LXXXI. A STUDY OF THE SYSTEM CARBONIC ACID, CARBON DIOXIDE AND WATER.

I. DETERMINATION OF THE TRUE DISSOCIATION- CONSTANT OF CARBONIC ACID.

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ALTHOUGH the quantitative equilibrium relations in the bicarbonate-carbonic acid systems are very accurately known in biological solutions, it is a remarkable fact that only little is known about the way in which this equilibrium is attained and about the velocities of the reactions occurring. Still it might be supposed that this equilibrium is not reached at once, as in purely ionic reactions, but that it is a very definite time-process under certain conditions. Thus the buffer-capacity of a bicarbonate system, after addition of a stronger acid, is calculated from the apparent dissociation-constant ($k_1 = 3 \times 10^{-7}$), but it is possible that initially the system is reacting according to the true constant of carbonic acid ($k_1 = 4 \times 10^{-4}$) during a biologically significant time, with a consequent large temporary increase of the resulting c_H . If the carbonic acid were produced continuously, it might then be acting continuously as a strong acid.

On the other hand, if we consider the hydration of CO_2 as a relatively slow time-reaction, the transport of this substance by the blood must be greatly influenced by its velocity of hydration, as was pointed out by Collingwood [1924].

Many questions present themselves in this way. What is the velocity of the hydration of carbonic acid *in vivo*; can H_2CO_3 exist there as a strong acid for a sufficiently long time? How is the velocity of dehydration influenced by temperature, $[\text{H}^+]$, $[\text{HCO}_3^-]$, other ions, CO_2 tension, etc.? We will try first to investigate the velocity of dehydration of carbonic acid in a pure bicarbonate solution, and to estimate its true dissociation-constant. Then we will study these properties in a more physiological solution and in the circulating blood.

The hydration of CO_2 and dehydration of H_2CO_3 .

Since the work of McBain [1912], Thiel [1913, 1916, 1922], Strohecker [1916], Thiel and Strohecker [1914, 1916], Pusch [1916] and especially of Faurholt [1922, 1924], it has been recognised that a solution of CO_2 in water

contains only a small amount of the rather strong acid, H_2CO_3 . If base is added to such a solution only a fraction, equivalent to the amount of H_2CO_3 present, is neutralised at once; the remaining large fraction is neutralised as a time-reaction with practically the velocity of the reaction $\text{CO}_2 + \text{OH}^- \rightarrow \text{HCO}_3^-$. Calculation of the true dissociation-constant from the fraction of pre-existing H_2CO_3 by Thiel and Strohecker, gave an approximate value of $K_{\text{H}_2\text{CO}_3} = 4.4 \times 10^{-4}$ (in a 0.008 mol. solution of carbonic acid at 4° , 1.23 % is present as H_2CO_3). The velocity of this hydration, as studied by Thiel and Strohecker, appeared to be dependent upon the p_{H} and to increase with increasing alkalinity.

Results of more detailed character have been obtained by Faurholt. His method makes use of the reaction between CO_2 and amines (the carbamino-reaction), so that in a mixture of $\text{CO}_2 + \text{H}_2\text{CO}_3$ the HCO_3^- can be precipitated as BaCO_3 , while the CO_2 is prevented from precipitation by addition of ammonia, methyl- or dimethyl-amine. Faurholt's conclusions are the following:

Hydration of carbon dioxide is occurring in two ways, viz.:

and

k_1 at $0^\circ = 0.0013$ and at $18^\circ = 0.0011$,

k_2 at $0^\circ = 10^{42.61}$ and at $18^\circ = 10^{43.24}$.

Both processes are occurring simultaneously, so the hydration is determined by

$$\frac{d[\text{CO}_2]}{dt} = -(k_1 + k_2[\text{OH}^-])[\text{CO}_2],$$

which indicates that the reaction is of monomolecular type if $[\text{OH}^-]$ is constant. If $p_{\text{H}} < 8$, $[\text{OH}^-]$ is so small that the reaction has practically the type $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3$; if $p_{\text{H}} > 10$ the second reaction $\text{CO}_2 + \text{OH}^- \rightarrow \text{HCO}_3^-$ is predominant. In the range p_{H} 8-10 the velocities of both reactions are important.

The dehydration of carbonic acid is taking place in two ways:

k_3 at $0^\circ = \pm 1$, at $18^\circ = 7.1$,

k_4 at $0^\circ = 10^{-5.53}$, at $18^\circ = 10^{-4.52}$.

Thus the dehydration of H_2CO_3 is a monomolecular process; Faurholt deduces that the dehydration of the bicarbonate is also monomolecular if the p_{H} is constant. Between p_{H} 8 and 10 both processes have velocities of similar magnitude, in more acid solutions $\text{H}_2\text{CO}_3 \rightarrow \text{H}_2\text{O} + \text{CO}_2$ predominates, in alkaline solutions the dehydration of HCO_3^- prevails. In acid fluids dehydration is practically complete; between p_{H} 5 and 8 CO_2 and carbonic acid can be present; in alkaline ranges hydration is nearly complete.

The time for 90 % establishment of equilibrium in strongly acid solutions is nearly independent of p_{H} ; at 0° this time is 1 sec., at 18° $\frac{1}{2}$ sec. At $p_{\text{H}} = \pm 8$, however, this time is maximal, at 0° about 700 sec., at 18° about 80 sec.; from p_{H} 9 the time decreases regularly. These times have been calculated for infinite dilution and must be modified for the presence of electrolyte concentrations. Faurholt gives a probable error of 50 %.

For equilibrium conditions he finds:

Although the value of the data given by Faurholt is certainly important for biological reactions, many of them are only approximate; especially in the physiological range of p_H and temperature many questions remain unsolved. In order to make an accurate estimation of the important true dissociation-constant of carbonic acid and the types and velocities of its dehydration under physiological conditions, we have directly recorded with a lag of $< \frac{3}{25}$ sec. the changes of p_H with an accuracy $> 0.005 p_H$ of a bicarbonate system, after quickly mixing it with acid.

METHOD.

It will be clear that a method intended not only to measure but also to record p_H continuously cannot be a compensation method with zero instrument, but must be suited to record the potential differences between the unknown electrodes and constant electrodes without any appreciable polarisation. Therefore we adapted a method similar to that of Goode [1922] and followed the changes of p_H accurately and with a time lag as short as possible by connecting a suitable type of hydrogen electrode system, compensated by a potentiometer circuit, with the grid of a common triode valve and recorded the changes of the (compensated) plate current with a suitable recording galvanometer. The method is illustrated by the diagram of Fig. 1.

Fig. 1.

The filament of a Philips B 406 valve (9) is heated by a 4 v. accumulator (1) through the circuit (2) and the resistance (3). The plate current flows through the anode battery (8), the circuit (5) and the galvanometer (6), and can be compensated by the shunt with resistance (4). In this scheme the compensating potential is taken from the heating potential, so that a large plate potential will take too much energy from the heating cells for compensation. Therefore the maximum plate potential in this scheme can only be 30 v., and the sensitivity of the galvanometer was about 4 mm. for 1 mv. tension on the grid. This sensitivity may be increased about tenfold by removing the shunt (4), bringing the plate potential to 80 v. and compensating the plate current by a separate 2 v. accumulator, with resistance (7). In such a system it is sometimes necessary to shunt a large capacity condenser across the anode battery.

The E.M.F. of the measured system is connected between the grid and the negative pole of the heating battery (circuit 10), and can be compensated and measured by the potentiometer (11).

As experimental electrodes we used the quinhydrone system with bright Pt electrodes, the reaction time of which is as short as possible. As second electrode we used a saturated calomel electrode, or an Ag—AgCl—KCl electrode, or a narrow tube with phosphate solution (equal parts of 1/15*N* KH_2PO_4 and $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$), quinhydrone and a long bright Pt wire which reaches to the cotton plug by which the bicarbonate and phosphate solutions are separated. This second electrode may be cemented to the moving electrode to ensure constant distance between the two, but this proved to be unnecessary. It is important that the resistance of the electrodes is small, in order to prevent the influence of induction potentials on the measured E.M.F. If this precaution is taken and if all parts of the apparatus which can come into contact with the grid circuit have been carefully insulated, the recording of the E.M.F. of the system under study is absolutely undisturbed.

An essential part of this research was the completeness of mixing of the reacting fluids in as short a time as possible. The following method was found to be the best.

The NaHCO_3 solution (100 cc. in a Dewar flask, diameter 3 cm., height 14 cm.) is stirred continuously by the Pt electrode itself which is a disc of 2 cm. diameter and moves up and down in the solution over a distance of 6 cm. twenty times a second. The connecting parts of this electrode are insulated with De Khotinsky cement, so that only the disc remains uncovered. The moving electrode is connected to the potentiometer circuit by very flexible wire and all contacts are soldered. The diluted HCl (0.95 cc.) is injected into the bicarbonate solution by means of an automatic 1 cc. syringe with strong springs; attached to the nozzle of the syringe is a straight glass tube of 0.3 cm. diameter which reaches half-way down the Dewar vessel. Through this tube the acid is injected automatically in less than 0.1 sec.

The variation in distance between the moving Pt electrode and the second constant electrode causes no deflection of the galvanometer if the resistance of these electrodes is not too high.

It might be supposed that the change of E.M.F. which follows upon the injection of HCl into the bicarbonate solution is initially caused by a temporary local concentration of free HCl at the electrode. If, however, the velocity of the mixing electrode is high enough (> ten movements per sec.) this possibility seems excluded by the following experiments:

(a) If the same amount of 0.01*N* HCl is injected into 100 cc. of another equimolar buffer solution (acetate-phosphate) a temporary rise of c_{H} is not noticed, but the E.M.F. is raised to its end-point in a time which is no greater than the lag of the recording system ($< \frac{3}{25}$ sec.) (mixing time and reaction time of the quinhydrone electrode).

(b) The temporary rise in the curve indicating the c_{H} of the bicarbonate solution (H_2CO_3 peak) does not occur in any $\text{HCO}_3' - \text{H}_2\text{CO}_3$ system in which

HCl is injected, but only in those systems, in which the p_H is less than 7.5¹. If this peak were caused by free HCl, we can find no reason why it should present itself only on the acid side of p_H 7.5. Moreover, if one causes such a peak by injecting HCl on a slowly moving electrode, the decrease of the curve is as rapid as the increase, while the decrease of the H_2CO_3 peak is much more gradual and corresponds exactly to the deduced velocity of dehydration.

Determination of the true dissociation-constant of H_2CO_3 .

In order to measure the true dissociation-constant of H_2CO_3 we recorded the p_H of 100 cc. 0.02N $NaHCO_3$, saturated with CO_2 , into which 0.95 cc. 0.01N HCl was quickly injected and mixed (see fig. 2 and Table I). The formation of H_2CO_3 causes here a rapid rise of $[H^+]$, which is followed at once by a slower decrease, owing to the dehydration process $H_2CO_3 \rightarrow H_2O + CO_2$. Our object now was to extrapolate from this registered curve of E.M.F. the initial c_H of the solution, as if no dehydration had occurred.

Fig. 2.

To do this, we may suppose the dehydration to be a monomolecular process at this reaction (p_H 5–6), which finally reaches a dynamic equilibrium with the inverse hydration of CO_2 .

Then the velocity of this reaction would be described by

$$-\frac{d[H_2CO_3]}{dt} = k_1[H_2CO_3] - k_2[CO_2];$$

but

$$[H_2CO_3] = \frac{[HCO_3^-] \cdot [H^+]}{K}$$

(K = the true dissociation-constant of H_2CO_3),

$$\text{therefore} \quad -\frac{[HCO_3^-]}{K} \cdot \frac{d[H^+]}{dt} = k_1 \frac{[HCO_3^-]}{K} \cdot [H^+] - k_2[CO_2].$$

$[HCO_3^-]$ is practically constant, as the small change in its concentration caused by the dehydration of H_2CO_3 may be neglected in regard to the total amount of $NaHCO_3$ in solution. $[CO_2]$ is also a constant as the solution is continuously kept saturated with the gas. So we have:

$$-\frac{d[H^+]}{dt} = k_1 \cdot [H^+] - k_2',$$

or by integration $[H^+] = C \cdot e^{-k_1 t} + D$, in which C and D are new constants; where D may be determined by the c_H at equilibrium, i.e. after infinite time.

¹ The influence of the reaction on the type of buffering of a bicarbonate solution will be described in the next paper of this series.

In this way we get $[H^+] = C \cdot e^{-k_1 t} + [H^+]_{\infty}$, or

$$\log ([H^+] - [H^+]_{\infty}) = -k_1' t + \log C \quad (k_1' = 0.434 \times k_1).$$

The experimental values of $\log ([H^+] - [H^+]_{\infty})$ should be a linear function of the time. All curves calculated in this way showed a convexity towards the plane axis, indicating that k_1 is not a constant but decreases during the reaction, which consequently cannot be monomolecular.

It was now natural to suppose that the hydrogen ions have a catalytic influence on this dehydration, as they have in many hydrations and dehydrations (formations of lactones and the inverse reactions, inversion of sucrose, etc.). Pusch [1916] made the same assumption for the hydration of CO_2 .

In this case the equation becomes

$$-\frac{d[H_2CO_3]}{dt} = k_1 [H_2CO_3] [H^+] - k_2 [CO_2] \cdot [H^+];$$

taking $[H_2CO_3] = \frac{[HCO_3^-] \cdot [H^+]}{K}$

(K = the true dissociation-constant of H_2CO_3),

we get $-\frac{d[H^+]}{dt} = k_1 [H^+]^2 - k_2 \cdot [CO_2] \cdot \frac{K}{[HCO_3^-]} \cdot [H^+].$

Let $k_2 \cdot [CO_2] \cdot \frac{K}{[HCO_3^-]} = k_3,$

then $-\frac{d[H^+]}{dt} = k_1 [H^+]^2 - k_3 [H^+].$

By integration $\ln \frac{k_1 [H^+] - k_3}{[H^+]} = -k_3 t + C$ in which C is an integration constant.

If $\frac{k_1}{k_3} = \alpha$ and $\frac{1}{[H^+]} = p$ then $\ln (\alpha - p) = -k_3 t + C. \dots (A)$

If α is known, $\log (\alpha - p)$ may be plotted as a function of time. α was calculated in the following way.

Substituting the determined values of p for equal intervals of time, then

$$\frac{\alpha - p_n}{\alpha - p_{n+1}} = \frac{\alpha - p_{n+1}}{\alpha - p_{n+2}} = \dots = e^{-k_3(t_n - t_{n+1})} = \beta,$$

as $t_n - t_{n+1} = t_{n+1} - t_{n+2} = \dots$

Thus $\alpha - p_n = \beta (\alpha - p_{n+1})$ or $p_n = \beta p_{n+1} + \alpha (1 - \beta).$

By taking p_n as a function of p_{n+1} , the formula must represent a straight line; β in this formula is the tangent of the angle with a plane axis of the constructed p_n/p_{n+1} line, which actually appears to be a straight line (Fig. 3). Thus β can be determined graphically; $\alpha (1 - \beta)$ is the portion which is cut from the vertical zero line. From the graph can be seen the portion which is cut from the plane axis of $p_{n+1} = 5.5$; from this portion $\alpha (1 - \beta)$ can be determined, and as β is known, α may be calculated.

This method of calculating α has the advantage that all observations on the experimental p_H curve may be used; this would not have been possible if α had been calculated from the limit-condition of the integral. The calculation of α in this way and its substitution in equation (A) gives in fact a very good straight line (Fig. 4). This forms a strong argument for our supposition that hydration and dehydration of carbonic acid are proportional to the $[H^+]$ of the system.

Fig. 3.

Fig. 4.

We are now able to extrapolate the $[H^+]$ of the bicarbonate solution immediately after injection of the HCl. As an example we take the curve reproduced in Fig. 2 which had been obtained by injecting 0.95 cc. of 0.01N HCl into 100 cc. 0.02N $NaHCO_3$ at 14° . The values calculated from this curve are combined in Table I.

Table I.

t.	cm.	Δ mv.	p_H^*	$p = \left(\frac{1}{[H^+]} \right) \cdot 10^5$	$(a - p) \cdot 10^5$	$10^5 \log(a - p)$
4	4.17	8.45	5.762	5.78	2.22	5.346
5	3.35	6.79	5.791	6.18	1.82	5.260
6	2.69	5.45	5.814	6.52	1.48	5.170
7	2.20	4.46	5.832	6.79	1.21	5.083
8	1.83	3.71	5.845	7.00	1.00	5.000
9	1.53	3.10	5.856	7.18	0.82	4.914
10	1.27	2.57	5.865	7.33	0.67	4.826
11	1.06	2.15	5.872	7.45	0.55	4.740
12	0.91	1.84	5.878	7.55	0.45	4.653
13	0.76	1.54	5.883	7.64	0.36	4.556

Calibration of the curve 10 mv. = 4.94 cm.

Initial p_H before addition of HCl = 5.91.

Graphical determination of $\beta = 1.221$.

Graphical determination of $\alpha = 8.00 \times 10^5$.

$k_3 = 2.16$ (Brigg. log., time in sec.)†.

So $k_1 = \alpha \times k_3 = 8.00 \times 10^5 \times 2.16 = 1.73 \times 10^6$.

Found by extrapolation $\log(a - p_0) = 5.68$ (see Fig. 4).

$p_0 = 3.16 \times 10^5$.

$H_0 = 3.16 \times 10^{-6}$.

* The absolute strength of these p_H values has not been determined in three decimals, but the relative differences in p_H , which are the only important values for the determination of α and β , may be given in three decimals.

† k_3 is, according to equation (A), equal to the tangent of the angle which the straight line of Fig. 4 makes with the plane axis.

The true dissociation-constant K was calculated as follows.

Concentration of H before injection of HCl	= H_1
„ „ H immediately after the injection	= H_0
„ „ H_2CO_3 before injection	= c_1
„ „ HCO_3^- before injection	= c_2
„ „ HCl (diluted to 100 cc.)	= c_3

After the injection of HCl the concentration of H_2CO_3 must be $c_1 + c_3$ and of $\text{HCO}_3^- = c_2 - c_3$.

Before injection we have $c_1 \times K = H_1 \times c_2$,
and after $(c_1 + c_3) \times K = H_0 \times (c_2 - c_3)$,

$$\text{so } K = \frac{H_0 \times (c_2 - c_3) - H_1 \times c_2}{c_3}.$$

In our example we had $H_1 = 1.23 \times 10^{-6}$, $H_0 = 3.16 \times 10^{-6}$, $c_2 = 2 \times 10^{-2}$, $c_3 = 9.5 \times 10^{-5}$, so K at $14^\circ = 4.0 \times 10^{-4}$.

We have made twelve similar experiments at temperatures between 14° and 18° and always found values between $K = 1.5$ and 4×10^{-4} . Researches in which the constancy of temperature is considered, and determinations at 37° are in progress.

The graphical determination of β gave a k_3 of 2.16; as $\alpha = \frac{k_1}{k_3}$ we find
 $k_1 = 8.00 \times 10^{-5} \times 2.16 = 1.73 \times 10^{-6}$.

Thus at $p_H = 6$, the dehydration constant of $\text{H}_2\text{CO}_3 = 1.73$, which indicates that the time for semi-completion of the dehydration is 0.17 sec. at 14° .

If it is permissible to extrapolate this value for a physiological p_H of 7.35, we get for semi-completion about 4 sec. So it seems quite probable that the existence of H_2CO_3 in a bicarbonate solution may occur for a biologically important time.

Hydration of CO_2 at p_H 7 seems to be a very slow reaction (cf. Collingwood [1924]).

The relation of the constants of hydration and dehydration is given by the equilibrium constant of $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3$:

$$\frac{k_{\text{hydration}}}{k_{\text{dehydration}}} = K_{\text{equilibrium}} = \frac{[\text{H}_2\text{CO}_3]}{[\text{CO}_2]} = (\text{approx.}) \frac{\text{apparent diss.-const. of carbonic acid}}{\text{true diss.-const. of carbonic acid}} = \frac{3 \cdot 10^{-7}}{4 \cdot 10^{-4}} = 7.5 \times 10^{-4}.$$

Another way of calculating the equilibrium constant is the following. According to Strohecker there is in a certain solution of carbon dioxide 99.44 % present in the form of CO_2 and 0.05 % as undissociated H_2CO_3 ; this gives for the equilibrium constant $\frac{0.05}{99.44} = 5 \times 10^{-4}$, which agrees fairly well with the calculation from the true and apparent dissociation-constant, but not with Faurholt's value. If we calculate the hydration constant of CO_2 from the relation

$$\text{hydration constant} = 7.5 \times 10^{-4} \times \text{dehydration constant},$$

we get at p_H 7.35

$$k_{\text{hydration}} = 7.5 \times 10^{-4} \cdot k_{\text{dehydration}} = 5.85 \times 10^{-5},$$

and the time for semi-completion of hydration is $\frac{0.301}{5.85 \times 10^{-5}} = 5.2 \times 10^3$ sec.

or about $1\frac{1}{2}$ hours. Moreover, the inverse reaction is retarding hydration, but it might be possible that the reaction $\text{CO}_2 + \text{OH}^- \rightarrow \text{HCO}_3^-$ is coming into play at this p_H .

The true dissociation-constant of H_2CO_3 being 4×10^{-4} , we may now estimate the buffer value of the bicarbonate solution, immediately after the addition of the acid.

Van Slyke's formula for molar buffering capacity is

$$\frac{dB}{cdp_{\text{H}}} = 2.3 \frac{k \cdot [\text{H}]}{(k + [\text{H}])^2}.$$

So the buffering capacity of the bicarbonate system in final equilibrium at p_{H} 7 is

$$2.3 \frac{3 \cdot 10^{-7} \cdot 10^{-7}}{16 \cdot 10^{-14}} = 0.43.$$

Immediately after addition of an acid, when the system is reacting according to its true constant, we get $2.3 \frac{4 \cdot 10^{-4} \cdot 10^{-7}}{(4 \cdot 10^{-4} + 10^{-7})^2} = 5.8 \times 10^{-3}$, a very small buffer value.

Also the second dissociation-constant (6×10^{-11}) gives a very small buffering capacity, viz. 13.8×10^{-4} . It must be concluded, therefore, that immediately after addition of an acid the bicarbonate system cannot exert buffering action at p_{H} 7.

Preliminary experiments with serum and blood indicate that the presence of free H_2CO_3 after addition of HCl cannot be demonstrated at all. Also there is quite another cause of changes occurring before attainment of equilibrium when HCl is added to ultrafiltrate. These problems will be dealt with in the next paper of this series.

SUMMARY.

1. The change in $[\text{H}^+]$ in a bicarbonate solution to which strong acid was added was studied by means of continuous observation of the p_{H} of the system with an accuracy of $0.005 p_{\text{H}}$ and a time lag not exceeding $\frac{3}{25}$ sec.
2. The true dissociation-constant of H_2CO_3 and the hydration and dehydration velocities were determined; they appeared to have biologically important values.
3. The velocity of dehydration was shown to be proportional to the $[\text{H}^+]$ of the system.
4. At p_{H} about 7 the bicarbonate system has practically no immediate buffering capacity.

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