \frac{[\text{HCO}_3^-]_A}{\alpha \cdot p\text{CO}_2 A}$$

and for B

$$7400 = pK_{1}\prime\prime + \log \frac{[\text{HCO}_3^-]_B - 10.0}{\alpha \cdot p\text{CO}_2 B}$$

Which give

$$[\text{HCO}_3^-]_A = \frac{10.0 \cdot p\text{CO}_2 A}{p\text{CO}_2 A - p\text{CO}_2 B}$$

or

$$pK_{1}\prime\prime = 7.400 + \log \left\{ \frac{p\text{CO}_2 A - p\text{CO}_2 B}{10} \right\}$$

With the given numbers we get $pK_{1}\prime\prime = 6.100$. The advantage of this method is that it allows a calculation of $pK_{1}\prime\prime$ at any arbitrary pH value on the basis of relatively few analyses.

The change in volume and ionic strength in B after addition of acid (or base) was compensated by an equivalent addition of water and sodium chloride to A.

**Practical procedure:**

7 different solutions were prepared.

One ml of plasma was pipetted into each of 7 test tubes by means of a syringe pipette. To each tube was added 250 µl of hydrochloric acid (0.15, 0.10 or 0.05 mol/l), or sodium chloride (0.15 mol/l), or sodium bicarbonate (0.05, 0.10 or 0.15 mol/l). The accurately titrated acids or bases were delivered by a constriction pipette. Sodium chloride was added to the acids and bases to give a total sodium concentration of 0.15 mol/l before the acids and bases were titrated. The base content of the samples rise stepwise by 10 meq/l.

C) In four experiments, the total-CO₂ concentration was determined by the method of van Slyke & Neill (Peters & van Slyke 1932) and bicarbonate was calculated as $[\text{HCO}_3^-] = [\text{total-CO}_2] - \alpha \cdot p\text{CO}_2$.

**RESULTS**

**Pure aqueous solutions:**

Under all experimental conditions, the $pK_{1}\prime\prime$ was found to decrease with increasing pH values. At the same pH value, the $pK_{1}\prime\prime$ was determined with a standard deviation (SD) = 0.008 for a single determination.

The results of the experiments performed...
Fig. 2. $pK_1''$ in aqueous solutions of NaCl ($\mu = 0.150$) at $38^\circ$C as a function of pH. The plotted points represent the mean of the $pK$ values measured at the respective pH values. The values are bracketed by $\pm 2 \times SDM = \pm 0.006$. The different symbols refer to solutions with a different bicarbonate concentration (meq/l): $\bullet = 3.9$, $\triangle = 13.9$, $\square = 23.9$, $\triangledown = 33.9$, $\Delta = 43.9$, $\circ = 53.9$. Each of these solutions was equilibrated with 4 different CO$_2$ concentrations. The dotted curve shows how an incorporation of CO$_3^{2-}$ in the determined bicarbonate will affect the calculated $pK_1''$ value. This is obviously insufficient to explain the experimental results, so the presence of undisassociated NaCO$_3$ is likely. If the dissociation constant of this compound is taken to be

$$K_{Na} = \frac{[Na^+][CO_3^{2-}]}{[NaCO_3]} = 0.075,$$

the unbroken curve shows how a co-determination of CO$_3^{2-}$ plus NaCO$_3$ as bicarbonate would affect the calculated $pK_1''$.

At an ionic strength of 0.150 and $38^\circ$C are shown in Fig. 2. The regression coefficient is $	ext{d}pK_1''/\text{d}pH = -0.027$, determined as the slope of the experimental curve at a pH of 7.40 and temperature, $pK_1'' = 6.136$ (SDM 0.002)$^1$. At the same ionic strength, 25$^\circ$C, and pH $< 6.5$, the $pK_1''$ was 6.189 (SDM 0.004). The regression coefficient $\text{d}pK_1''/\text{d}pH$ was somewhat greater at 25$^\circ$ than at $38^\circ$C.

At an ionic strength of 0.025 and $38^\circ$C, the $pK_1''$ was found to be 6.25 by extrapolation to pH 6.5. At this ionic strength, the fall in $pK_1''$ with rising pH was somewhat less than at the ionic strength of 0.150.

In solutions containing potassium in place of sodium, a similar (but slightly greater) fall in $pK_1''$ with rising pH was found.

**Plasma:**

At temperatures of $38^\circ$ and 25$^\circ$C, the $pK_1''$ fell with rising pH. At a given pH value, the $pK_1''$ was determined with the same accuracy as in pure bicarbonate solutions (SD 0.008).

The results of the measurements at $38^\circ$C are shown in Fig. 3. At pH 7.0, 7.2, 7.4 and 7.6, the $pK_1''$ was found to be 6.113, 6.107, 6.099 and 6.089 respectively (SDM 0.0015). The regression coefficient is estimated to be $\text{d}pK_1''/\text{d}pH = -0.047$ at pH 7.40.

At 25$^\circ$C and pH 7.0, 7.2, 7.4 and 7.6,

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$^1$ Standard deviation of mean.
A similar variation of $\text{pK}_1''$ with pH as found here, was briefly reported by Van Heijst & Maas (1961), without any explanation. After finishing this work personal communication with Dr. Maas revealed that the results of our independent investigations are very close whereas our conclusions are somewhat different. The variation is surprising, because it is impossible to explain on the basis of the previously mentioned theory. **The reason must therefore be a pH variable "error" in the determination of pH, $\text{pCO}_2$ or $\text{HCO}_3$.**

**The pH determination** might be erroneous in the presence of a pH-variable residual liquid-junction potential. This is, however, improbable as the residual liquid-junction potential should be minimal when using a saturated KCl-bridge (Bates 1954, p. 58).

A second reason for a pH variable error would be the alkaline error of the glass electrode which is due to the co-determination of alkali metallic ions. This effect should be far greater for Na$^+$ than for K$^+$, but was found to be slightly greater for K$^+$ than for Na$^+$. Further, the magnitude of the sodium error calculated from Dole's formula (Bates 1955, p. 240), is insufficient as the only reason for the measured variations.

**The $\text{pCO}_2$ determination** is most unlikely to be erroneous, as it is determined by equilibration. Also a pH-variability of the absorption coefficient $\alpha$ is less probable.

**The bicarbonate determination** will be erroneous in the presence of significant amounts of CO$_3^-$ or undissociated NaCO$_3$. The existence of ion-pair formation of this type is well known (cf. Robinson & Stokes...
Titration methods for the determination of $\text{HCO}_3^-$ give the equivalent concentration

$$[\text{HCO}_3^-, \text{titrated}] = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + 2[\text{NaCO}_3^-] \quad (4)$$

By means of the equilibrium constants we have

$$[\text{CO}_3^{2-}] = \frac{K_1'}{a_{H^+}[\text{HCO}_3^-]}$$

and

$$[\text{NaCO}_3^-] = \frac{K_3' [\text{Na}] [\text{CO}_3^{2-}]}{a_{H^+} K_{Na}^-} = \frac{K_3'[\text{Na}^+]}{K_{Na}^-} [\text{HCO}_3^-]$$

By insertion in (4) we get

$$[\text{HCO}_3^-, \text{titrated}] =$$

$$[\text{HCO}_3^-] \left( 1 + 2 \frac{K_1'}{a_{H^+}} + 2 \frac{K_3'}{a_{H^+}} K_{Na}^- \right)$$

And by insertion in equation (1) we get the measured $pK_1''$ expressed by the theoretical $pK_1'$:

$$pK_1'' = pK_1' - \log \left( 1 + 2 \frac{K_1'}{a_{H^+}} + 2 \frac{K_3'}{a_{H^+}} K_{Na}^- \right)$$

According to this equation, the measured $pK_1''$ will be lower than the real $pK_1'$ by a pH-variable quantity. The equation will explain the results shown in Fig. 2 if the following values are inserted: $[\text{Na}^+] = 0.150$ mol/l, $K_3' = 10^{-9.80}$ (for an ionic strength of 0.150 and 38°C according to Hastings & Sandroy (1925)) and $K_{Na}^- = 0.075$ (an ad hoc adjusted value). As shown in Fig. 2, the presence of $\text{CO}_3^{2-}$ alone will not explain the results. The magnitude of $K_{Na}^- = 0.075$ must be accepted with some reservation, but for comparison, the $K_{Na}^-$ for NaSO₄ has been found to be 0.19 at 25°C (Jenkins & Monk 1950). The somewhat greater variation of $pK_1'$ with pH in solutions containing K⁺ instead of Na⁺ indicates that $K_{Na}^- > K_{Na}^-$, which is also the case for the sulfates.

Gasometric methods for the determination of $\text{HCO}_3^-$ give the molar concentration and not the equivalent concentration

$$[\text{HCO}_3^-, \text{gasometric}] = [\text{HCO}_3^-] + [\text{CO}_3^{2-}] + [\text{NaCO}_3^-]$$

The $pK_1''$ is given by equation (5) provided that the factors 2 are omitted. It is seen that the variation of the measured $pK_1''$ with pH will be only approximately half of the variation determined by titrimetric methods.

2) The variation of $pK_1'$ with the ionic strength and temperature:

When pH < 6.5 the amounts of $\text{CO}_3^{2-}$ and $\text{NaCO}_3^-$ are negligible with the bicarbonate concentrations used. Thus $pK_1''$ approaches the $pK_1'$ as the pH falls to 6.5.

The two values found for $pK_1'$ (6.136 and 6.250) at ionic strengths of 0.150 and 0.025 (38°C) respectively fit the equation

$$pK_1' = pK_1 - 0.495 \sqrt{\mu}$$

There is a good agreement between this equation and the previously mentioned equation (3) of Hastings & Sandroy (1925). Extrapolation to $\mu = 0$ gives $pK_1 = 6.328$.

The temperature coefficient for $pK_1'$ ($\mu = 0.150$) calculated from the two results at 38° and 25°C respectively is $\Delta pK_1'/\Delta T = -0.0041$ which is in good agreement with the coefficient of $-0.0038$ calculated on the basis of the results of Harned & Davis (1943).

Plasma:

1) The variation of $pK_1''$ with pH:

At 38°C and a pH of 7.40, the $pK_1''$ was found to be 6.099 with a regression coefficient of $\Delta pK_1''/\Delta \text{pH} = -0.047$. The value for $pK_1''$ is slightly higher than the
pK'' value (6.086) of Severinghaus et al. (1956), but considerably lower than the pK'' value (6.13) of Gambino (1961). The regression coefficient is very close to the result (−0.044) of Severinghaus et al. (1956), but considerably lower than the regression coefficient, −0.092, for spinal fluid according to Alexander et al. (1961). The results are, however, in excellent agreement with recent investigations by Maas (1962).

The factors which might influence the pK'' value of plasma are the same as in the case of pure bicarbonate solutions, but the presence of proteins add further reasons for a pH variation of the measured pK''.

The presence of a considerable liquid-junction potential in colloid solutions due to the different diffusion rate for K+ and Cl− is well known. Due to the pH variability of the negative charge of the proteins, a pH variability of the liquid-junction potential would not be improbable. The present experiments, however, allow only a very rough estimate of the magnitude of this effect to be made, as the difference between the measured pK'' variation and the variation calculated from the formula derived below.

The bicarbonate concentration is determined by a special titration method as previously mentioned. The validity of this depends on the constancy of the protein dissociation at a constant pH and ionic strength, irrespective of the pCO2 value. It can not be excluded, however, that a binding of CO2 to the proteins as carbamino-CO2, will affect the ionization of neighboring acid or basic groups. If the binding of CO2 increases the acid dissociation of the proteins (cf. Warburg 1922, p. 305), the pK'' values determined by the titration method will be too low. The agreement with the 4 gasometrically determined pK'' values makes a greater effect of this type less probable.

Due to the presence of proteins, the determination of bicarbonate in plasma will be more erroneous than in pure bicarbonate solutions. First, the concentration determined must be corrected from the mean concentration of the system to the concentration in the water “phase”:

\[\text{[Mean conc.]} = \text{[conc. water phase]} \times (1- q)(1-d), \text{q being the magnitude of the protein “phase” (v/v) and d being the distribution ratio, } d = \frac{\text{[conc. protein phase]}}{\text{[conc. water phase]}} \text{ (Warburg 1922, p. 177).}\]

Second, the titrated bicarbonate will include carbamino-CO2 together with CO3− and NaCO3−:

\[\text{[HCO3−, titrated]} = \text{[HCO3−]} + 2[\text{CO3−}] + 2[\text{NaCO3−}] + [\text{Pr-NH-COO−}]\]

By means of the equilibrium constant for carbamino-CO2 (Stadie & O’Brien 1937) we have

\[\text{[Pr-NH-COO−]} = \frac{K^{\text{Am}}_{\text{Pr-}}} {K_{1}} \text{[Pr−] \text{[HCO3−]}}\]

According to Van Slyke, Hastings, Hiller & Sendroy (1928) [Pr−] = 0.00104 (Pr−) (pH = 5.08), (Pr−) being the protein conc. g/100 ml, [Pr−] being Eq/l.

By insertion in equation (1) we get in analogy to the formula for pK'' in pure solutions (5)

\[\text{pK''} = \text{pK''}_{1} + \log \frac{1-q(l-d)\text{CO}_2}{1-q(l-d)\text{HCO}_3^{-}}\]

\[\log \left(1+2\frac{K_{a}^{\text{H+}}}{K_{a}^{\text{H+}}} + 2\frac{K_{a}^{\text{H+}}}{K_{a}^{\text{H+}}} [\text{Na}^{+}] + 2\frac{K_{a}^{\text{H+}}}{K_{a}^{\text{H+}}} + \frac{K^{\text{Am}}_{\text{Pr-}}}{K_{1}}\right)\] (6)

+ liquid junction error.
The following constants valid for 38° C, can be inserted into the equation:

\[ pK' = 6.136 \] according to the present work.

\[ q \] is taken to be 0.06 (v/v) at a protein concentration of 70 g/l. \( d_{\text{HCO}_3} \) is taken to be zero and \( d_{\text{CO}_2} \) is taken to be 1 (Van Slyke, Wu & McLean 1923), but these figures must be accepted with reservation. By insertion we get

\[ \log \frac{1-q(1-d_{\text{CO}_2})}{1-q(1-d_{\text{HCO}_3})} = 0.027 \]

\[ [\text{Na}^+] = 0.150 \text{ M} \text{ol/l}. \]

\[ K'_2 = 10^{-8.80} \text{ (Hastings & Sendroy 1925)}. \]

\[ K'_\text{Na} = 0.075 \], the value fitting the formula for \( pK'' \) in pure bicarbonate solutions.

\[ K'_\text{Am} = 6 \cdot 10^{-7} \text{ according to Stadie & O'Brien (1937)}. \]

The liquid-junction potential error is taken to be 0.030, but this value must be accepted with reservation.

Using these constants, the formula should fit the experimental results (Fig. 3). It is seen that the experimental curve is somewhat steeper than the calculated curve. The reasons might be several, e.g. the formation of other \( \text{CO}_2 \) compounds than \( \text{NaCO}_3^- \), or the presence of a pH variable liquid-junction potential.

By extrapolation to a pH of about 6.0 the amounts of \( \text{CO}_3^- \), \( \text{NaCO}_3^- \) and \( \text{Pr-NH-COO}^- \) will be negligible. The \( pK'_1 \) will thus approach the \( pK'_1 \) as the pH falls towards 6.0. From Fig. 3 the \( pK'_1 \) for plasma is found to be about 6.13.

Gasometric methods give the molar concentrations according to \( [\text{HCO}_3^- \text{ gasometric}] = [\text{HCO}_3^-] + [\text{NaCO}_3^-] + [\text{CO}_3^-] \) and \( [\text{Pr-NH-COO}^-] \). The equation for \( pK'' \) is equation (6), when the factors 2 are omitted. At a pH = 7.40 and 38° C the \( pK'' \) will be 0.005 higher than the \( pK'' \), i.e. \( pK'' = 6.104 \). Actually the \( pK'' \) was found to be 6.108 at a pH of 7.37 by four determinations.

2) The variation of \( pK'' \) with temperature:

On the basis of the \( pK'' \) values at 38° and 25° C respectively, the temperature coefficient \( \Delta pK''/\Delta T \) can be calculated to be ---0.0035 at a pH of 7.4, and ---0.0039 at a pH of 7.0. The corresponding values according to Severinghaus et al. (1956) are ---0.0054 and ---0.0060 respectively. No explanation can be offered for this discrepancy.

CONCLUSION

\( pK'_1 \), the "thermodynamic" dissociation exponent using molar activities in Henderson-Hasselbalch's equation, is 6.328 at 38° C. The temperature coefficient is \( \Delta pK'_1/\Delta T = -0.0041 \) on the basis of results at 38° and 25° C respectively. \( pK_1 \) does not vary with ionic strength or pH.

\( pK'_1 \), the dissociation exponent using molar concentrations in Henderson-Hasselbalch's equation (with the exception of pH) varies with the ionic strength of pure bicarbonate solutions as follows \( pK'_1 = pK_1 - 0.495 \sqrt{\mu} \). For plasma \( pK'_1 \) is approximately 6.13 at 38° C. \( pK'_1 \) does not vary with pH.

\( pK'' \) is the dissociation exponent when the apparent bicarbonate concentration determined titrimetrically is used. The bicarbonate
determined by titration is equal to $[\text{HCO}_3^-] + 2[\text{CO}_2^+] + 2[\text{NaCO}_3] + [\text{Pr-NH-COO}^-]$. The pK$_1''$ varies with pH. For pure sodium bicarbonate solutions pK$_1''$ is defined by equation (5). For plasma at 38°C the pK$_1''$ is 6.113, 6.107, 6.099 and 6.089 at pH 7.0, 7.2, 7.4 and 7.6 respectively. The temperature coefficient of the plasma pK$_1''$, calculated from values determined at 38°C and 25°C, was found to be $\Delta \text{pK}_1''/\Delta T = -0.0035$ and $-0.039$ at pH 7.4 and 7.0 respectively.

pK$_1''$ is the dissociation exponent when the apparent bicarbonate concentration is determined gasometrically. The bicarbonate determined by gasometry is equal to $[\text{HCO}_3^-] + [\text{CO}_2^-] + [\text{NaCO}_3] + [\text{Pr-NH-COO}^-]$. For pure sodium bicarbonate solutions pK$_1''$ is given by equation (5) when the factors 2 of the equation are omitted. For plasma pK$_1''$ at 38°C and pH = 7.40 was calculated to be 6.104.

It is necessary to take the pH variation of pK$_1''$ and pK$_1''$ into account in cases where great absolute accuracy is required e.g. on calculation of the arterial pCO$_2$ for comparison with the alveolar pCO$_2$. For routine examinations of the acid-base disturbances in patients, the accuracy obtained by using a constant pK irrespective of the pH variation will, however, be completely satisfactory.

SUMMARY

The first dissociation exponent for carbonic acid was determined at 38° and 25°C in pure aqueous solutions of ionic strengths of 0.150 and 0.025, and also in plasma. Apparently the dissociation exponent decreases with an increase in pH in both pure solutions and plasma. It is concluded that the most important reason for this variation is the presence of undissociated NaCO$_3^-$ and CO$_3^{2-}$, and in plasma also carboxamino-CO$_2$, which are all erroneously determined as bicarbonate.

pK$_1'$ was chosen to designate the value to be used in Henderson-Hasselbalch's equation provided that the real bicarbonate concentration is inserted. pK$_1'$ is then invariable with pH and found to be about 6.13 for plasma at 38°C.

pK$_1''$ designates the value to be used when the apparent bicarbonate concentration is determined titrimetrically. pK$_1''$ designates the value to be used when the apparent bicarbonate concentration is determined gasometrically. Both pK$_1''$ and pK$_1''$ vary inversely with pH. For plasma at 38°C and pH = 7.40, pK$_1''$ was found to be 6.099 while pK$_1''$ was calculated to be 6.104.

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