An Overall Equation for the First Ionization Equilibrium of Carbonic Acid *

A. H. J. Maas, B. F. Visser, A. M. van Leeuwen,

and J. Th. G. Overbeek

Departments of Cardiology, Cardiac Surgery and Lung Diseases,
University Hospital, Utrecht; Lung Function Laboratory, Binnengasthuis,
Amsterdam; Van 't Hoff Laboratory, Utrecht — The Netherlands

* This communication is based on a part of a dissertation presented by A. H. J. Maas to the University of Utrecht in partial fulfilment of the requirements for the degree of Doctor of Philosophy. January 1967.

Summary. This article deals with the equilibrium of carbonic acid in sodium-bicarbonate solutions and in the body fluids to be considered as such: cerebrospinal fluid and blood plasma. The main problem is how to choose an equation for the 1st ionization equilibrium of carbonic acid.

The thermodynamic theory of equilibrium is given clearly distinguishing a true constant $\bar{K}$ (product of activities) and the practical coefficients K (product of concentrations) and K' (product of activities and concentrations). By virtue of this theory it is shown that the 1st ionization equilibrium of carbonic acid in the solutions above-mentioned may best be described by the overall 1st ionization equation as related to the CO_2 in the gas phase—the modified Henderson-Hasselbalch equation—:

$$\mathrm{pH} = pK'_{1g} - \log P_{CO_2} + \log [HCO_3^-].$$

Key-Words: Carbonic Acid-Bicarbonate Equilibrium — Activity of Bicarbonate Ion — Henderson-Hasselbalch Equation.

Schlüsselwörter: Kohlensäure-Bicarbonat-Gleichgewicht — Aktivität von Bicarbonat-Ion — Henderson-Hasselbalch-Gleichung.

The carbonic acid equilibrium of biological fluids is described by the Henderson-Hasselbalch equation as applied to the carbonic acid/bicarbonate buffer system. This useful equation gives the relation between acidity, carbonic acid and bicarbonate concentration of a solution. However, little emphasis has been placed on an exact formulation which accounts for the non-ideality of the system.

In discussing the carbonic acid equilibrium system, we intend to confine ourselves to sodium bicarbonate solutions and body fluids, viz.: cerebrospinal fluid (low-protein) and blood plasma (high protein). These will be regarded as homogeneous media to which we apply the thermodynamic theory of equilibrium. The clinically important bicarbonate

buffer system will receive special attention. It will be demonstrated that the (overall) equation for the 1st ionization equilibrium of carbonic acid as related to the partial pressure of CO_2 in the gas phase, has advantages over the conventional Henderson-Hasselbalch equation.

1. Definition of the Equilibrium Constant and Equilibrium Coefficients

If at constant T and P a chemical reaction occurs between the various components of the system, which reaction can be described in a general fashion as

$$aA + bB + \ldots \leftrightharpoons lL + mM + \ldots \tag{1,1}$$
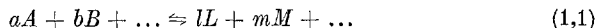
where $A, B \ldots L, M$ indicate the chemical components, and $a, b \ldots l, m \ldots$ the stoichiometric coefficients, then according to thermodynamics the condition of equilibrium is described by:

$$\frac{a_L^l \, a_M^m \, (\text{or } F_L^l \, F_M^m) \ldots}{a_A^a \, a_B^b \, (\text{or } F_A^a \, F_B^b) \ldots} = \overline{K} . \tag{1,2}$$

in which $\overline{K}$ is called the true equilibration constant. If we split the fugacities $F_L^l, F_M^m \ldots F_A^a, F_B^b \ldots$ and activities $a_L^l . a_M^m \ldots a_A^a, a_B^b \ldots$ in fugacity coefficient g and partial pressure P, and in activity coefficient f and concentration C resp., then (1,2) can be written:

$$\frac{C_L^l \, C_M^m \, (\text{or } P_L^l \, P_M^m) \, f_L^l \, f_M^m \, (\text{or } g_L^l \, g_M^m) \ldots}{C_A^a \, C_B^b \, (\text{or } P_A^a \, P_B^b) \, f_A^a \, f_B^b \, (\text{or } g_A^a \, g_B^b) \ldots} = \overline{K} . \tag{1,3}$$

The law of mass action as applied in the classical theory of equilibrium, is for reaction (1,1):

$$\frac{C_L^l \, C_M^m \, (\text{or } P_L^l \, P_M^m) \ldots}{C_A^a \, C_B^b \, (\text{or } P_A^a \, P_B^b) \ldots} = K . \tag{1,4}$$

In this equation, K (concentration-equilibrium coefficient) is not constant but a function of the concentrations. The relation between K and $\overline{K}$ is obviously:

$$K = \overline{K} \, \frac{f_A^a \, f_B^b \, (\text{or } g_A^a \, g_B^b) \ldots}{f_L^l \, f_M^m \, (\text{or } g_L^l \, g_M^m) \ldots} . \tag{1,5}$$

This re-emphasizes that K equals $\overline{K}$ (and thus is constant) only in highly diluted solutions and ideal gases, because in this special case activity coefficients and fugacity coefficients equal l.

By introducing activities (fugacities) instead of concentrations (pressures), the form of the ideal law has been retained but in actual experiments this formulation cannot be applied without qualification. In calculating the position of the chemical equilibrium the activity coefficients (fugacity coefficients) are often unknown, so that concentrations (pressures) have to be used to begin with. However, if we consider equilibria in which the composition of the solutions remains virtually constant (and in which in particular the ionic strength is constant), then the activity coefficients, although unknown are at least *constant*, so that K can be regarded as constant for practical purposes. This practical coefficient will be represented by K plain.

There is a third alternative: For some components the activity is introduced in the formula, for the other components the concentration. This is the case in H^+

ion equilibria for which the a_H is determined directly (at least approximately so) so that it can be included as such in the mass action product.

For these equilibria we intend to use a practical coefficient K' which is defined:

$$K' = K \frac{f_A^a \, f_B^b \ (\text{or } g_A^a \, g_B^b) \text{ except } f_H \cdots}{f_L^l \, f_M^m \ (\text{or } g_L^l \, g_M^m) \text{ except } f_H \cdots} \tag{1,6}$$

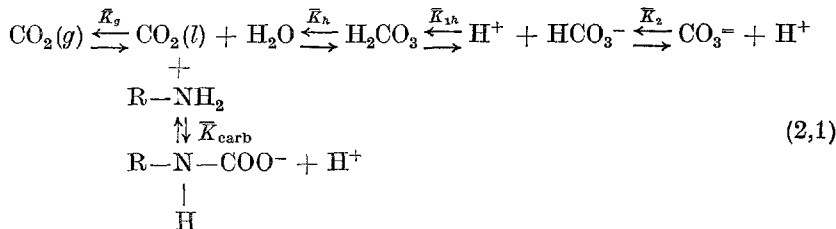## 2. The Carbonic Acid Equilibrium. Formulation of Equilibrium Constants

When carbon dioxide is in equilibrium with pure water, less than 0.5% (Buytendijk, 1927) of the dissolved CO_2 is present in the hydrated form, mainly as H_2CO_3 (which is partly dissociated so that the solution also contains traces of HCO_3^- and $CO_3^=$).

Table 1. *Approximate concentration (in mmole/l) of various forms in which carbonic acid is present in plasma and cerebrospinal fluid at* $P_{CO_2} = 40$ *mm Hg, pH = 7.40 and temperature of 38° C*

Carbonic acid as	Chemical formula	mmole/l
dissolved CO_2	CO_2	1.2
hydrated CO_2	H_2CO_3	0.05
bicarbonate ion	HCO_3^-	25
carbonate ion	$CO_3^=$	0.1
carbamate ion (only in plasma)	$R-N-COO^-$ $\mid$ H	<0.2

At an approximately neutral pH, however, saline solutions and physiological fluids such as plasma or cerebrospinal fluid contain by far the greater part of the CO_2 in the form of HCO_3^--ions. In plasma, a small quantity of CO_2 is also bound to protein in the form of carbamate. Table 1 presents the approximate concentrations of the various forms in which carbonic acid is present in plasma and cerebrospinal fluid. This table shows that we are dealing chiefly with bicarbonate ions, and only to a small extent with dissolved carbonic acid.

Between these carbon dioxide derivatives and the CO_2 present in the gas phase, there exist equilibria according to the diagram below, in which the corresponding true equilibrium constants are also indicated. Since we are concerned with properties of the carbonic acid *equilibrium*, we need not discuss details of kinetics.

$$CO_2(g) \overset{\overline{K}_g}{\rightleftharpoons} CO_2(l) + H_2O \overset{\overline{K}_h}{\rightleftharpoons} H_2CO_3 \overset{\overline{K}_{1h}}{\rightleftharpoons} H^+ + HCO_3^- \overset{\overline{K}_2}{\rightleftharpoons} CO_3^= + H^+$$

$$+$$
$$R-NH_2$$
$$\updownarrow \overline{K}_{carb} \tag{2,1}$$
$$R-N-COO^- + H^+$$
$$\mid$$
$$H$$

The equilibrium constants will now be successively defined in accordance with the thermodynamic theory of equilibrium. The concentration, indicated by [ ], will be expressed in mole/litre or gram-ion/litre, and pressure in mm Hg unless stated otherwise. The relation between the true constants and the practical coefficients will be found by writing the fugacities and the activities of the components concerned in factors.

a) Solubility constant.

The constant $\overline{K}_g$ is defined by the equation:

$$\overline{K}_g = \frac{a_{CO_2}}{F_{CO_2}} = \frac{f_{CO_2}}{g_{CO_2}} \times \frac{[CO_2]}{P_{CO_2}} = \frac{f_{CO_2}}{g_{CO_2}} \times K_g \qquad (2,2)$$

in which K_g is the solubility coefficient of Henry's classical law.

b) Hydration constant.

The hydration constant $\overline{K}_h$ is defined by the equation:

$$\overline{K}_h = \frac{a_{H_2CO_3}}{a_{CO_2} a_{H_2O}} = \frac{f_{H_2CO_3}}{f_{CO_2} a_{H_2O}} \times \frac{[H_2CO_3]}{[CO_2]} = \frac{f_{H_2CO_3}}{f_{CO_2} a_{H_2O}} K_h . \qquad (2,3)$$

Following the usual convention, a_{H_2O} is expressed in the rational concentration scale (mole fraction scale).

c) The 1st ionization constant is given by

$$\overline{K}_{1h} = \frac{a_H a_{HCO_3}}{a_{H_2CO_3}} = \frac{f_H f_{HCO_3}}{f_{H_2CO_3}} \times \frac{[H^+][HCO_3^-]}{[H_2CO_3]} = \frac{f_H f_{HCO_3}}{f_{H_2CO_3}} \times K_{1h}. \qquad (2,4)$$

The suffix h in $\overline{K}_{1h}$ and K_{1h} indicates that the ionization equilibrium has been related to hydrated carbonic acid.

d) The 2nd ionization constant is given by

$$\overline{K}_2 = \frac{a_H a_{CO_3}}{a_{HCO_3}} = \frac{f_H f_{CO_3}}{f_{HCO_3}} \times \frac{[H^+][CO_3^-]}{[HCO_3^-]} = \frac{f_H f_{CO_3}}{f_{HCO_3}} \times K_2 . \qquad (2,5)$$

e) The constant of the carbamino-equilibrium.

Using for H_2N-R the symbol Am, and for $\overset{\displaystyle R}{\overset{\displaystyle |}{-OOC-N-R}}$ the symbol carb⁻ the constant $\overline{K}_{carb}$ of the carbamino-equilibrium is described by the equation:

$$\overline{K}_{carb} = \frac{a_{carb} a_H}{a_{Am} a_{CO_2}} = \frac{f_{carb} f_H}{f_{Am} f_{CO_2}} \times \frac{[carb^-][H^+]}{[Am][CO_2]} = \frac{f_{carb} f_H}{f_{Am} f_{CO_2}} \times K_{carb} . \qquad (2,6)$$

For the sake of orientation we present in Table 2 the values of the various constants at 25° C and 38° C.

Table 2. *Values for solubility constant, hydration constant, ionization constants and carbamino-equilibrium constant of carbonic acid at 25° C and 38° C*

Equilibrium constants	25° C	38° C	Authors
$\overline{K}_g$ (mmole/l/mm Hg)	438×10^{-4}	321×10^{-4}	Bartels (1960) Austin (1963)
$\overline{K}_h$	about 1.8×10^{-3}	about 1.7×10^{-3}	Kern (1961)
$\overline{K}_{1h}$ (mole/l)	2.5×10^{-4}	3×10^{-4}	Roughton (1941)
$\overline{K}_2$ (mole/l)	4.69×10^{-11}	5.86×10^{-11}	Harned and Scholes (1941)
K'_{carb}		6.4×10^{-7}	Stadie and O'Brien (1937)

The constant $\overline{K}_g$ was calculated with the aid of formula: $S_0 = \overline{K}_g (1 + \overline{K}_h)$ from the value of the solubility of CO_2 in pure water (S_0), which equals the sum of the dissolved CO_2 (at the unit of P_{CO_2}) and the hydrated CO_2. In this calculation the hydrated CO_2 was supposed to be 0,2% of the total, which means that $\overline{K}_h$ was supposed to be 2×10^{-3}.

From the value of $\overline{K}_{1h}$ it follows that carbonic acid is a fairly strong acid, about comparable to formic acid [($\overline{K}_{formic\ acid} = 1.77 \times 10^{-4}$ (25°C); Hodgman, 1962]. The table includes a practical constant of the carbamino-equilibrium

$$K'_{carb} = \frac{[Carb^-] \, a_H}{Am \, [CO_2]}$$

which is valid for plasma only.

3. The Overall 1st Ionization Equilibrium as Related to the CO₂ in the Liquid Phase

The 1st ionization constant $\overline{K}_{1h}$ is ill-suited for establishing the position of the 1st ionization equilibrium of carbonic acid, because it is difficult to determine $[H_2CO_3]$.

The bicarbonate/carbonate equilibrium may be left out of consideration because in biological fluids (pH between 6—8) the carbonate concentration is very low and also difficult to determine. In practice, therefore, the 1st ionization equilibrium is related to the sum of freely dissolved CO_2 and the hydrated CO_2. The starting-point in that case is the overall 1st ionization equilibrium, related to the dissolved carbon dioxide:

$$CO_2(l) + H_2O \rightleftarrows H^+ + HCO_3^-. \tag{3,1}$$

The constant valid for this equilibrium is $\overline{K}_{1l}$:

$$\overline{K}_{1l} = \frac{a_H a_{HCO_3}}{a_{H_2O} a_{CO_2}} = \frac{a_H f_{HCO_3} [HCO_3^-]}{a_{H_2O} f_{CO_2} [CO_2]}. \tag{3,2}$$

The suffix l in $\overline{K}_{1l}$ refers to the CO_2 in the liquid.

Instead of the concentration of physically dissolved CO_2 ($[CO_2]$), one determines the concentration of *carbonic acid* ([carbonic acid]), which

equals the sum of the concentrations of physically dissolved CO_2 and hydrated CO_2:

$$[\text{carbonic acid}] = [CO_2] + [H_2CO_3]. \tag{3,3}$$

For this purpose we make use of the coefficients S of the solubility of the neutral (non-ionized) forms of carbonic acid and which is defined as follows:

$$S = \frac{[\text{carbonic acid}]}{P_{CO_2}} = \frac{[CO_2] + [H_2CO_3]}{P_{CO_2}}. \tag{3,4}$$

Because, according to (3,3):

$$[H_2CO_3] = \frac{a_{H_2O} f_{CO_2} [CO_2] \overline{K}_h}{f_{H_2CO_3}}. \tag{3,5}$$

S can also be written as:

$$S = \frac{[CO_2]}{P_{CO_2}} \left(1 + \frac{a_{H_2O} f_{CO_2} \overline{K}_h}{f_{H_2CO_3}} \right) \tag{3,6}$$

or in a simplified form:

$$S = \frac{[CO_2]}{P_{CO_2}} (1 + \varDelta). \tag{3,7}$$

If we replace $[CO_2]$ by $S\, P_{CO_2}/(1 + \varDelta)$ [cf (3,7)], then (3,2) is changed to the *conventional* form:

$$\overline{K}_{1l} = \frac{a_H f_{HCO_3} [HCO_3^-] (1 + \varDelta)}{a_{H_2O} f_{CO_2} S\, P_{CO_2}}. \tag{3,8}$$

For solutions of virtually constant ionic strength (e.g. plasma, cerebrospinal fluid), activity coefficients are about constant, and the following practical coefficient can be defined:

$$K'_{1l} = \overline{K}_{1l} \frac{f_{CO_2} a_{H_2O}}{f_{HCO_3} (1 + \varDelta)} = \frac{a_H [HCO_3^-]}{S\, P_{CO_2}}. \tag{3,9}$$

4. The Overall 1st Ionization Equilibrium as Related to the CO_2 in the Gas Phase

If there is equilibrium between the CO_2 in the gas phase and that in the liquid phase, we can consider the overall equilibrium:

$$CO_2(g) + H_2O \rightleftarrows HCO_3^- + H^+ . \tag{4,1}$$
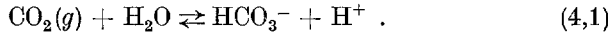
This equilibrium obeys the equation

$$\overline{K}_{1g} = \frac{a_H f_{HCO_3} [HCO_3^-]}{a_{H_2O}\, g_{CO_2}\, P_{CO_2}} \tag{4,2}$$

in which $\overline{K}_{1g}$ is the overall constant of the 1st ionization equilibrium, related to the CO_2 in the gas phase.

For practical purpose we define a practical coefficient K'_{1g}:

$$K'_{1g} = \frac{a_{H_2O} g_{CO_2}}{f_{HCO_3}} \times \overline{K}_{1g} = \frac{a_H [HCO_3^-]}{P_{CO_2}} \, . \tag{4,3}$$

Apart from the coefficient g_{CO_2} ($g_{CO_2} = 1 - 0.003 \, P_{CO_2}$ [atm]; Lewis and Randall, 1923, page 192), which for physiological carbon dioxide pressures ($< 0,2$ atm) can be assumed to equal 1, the ratio a_{H_2O}/f_{HCO_3} determines the variability of K'_{1g} with the composition of the solution.

To understand the way in which the two factors a_{H_2O} and f_{HCO_3} influence one another, we shall therefore consider how, for physiological solutions, they change with ionic strength and protein concentration.

The *ionic strength I* is defined by the equation:

$$I = \frac{1}{2} \, \Sigma \, z_i^2 \times C_i \tag{4,4}$$

in which C_i refers to the molar concentration (moles/litre) and z_i to the valence of the ion type. For plasma and cerebrospinal fluid, the ionic strength $I = 0.160$.

a_{H_2O}:

From vapour tension determinations we calculate for $I < 0.2$ (Robinson and Stokes, 1959, page 476):

$$a_{H_2O} = 1 - 0.035 \, I. \tag{4,5}$$

From determinations of freezing-point depressions (U), Lewis and Randall (1923) found the relation:

$$\log a_{H_2O} = -0.0042 \, U. \tag{4,6}$$

Saline solutions of $I = 0.160$, which can be compared with cerebrospinal fluid and plasma show the same freezing-point depression of $0.55°$ C (Dorhout Mees, 1958). From both relations it is found that $a_{H_2O} = 0.994$ to 0.995, so that it is justified to accept (4,5) for cerebrospinal fluid and plasma.

f_{HCO_3}:

Although single ion activities are experimentally accessible and f_{HCO_3} occurring in our equations actually stands for $(f_{HCO_3} \times f_H)/f'_H$ where f_H is the value of the activity coefficient of the hydrogen ions as included in the definition of the pH $= -\log f'_H[H^+]$, reasonable estimates can be made for our f_{HCO_3} which give an impression about the sign and the order of magnitude of the variations occuring in it.

The activity coefficient is a measure of the total effect of the environment on the bicarbonate ion, and indicates the extent to which the HCO_3^--ions in solution deviate from ideal behaviour. In protein solutions,

two factors must be taken into account, namely: the salt effect and the protein effect. According to the limiting law of Debye and Hückel, the activity coefficient f_i of an ion is a function of ionic strength

$$\log f_i = - A \sqrt{I} \qquad (4,7)$$

in which A is a constant, which can be calculated from the theory. In general, this relation is not valid for $I > 0.010$ mole l^{-1}. HASTINGS and SENDROY (1925) demonstrated, however, that in the case of the bicarbonate ion even at a physiological salt concentration ($I = 0,160$), a linear relationship with $\sqrt{I}$, but with an empirical constant exists. Consequently we chose for f_{HCO_3} an approximating formula of the form:

$$f_{HCO_3} = B - \gamma \sqrt{I} - \delta \,[Pr] \qquad (4,8)$$

in which $\gamma \sqrt{I}$ gives the contribution of salt and $\delta \,[Pr]$ the contribution of protein; B, γ and δ are constants for which an estimate will be given below.

In a hemoglobin solution, the following equation is valid for the activity coefficient (STADIE and HAWES, 1928):

$$\log f_{HCO_3} = -\varrho \,[Hb] - (0.54 - \sigma \,[Hb]) \sqrt{I} \qquad (4,9)$$

ϱ and σ vary somewhat with the type of Hb, but are on the average equal to 0.02 ± 0.005. The [Hb] has been expressed in mmole/l. Assuming that this formula is valid also for the solution of plasma proteins, we write (4,9) in the form:

$$\ln f_{HCO_3} = 2.3 \, \{ -0.02 \,[Pr] - (0.54 - 0.02 \,[Pr]) \sqrt{I} \} \; . \quad (4.10)$$

Assuming further that the average molecular weight of plasma protein equals 70.000, then it follows from (4,10) that at Pr = 7 g/100 ml (i.e. [Pr] = 1 mmole/l) and $I = 0.160$:

$$f_{HCO_3} = 0.592.$$

To compare the variations of f_{HCO_3} with salt concentration and protein content in the physiological range, we calculate γ with the differential formula:

$$\gamma = - \left(\frac{d f_{HCO_3}}{d \sqrt{I}} \right)_{[Pr] = 1} = - \left(\frac{d \ln f_{HCO_3}}{d \sqrt{I}} \right)_{[Pr] = 1} \times f_{HCO_3}$$
$$= - 2.3 \, (-0.54 + 0.02) \, 0.592 = 0.708.$$

Equally we find for δ:

$$\delta = - \left(\frac{d f_{HCO_3}}{d \,[Pr]} \right)_{\sqrt{I} = 0.4} = - \left(\frac{d \ln f_{HCO_3}}{d \,[Pr]} \right)_{\sqrt{I} = 0.4} \times f_{HCO_3}$$
$$= - 2.3 \, (-0.02 + 0.008) \, 0.592 = 0.016.$$

If these values of γ and δ are introduced in (4,8) we find with f_{HCO_3} = 0.592 for $I = 0.16$ and $[Pr] = 1$ the value $B = 0.892$.

For the activity coefficient f_{HCO_3} in plasma we can, therefore write *by approximation:*

$$f_{HCO_3} = 0.892 - 0.708\,\sqrt{I} - 0.016\,[Pr]. \tag{4,11}$$

After substitution of (4,6) and (4,11) in (4,3) we find for K'_{1g} the equation:

$$K'_{1g} = \overline{K}_{1g} \times \frac{1 - 0.035\,I}{0.892 - 0.708\,\sqrt{I} - 0.016\,[Pr]}. \tag{4,12}$$

From this equation we may conclude that the variability of K'_{1g} with the ionic strength is determined mainly by the $\sqrt{I}$ term of the coefficient f_{HCO_3}.

In comparison with the ionic strength, the influence of the protein concentration on K'_{1g}, is very small.

5. Choice of an Equation for the Bicarbonate Equilibrium in Biological Fluids

In order to compare our practical equations of Sections 3 and 4 we take the negative logarithm of these equations and apply the notation $-\log = p$.

This gives us for (3,9):

$$pa_H = pK'_{1l} + \log\frac{[HCO_3{}^-]}{S\,P_{CO_2}} = pK'_{1l} - \log S + \log\frac{[HCO_3{}^-]}{P_{CO_2}} \tag{5,1}$$

which is the well-known *"Henderson-Hasselbalch"* equation and for (4,3):

$$pa_H = pK'_{1g} + \log\frac{[HCO_3{}^-]}{P_{CO_2}} \tag{5,2}$$

which we call the *modified Henderson-Hasselbalch* equation. Since this equation contains one term less than equation (5,1) (namely the solubility coefficient S), the modified Henderson-Hasselbalch equation is the most suitable equation for description of the carbonic acid/bicarbonate buffer system.

6. The Practical Consequences of the Modified Henderson-Hasselbalch Equation

As we have seen, the practical overall equation (modified Henderson-Hasselbalch equation) contains three variables: pa_H, P_{CO_2}, and $[HCO_3{}^-]$, and a coefficient pK'_{1g}. In order to be able to apply this equation, it is necessary to know the value of the coefficient over the largest possible range of application and to determine two of the three variables, e.g.

pH (i.e. approximately pa_H) and P_{CO_2}. To establish the coefficient, however, it is necessary to know all three variables.

In a subsequent publication we shall therefore determine from measurements of pH, [HCO_3] and P_{CO_2}, the overall coefficient $p\widetilde{K}'_{1g}$ (i.e. approximately pK'_{1g}, because pH in consequence of the residual liquid-junction potential in the measurement is not exactly identical to pa_H).

References

AUSTIN, W. H., E. LACOMBE, P. RAND, and M. CHATTERJEE: Solubility of carbon dioxide in serum from 15° C to 38° C. J. appl. Physiol. **2**, 301—304 (1963).

BARTELS, H., u. R. WRBITSKY: Bestimmung des CO_2-Absorptions-Coeffizienten zwischen 15° und 38° C in Wasser und Plasma. Pflügers Arch. ges. Physiol. **271**, 162—168 (1960).

BUXTENDIJK, F. J., R. BRINKMAN, and H. W. MOOK: LXXXI. A study of the system carbonic acid, carbon dioxide and water. I. Determination of the true dissociation constant of carbonic acid. Biochem. J. **21**, 576—584 (1927).

DORHOUT MEES, E. J.: Onderzoek naar de oorzaken van verminderde urine-concen-tratie bij nierziekten. University Utrecht, Thesis, p. 80 (1958).

HARNED, H. S., and S. R. SCHOLES: The ionization constant of HCO_3^- from 0—50° C. J. Amer. chem. Soc. **63**, 1706—1709 (1941).

HASTINGS, A. B., and J. SENDROY, JR.: The effect of variation ionic strength of the apparent first and second dissociation constants of carbonic acid. J. biol. Chem. **65**, 445—455 (1925).

HODGMAN, CH. D.: Handbook of Chemistry and Physics, o. 1754. Cleveland. Ohio: The chemical rubber scientific publishing Co. 1963.

KERN, D. M.: The hydration of carbon dioxide. J. chem. Educ. **37**, 14—23 (1961).

LEWIS, G. N., and M. RANDALL: Thermodynamics and the free energy of chemical substances, p. 284. New York: Mc. Graw-Hill Book Company Inc. 1923.

ROBINSON, R. A., and R. H. STOKES: Electrolyte solutions. p. 476, Appendix 8.3. (calculated from table 1). London: Butterworths (1959).

ROUGHTON, F. J. W.: The kinetics and rapid thermochemistry of carbonic acid. J. Amer. chem. Soc. **63**, 2930—2934 (1941).

STADIE, W. C., and E. R. HAWES: Studies on the oxygen-, acid-, and base-com-bining properties of blood. IV. The apparent first dissociation constant pK'_1, of carbonic acid and the activity coefficient of the bicarbonate ion in solution of hemoglobin, methemoglobin, cyanhemoglobin, and nitric oxide hemoglobin, at varying ionic strengths. J. biol. Chem. **77**, 266—301 (1928).

—, and H. O'BRIEN: The carbamate equilbrium II. The equilibrium of oxyhemo-globin and reduced hemoglobin. J. biol. Chem. **117**, 439—470 (1937).

Dr. A. H. J. MAAS
Kliniek voor Hart- en Vaatziektenchirurgie
Academisch Ziekenhuis
Catharijnesingel 101, Utrecht (Holland)

.