Facilitated Diffusion of CO₂ across Albumin Solutions

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ABSTRACT The steady-state CO₂ flux across thin layers of 30 g/100 ml albumin solutions was measured in two different CO₂ partial pressure ranges (boundary Pco₂ values 3 and 8 torr, and 160 and 650 torr, respectively). From the data the apparent diffusion coefficient for CO₂, Dco₂, was calculated. In the high Pco₂ range a value of Dco₂ was found which is to be expected on the basis of diffusion of dissolved CO₂ only. In the low Pco₂ range Dco₂ was about 100% higher than in the high Pco₂ range, when carbonic anhydrase was present and the pH was 7.7. Dco₂ depended on the concentration of carbonic anhydrase. It increased with increasing pH. It is concluded that an additional diffusion of bound CO₂ (facilitated CO₂ diffusion) occurs in the low Pco₂ range and that this transport involves the hydration of CO₂. From the diffusion coefficients in the two Pco₂ ranges the rate of facilitated diffusion was determined. Approximate calculations show that this rate (at pH ≤ 7.7) can be explained on the basis of the proposed mechanism of facilitated CO₂ diffusion: bicarbonate diffusion and simultaneous proton transport by albumin diffusion. The view that facilitated CO₂ diffusion is mediated by the diffusion of albumin is supported by the experimental finding of a considerable suppression of the facilitated CO₂ flux in the presence of gelatinized agar-agar.

INTRODUCTION

In 1966, Longmuir et al. showed that the diffusion constant of CO₂ in hemo-lyzed blood may be twice the diffusion constant in water and concluded that facilitated diffusion of CO₂ occurs in addition to free diffusion. Enns et al. (1966) and Moll and Gros (1966) demonstrated facilitated diffusion of CO₂ in layers of red cells. Gros (1969) found evidence for facilitated diffusion of CO₂ in highly concentrated hemoglobin solutions free of other buffers.

The molecular mechanism of facilitated CO₂ diffusion in protein solutions has not been fully elucidated so far. Gros (1969) and Gros and Moll (1972) found that facilitated CO₂ diffusion in hemoglobin solutions is suppressed by addition of acetazolamide. It was concluded from this that facilitated CO₂ diffusion in hemoglobin solutions is based on bicarbonate diffusion and simul-
taneous proton transport. This mechanism is illustrated in Fig. 1. On the basis of this concept a flux of hydrogen ions had to be postulated, which was more than 1,000 times greater than the estimated flux of free protons under the pertinent conditions (Gros and Moll, 1972). Thus, a powerful mechanism of proton transport seems to act in hemoglobin solutions. Since this mechanism may be of general physiological significance in intracellular proton transfer processes, we were especially interested in the physicochemical basis of this H⁺ flux.

Part of the proton flux, postulated from experimental data obtained in hemoglobin solutions, could be explained by a hemoglobin-facilitated proton diffusion, i.e. by diffusion of buffered protons. Another part of the proton flux, however, remained unexplained. It was speculated that rotary diffusion of hemoglobin molecules may account for this part of H⁺ transport.

For the analysis of the facilitated diffusion of CO₂ and the linked proton transport in protein solutions it may be useful to study the CO₂ transport in albumin solutions. The rate of translational diffusion of albumin and of hemoglobin is similar at the same protein concentrations (Polson, 1939; Keller et al., 1971); therefore, the proton transport by translational diffusion of the protein molecules should be similar. On the other hand, the rotation velocity of the albumin molecule, which has a spindle shape, should be lower than that of the spheroid hemoglobin molecule, especially at high protein concentrations. Accordingly, a better agreement between the observed CO₂ fluxes and the CO₂ fluxes to be postulated on the basis of protein diffusion should be expected in albumin solutions.

It is the aim of this paper to answer the following questions on facilitated CO₂ diffusion: (a) Does it occur in albumin solutions? (b) If so, can it be ex-
explained on the basis of HCO$_3^-$ diffusion and simultaneous proton transport by (translational) diffusion of albumin?

To prove qualitatively facilitated diffusion (a) we investigated whether the CO$_2$ diffusion coefficient depends on the CO$_2$ partial pressure range, the carbonic anhydrase activity, and the pH value of the solution. For a specific approach to the role of albumin diffusion (b) the effect of agar-agar on the CO$_2$ flux across albumin solutions was studied. Gelatinized agar-agar forms a network whose pores may be in part too small for the penetration of macro-molecules but large enough for the penetration of smaller molecules such as CO$_2$ or HCO$_3^-$. Indeed, Schantz and Lauffer (1962) found that the diffusion of bovine serum albumin was more restricted by agar-agar than the diffusion of KCl. The size of the effect of agar-agar on the rate of facilitated diffusion should, therefore, indicate if facilitated diffusion is based on the diffusion of small molecules only or if protein diffusion is involved. To answer quantitatively the second question we evaluated the facilitated CO$_2$ diffusion to be expected on the basis of the protein diffusion and compared the result with the experimental data.

METHODS

Principle

Albumin solutions were soaked into filters with a thickness of 160–180 μm (Millipore filters, Millipore Corp., Bedford, Mass.) or placed between silicone membranes stretched out at a distance of 600 μm. These layers were put as separating diaphragms between two chambers where gases with different CO$_2$ partial pressures were passed through. The concentrations of the gases leaving the chambers were determined after steady-state conditions were reached. From the CO$_2$ concentrations and flow rates of the gases the CO$_2$ partial pressure difference and the CO$_2$ transfer across the layer were calculated. Using the solubilities of CO$_2$ in the solutions (see p. 359) the CO$_2$ partial pressure difference was converted into the concentration difference of dissolved CO$_2$ across the layer. These data made it possible to compute the apparent CO$_2$ diffusion coefficient, i.e. the CO$_2$ transfer per concentration gradient of dissolved CO$_2$ and unit area.

Arrangement

We used essentially the same arrangement for the measurements as in a previous paper (Gros and Moll, 1971). The volumetric gas analysis was replaced by gas chromatography.

Materials

Albumin solutions were prepared from pure bovine serum albumin (Serva Company, Heidelberg) and saline. The sum of the molar concentrations of Na$^+$, K$^+$, and Cl$^-$ was adjusted to 0.26 mol/liter. The ionic strength of all solutions, therefore, was 0.13 mol/liter, plus the ionic strength caused by bicarbonate and protein. The pH value of the
solutions was adjusted by 0.1 N NaOH or 0.1 N HCl. The protein concentration was adjusted in most solutions to 30 g/100 ml.

Commercial bovine carbonic anhydrase (Serva) was added to part of the solutions (the activity was 11 at a concentration of 1 mg/liter according to the suppliers). In order to prevent inactivation of the enzyme by heavy metal ions EDTA (0.4 mmol/liter) was added.

For the inhibition of carbonic anhydrase Diamox (Lederle Company, Munich) was used in a concentration of 0.1 g/100 ml. According to Maren (1967) this should be sufficient for a >99.999 % inhibition of a carbonic anhydrase concentration of 0.1 g/100 ml.

In order to prepare layers of 11 g/100 ml albumin solution in gelatinized agar-agar 3 g agar-agar (Serva) were dissolved in 100 ml boiling saline, cooled to 45°C and mixed with an equal volume of 22 g/100 ml albumin solution of the same temperature. The warm solution was filled in between two silicone membranes which were stretched out at a distance of 600 μm. The solution gelatinized when cooled to room temperature. The pH value of the original 22 g/100 ml albumin solution was 7.3 at Pco2 = 40 torr.

Analytical Procedures

Na+ and K+ were determined by flamephotometry, Cl− by coulometric titration. The protein concentration was obtained from the dry weight minus the weight of the salts. The CO2 concentrations were determined in a gaschromatograph (carrier gas: helium 40 ml/min, column: Porapak, OD ¼ inch, length 126 cm).

Calibration of the Filters

The CO2 diffusion rate across layers of water soaked into filters was only 65 % of the value determined in a filter-free layer of equal dimensions. This seems to be due to the solid part of the filter, which reduces the cross-sectional area by 20 %, and to the windings of the channels in the filter, which extend the diffusion path (Gros and Moll, 1971). We corrected for these effects by multiplying the filter area by 0.8 and the filter thickness by 1.23.

Solubility Coefficient of CO2

In order to calculate CO2 diffusion coefficients from the measurements, it was necessary to calculate concentrations of dissolved CO2 in the investigated solutions from the CO2 partial pressures (see p. 358). The solubility coefficient of CO2, α, in water at 22°C is 0.829 atm⁻¹ (Handbook of Chemistry and Physics, 1954-55, p. 1608). However, no data are available describing the effect of albumin and of agar-agar on the solubility of CO2. We assumed that the effect of 1 g albumin or agar-agar per liter solution on the value of α is the same as that of 1 g hemoglobin/liter solution as described by Van Slyke et al. (1928). Accordingly, the calculation of the CO2 diffusion coefficients is based on the following values for α: 0.66 atm⁻¹ for 30 g/100 ml albumin solution, 0.76 atm⁻¹ for 11 g/100 ml albumin solution, 0.82 atm⁻¹ for 1.5 g/100 ml agar-agar, and 0.75 atm⁻¹ for 11 g/100 ml albumin in 1.5 g/100 ml agar-agar.
RESULTS

CO₂ Diffusion Coefficient in a High CO₂ Partial Pressure Range (Boundary CO₂ Partial Pressures 160 and 650 Torr)

The CO₂ diffusion coefficient in 30 g/100 ml albumin solutions was measured in the high CO₂ partial pressure range with and without carbonic anhydrase, with and without acetazolamide, and at various pH values (5.9, 7.2, 7.3, 8.0). The temperature was 22°C. The measured diffusion coefficients are nearly the same under all these conditions (see Table I). The values range from 7.3 to 8.1 \( \times 10^{-6} \) cm²s⁻¹. The average of all values is 7.6 \( \times 10^{-6} \) cm²s⁻¹.

TABLE I
DIFFUSION COEFFICIENTS OF CO₂ IN THE HIGH PARTIAL PRESSURE RANGE

| CO₂ diffusion coefficient \( \times 10^{-6} \) \( \text{cm}^2 \text{s}^{-1} \) | pH | Carbonic anhydrase | 0.1 g/100 ml | 0.1 g/100 ml |
|-----------------------------|----|--------------------|--------------|--------------|
| 7.3 \( \times 10^{-6} \) (±1.4%) | 7.2 | --                 | --           | --           |
| 7.3 \( \times 10^{-6} \) (±0.7%) | 7.2 | 0.1                | --           | --           |
| 7.4 \( \times 10^{-6} \) (±1.0%) | 7.2 | --                 | 0.1          | --           |
| 8.1 \( \times 10^{-6} \) (±1.9%) | 5.9 | 0.1                | --           | --           |
| 7.9 \( \times 10^{-6} \) (±1.6%) | 7.3 | 0.1                | --           | --           |
| 7.4 \( \times 10^{-6} \) (±2.4%) | 8.0 | 0.1                | --           | --           |

CO₂ diffusion coefficients (with standard error as percentage of the mean) in 30 g/100 ml albumin solutions when the boundary CO₂ partial pressures were about 160 and 650 torr. Temperature 22°C. The layers were prepared from Millipore filters, the given pH values refer to a Pco₂ of 40 torr.

CO₂ Diffusion Coefficient in a Low CO₂ Partial Pressure Range (Boundary CO₂ Partial Pressures About 3 and 8 Torr)

Table II shows the apparent CO₂ diffusion coefficients in 30 g/100 ml albumin solutions measured at 22°C in the low partial pressure range. All values obtained in this low partial pressure range are significantly higher than those measured in the high partial pressure range.

The diffusion coefficient increases after addition of carbonic anhydrase. Table II shows this to be true in all examined pH ranges. In the solution which has a pH = 7.2 at Pco₂ = 40 torr the diffusion coefficient reaches nearly twice the value in the high partial pressure range when 0.1 g/100 ml carbonic anhydrase is present. A further increase of the carbonic anhydrase concentration to 0.2 g/100 ml led in none of the examined pH ranges to a further increase of the diffusion coefficient.

The diffusion coefficient in the low partial pressure range is shown to depend
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on the pH of the solution (Table II). When the pH is decreased the CO₂ diffusion coefficient drops; when the pH is increased the diffusion coefficient rises. The diffusion coefficient amounts to about six times its value in the high partial pressure range when the pH of the albumin solution is 8.0 at Pco₂ = 40 torr.

**Effect of Gelatinized Agar-Agar on the CO₂ Diffusion Coefficient**

In order to examine the possible role of protein diffusion in facilitated CO₂ diffusion the CO₂ transfer in 11 g/100 ml albumin solutions was measured in

| CO₂ diffusion coefficient | Facilitated diffusion (as fraction of free diffusion) | pH | Carbonic anhydrase | Boundary CO₂ partial pressures |
|---------------------------|-----------------------------------------------------|----|--------------------|-------------------------------|
| 10.0 \( \cdot 10^{-6} \) (±1.8%) | 0.4 | 7.2 | — | 7.5 / 2.7 |
| 14.2 \( \cdot 10^{-6} \) (±2.0%) | 0.9 | 7.2 | 0.1 | 7.5 / 2.9 |
| 9.0 \( \cdot 10^{-6} \) (±1.1%) | 0.1 | 5.9 | — | 7.6 / 2.5 |
| 10.0 \( \cdot 10^{-6} \) (±1.3%) | 0.2 | 5.9 | 0.1 | 7.5 / 2.7 |
| 11.4 \( \cdot 10^{-6} \) (±1.8%) | 0.4 | 7.3 | — | 7.7 / 2.8 |
| 15.4 \( \cdot 10^{-6} \) (±2.1%) | 0.9 | 7.3 | 0.1 | 7.3 / 4.2 |
| 15.4 \( \cdot 10^{-6} \) (±4.6%) | 0.9 | 7.3 | 0.2 | 7.3 / 4.2 |
| 14.3 \( \cdot 10^{-6} \) (±1.8%) | 0.9 | 8.0 | — | 7.6 / 3.2 |
| 48 \( \cdot 10^{-6} \) (±3.3%) | 5.5 | 8.0 | 0.1 | 7.1 / 5.1 |
| 47 \( \cdot 10^{-6} \) (±2.9%) | 5.4 | 8.0 | 0.2 | 7.1 / 5.1 |

CO₂ diffusion coefficients (with standard error as percentage of the mean) in 30 g/100 ml albumin solutions when the boundary CO₂ partial pressures were between 3 and 8 torr. Temperature 22°C. The layers were prepared from Millipore filters. The given pH values refer to a Pco₂ of 40 torr. Facilitated diffusion is expressed as \((D_f - D_h)/D_h\) where \(D_h\) means the diffusion coefficient in the high partial pressure range, \(D_f\) the diffusion coefficient in the low partial pressure range.

presence and absence of 1.5 g/100 ml agar-agar. The data obtained from 600-μm thick layers are compiled in Table III. It can be seen that agar-agar has a small effect on the CO₂ diffusion coefficient in water: in both partial pressure ranges it is reduced by about 6%. Similarly, there is an effect of only 6% on the CO₂ diffusion coefficient in albumin solutions in the high partial pressure range. However, there is a large effect in the low partial pressure range: the CO₂ diffusion coefficient in albumin solutions containing carbonic anhydrase is reduced to 60% of its previous value in the presence of gelatinized agar-agar.
TABLE III

EFFECT OF AGAR-AGAR ON THE CO₂ DIFFUSION COEFFICIENT

| Material                  | CO₂ diffusion coefficient (140 torr/650 torr) | CO₂ diffusion coefficient (2 torr/8 torr) | Facilitated diffusion (as fraction of free diffusion) |
|---------------------------|---------------------------------------------|----------------------------------------|-----------------------------------------------------|
| Water                     | 17.2 \( \times \) \( 10^{-6} \) (±4.0%)    | 17.5 \( \times \) \( 10^{-6} \) (±2.2%)  | 0.0                                                 |
| 1.5 g/100 ml agar-agar     | 16.3 \( \times \) \( 10^{-6} \) (±1.5%)    | 16.5 \( \times \) \( 10^{-6} \) (±2.2%)  | 0.0                                                 |
| 11 g/100 ml albumin        | 12.7 \( \times \) \( 10^{-6} \) (±4.1%)    | 33.8 \( \times \) \( 10^{-6} \) (±1.8%)  | 1.7                                                 |
| 11 g/100 ml albumin in     | 12.0 \( \times \) \( 10^{-6} \) (±2.0%)    | 20.7 \( \times \) \( 10^{-6} \) (±2.1%)  | 0.7                                                 |
| 1.5 g/100 ml agar-agar     |                                            |                                        |                                                     |

CO₂ diffusion coefficients in \( \text{cm}^2 \text{s}^{-1} \) (with standard error as percentage of the mean) in 600-\( \mu \text{m} \) thick filter-free layers of water and 11 g/100 ml albumin solutions, with and without 1.5 g/100 ml gelatinized agar-agar. All albumin solutions contained 0.1 g/100 ml carbonic anhydrase. Diffusion coefficients were determined in a high and a low partial pressure range. Temperature 22°C. Facilitated diffusion is expressed as \( (D_f - D_0)/D_0 \) (see Table II).

DISCUSSION

Lack of Evidence for Facilitated Diffusion of CO₂ in the High Partial Pressure Range

The diffusion coefficient of CO₂ in 30 g/100 ml albumin solutions is found to be 7.6 \( \times \) \( 10^{-6} \) cm² s⁻¹, when the boundary CO₂ partial pressures are 160 and 650 torr. This value is 44% the value in water (Table III) and nicely fits the value to be expected on the basis of the CO₂ diffusion coefficient in water and the depressive effect of proteins on the diffusion of CO₂ (see Gros and Moll, 1971). The diffusion coefficient is not affected by the addition of carbonic anhydrase nor by a change of pH. The effect of agar-agar on the CO₂ diffusion coefficient is as small as the effect of agar-agar on the diffusion of CO₂ in water, where only free diffusion of CO₂ occurs, and similar to the effect on the diffusion of other small molecules (Schantz and Lauffer, 1962). All these findings strongly indicate that no measurable facilitated diffusion occurs, i.e. that the diffusion coefficient found in this CO₂ partial pressure range describes the diffusion of dissolved CO₂ only.

This conclusion is important for the following considerations: The diffusion coefficient of dissolved CO₂ being known, the diffusion rate of dissolved CO₂ occurring in the low partial pressure range can be estimated. Subtracting this value from the measured total diffusion rate in the low partial pressure range, the rate of facilitated CO₂ diffusion is obtained.

Evidence for Facilitated Diffusion of CO₂ in the Low Partial Pressure Range

The diffusion coefficient of CO₂ in the low partial pressure range differs in the following respects from the value obtained in the high partial pressure range:
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(a) It is higher than in the high partial pressure range; (b) it depends on the pH value; (c) it depends on carbonic anhydrase concentration; (d) it is decreased in the presence of agar-agar to a far higher degree than is expected on the basis of the effect of agar-agar on the free diffusion on CO$_2$. All these findings are not compatible with the well-known behavior of the diffusion of dissolved gases. It must be concluded that besides free diffusion of CO$_2$, facilitated transport of CO$_2$ occurs in the low partial pressure range.

**Mechanism of Facilitated Diffusion of CO$_2$ in Albumin Solution**

In Fig. 2 the concept of facilitated CO$_2$ diffusion by simultaneous translational diffusion of HCO$_3^-$ and albumin is illustrated. According to this concept HCO$_3^-$ and H$^+$ are produced by hydration of CO$_2$ at the side of high P$_{CO_2}$.

The protons are buffered by albumin. Proton-loaded albumin diffuses with HCO$_3^-$ to the other side of the layer where HCO$_3^-$ and H$^+$ recombine and yield CO$_2$. Unloaded albumin diffuses in the reverse direction to pick up new protons. In other words, albumin facilitates the diffusion of H$^+$. It is a purpose of the present paper to get evidence if this mechanism is the main basis of the facilitated CO$_2$ diffusion in albumin solutions.

**QUALITATIVE CONSIDERATIONS** From a qualitative point of view the experimental observations a–d (see above) fit the characteristics of this concept: (1) The proposed concept implies that facilitated CO$_2$ diffusion depends on the velocity of the hydration of CO$_2$, we observed that the CO$_2$ diffusion rate depends on the carbonic anhydrase concentration (c). (2) According to the above concept the facilitated CO$_2$ diffusion is related to the gradient of buffered protons which is high in the low CO$_2$ partial pressure range and rises with the pH, we observed an increased diffusion rate of CO$_2$.
in the low partial pressure range (a) and found that the diffusion rate rises further with the pH (b).

(3) The proposed mechanism is based on albumin diffusion, we observed that the diffusion rate of CO$_2$ is markedly decreased when the albumin diffusion is restricted by agar-agar (d).

The dependency of facilitated CO$_2$ diffusion on the CO$_2$ hydration velocity and on the CO$_2$ partial pressure range has been verified experimentally in all examined pH ranges. However, its dependency on the diffusivity of albumin has been verified at pH close to 7.2 at P$_{CO_2}$ = 40 torr (see p. 359) only. Thus, we may conclude that, for albumin solutions of pH $\approx$7.2 at P$_{CO_2}$ = 40 torr, all mentioned criteria of the mechanism proposed in Fig. 2 are qualitatively fulfilled by the experimental data.

### QUANTITATIVE CONSIDERATIONS

In the following it shall be shown that the experimental data obtained in albumin solutions of pH = 7.2 at P$_{CO_2}$ = 40 torr fit the concept of Fig. 2 even from a quantitative point of view.

**The Observed Rate of Facilitated CO$_2$ Diffusion**

As can be derived from the diffusion coefficient ($D_{CO_2} = 14.2 \cdot 10^{-6}$ cm$^2$s$^{-1}$) and the effective thickness of the layer ($d = 224 \cdot 10^{-4}$ cm), the total flux (diffusion rate/area)

$$F = D_{CO_2} \cdot \frac{\Delta P \cdot \alpha}{d},$$

was $114 \cdot 10^{-12}$ mol cm$^{-2}$s$^{-1}$ when the boundary CO$_2$ partial pressures were 7.5 and 2.9 torr and $\alpha$, the solubility of CO$_2$, was 0.66 atm$^{-1}$. According to the diffusion coefficient in the high CO$_2$ partial pressure range ($7.3 \cdot 10^{-6}$ cm$^2$s$^{-1}$) the flux of dissolved CO$_2$ was $58 \cdot 10^{-12}$ mol cm$^{-2}$s$^{-1}$ under these conditions.

The facilitated flux of CO$_2$ is the difference between the total flux and the flux of the dissolved CO$_2$, i.e. $114 - 58 = 56 \cdot 10^{-12}$ mol cm$^{-2}$s$^{-1}$. Hence, the facilitated CO$_2$ diffusion occurred at about the same rate as the diffusion of dissolved CO$_2$.

**The Diffusion Coefficient of Albumin Necessary to Explain the Observed Facilitated CO$_2$ Diffusion**

Since a facilitated flux of CO$_2$ based on bicarbonate diffusion requires equivalent fluxes of HCO$_3^-$ and protons, we have to postulate a flux of buffered protons, $F_{fac}$, equal to the measured facilitated flux. At this, the free diffusion of protons is neglected because its contribution to the total H$^+$ flux amounts to about 0.1% only. (This conclusion results from the extremely low proton concentration in the present solution.)

$F_{fac}$ may be derived from the difference of the boundary concentrations of protons buffered by albumin, $\Delta [H^+]_b$, the diffusion coefficient of albumin, $D_{Alb}$, and the thickness of the layer, $d$, by the following equation:

$$F_{fac} = D_{Alb} \cdot \frac{\Delta [H^+]_b}{d}.$$
Inversely, from Eq. 2 the diffusion coefficient of albumin necessary to explain the measured facilitated CO₂ transfer is given by:

\[ D_{Alb} = \frac{F_{fac} \cdot d}{\Delta[H^+]} \]  \hspace{1cm} (2 a)

\( \Delta[H^+] \) can be obtained from the concentration of albumin, \([Alb] \), the buffer capacity, \( \beta \), and the difference of the boundary pH values, \( \Delta \text{pH} \):

\[ \Delta[H^+] = [Alb] \cdot \beta \cdot \Delta \text{pH} \]  \hspace{1cm} (3)

\([Alb]\) is 4.4 \( \cdot \) 10⁻³ mol/liter in the present study (mol wt 69,000). \( \beta \) of the investigated albumin solution was determined experimentally by measurements of pH and concentration of bound CO₂ after equilibration with different CO₂ partial pressures. A value of \( \beta = 7.64 \) was obtained. The boundary pH values are obtained from the known boundary CO₂ partial pressures and the calculated boundary HCO₃⁻ concentrations applying the Henderson-Hasselbalch equation. This proceeding supposes the equilibrium between CO₂, H⁺, and HCO₃⁻ to be established everywhere in the layer. This should be justified in the present case of albumin solution containing 0.1 g/100 ml carbonic anhydrase.¹

A major problem of the above approach is to evaluate the boundary HCO₃⁻ concentrations established, if the proposed mechanism is actually the basis of the measured facilitated diffusion. A partial pressure gradient of CO₂ in the sheet leads first to a gradient of bicarbonate and buffered protons of equal size. This initial concentration gradient can be read from the CO₂ binding curve of the albumin solution. The concentration gradient leads to a diffusion of bicarbonate and buffered protons. Since the diffusivity of bicarbonate is about 30 times the diffusivity of albumin a gradient of electrical potential occurs. The latter induces a shift of the ions present in the layer in such a way that concentration gradients of Cl⁻, Na⁺, and total albumin (which has an average charge of about -20 under the present conditions) are established. During these ionic movements electroneutrality is maintained by a corresponding movement of bicarbonate ions leading to a reduction of the initial bicarbonate concentration gradient. After reaching steady-state conditions the fluxes of bicarbonate and buffered protons are equal and no net fluxes of Cl⁻, Na⁺, and total albumin occur anymore. Only a small potential difference seems to occur in the steady state; using the boundary concentrations of HCO₃⁻ derived below it can be shown that for a potential difference of ~0.05 mV boundary concentrations of the ions in the layer result, which (a) fulfill the

¹ The rates of facilitated diffusion measured at carbonic anhydrase concentrations of 0.1 g/100 ml are maximum values, which are not limited by the CO₂ hydration velocity: A further increase of carbonic anhydrase concentration leads to no further increase of CO₂ transfer. In the following theoretical treatment the CO₂ hydration velocity is therefore assumed to be infinite.
condition of electroneutrality, and (b) satisfy the Nernst equation (Nernst, 1888) as applied to the boundary concentration ratios of Cl\(^-\), Na\(^+\), and total albumin, respectively.

A potential difference of 0.05 mV has no appreciable effect on the bicarbonate flux (nor has it on the albumin flux). Therefore, the bicarbonate concentration difference, \(\Delta [\text{HCO}_3^-]\), may simply be calculated using Fick's diffusion equation:

\[
\Delta [\text{HCO}_3^-] = \text{measured facilitated CO}_2 \text{ flux} \cdot \frac{d}{D_{\text{HCO}_3^-}}, \tag{4}
\]

where \(D_{\text{HCO}_3^-}\) is the diffusion coefficient of bicarbonate. Using \(D_{\text{HCO}_3^-} = 4.0 \cdot 10^{-6} \text{ cm}^2 \text{s}^{-1}\) (see Appendix), facilitated flux = \(56 \cdot 10^{-12} \text{ mol cm}^{-2} \text{s}^{-1}\), and \(d = 224 \cdot 10^{-4} \text{ cm}\), \(\Delta [\text{HCO}_3^-]\) turns out to be \(0.31 \cdot 10^{-3} \text{ mol/liter}\), which is only \(\frac{1}{14}\) of the value to be expected from the \(\text{CO}_2\) binding curve.

The initial average bicarbonate concentration in the layer should be \(6.5 \cdot 10^{-3} \text{ mol/liter}\) according to the \(\text{CO}_2\) binding curve. We assume this value to be the same after the reduction of the bicarbonate concentration difference. Therefore the \(\text{HCO}_3^-\) boundary concentrations should be \(6.5 \pm \frac{1}{2} \cdot 0.31 \cdot 10^{-3}\) mol/liter, i.e. \(6.66 \cdot 10^{-3}\) and \(6.35 \cdot 10^{-3}\) mol/liter.

From the boundary \(\text{CO}_2\) partial pressures given above the boundary pH values are calculated to be 7.45 and 7.84 according to the Henderson-Hasselbalch equation (\(pK' = 6.09\), as determined from the \(\text{CO}_2\) binding curve; \(\alpha = 0.66 \text{ atm}^{-1}\)). The pH difference, 0.39, is about three times the value to be expected from the \(\text{CO}_2\) binding curve. Since the process of facilitated \(\text{CO}_2\) diffusion obviously is not limited by the transport of \(\text{HCO}_3^-\) but by the transport of protons, this reveals an important aspect of the present mechanism: the reduction of the initial bicarbonate concentration difference results in a considerable enhancement of the pH difference.

Inserting the value of \(\Delta \text{pH}\) into Eq. 3 \(\Delta [\text{H}^+]\) turns out to be \(13 \cdot 10^{-4}\) mol/cm\(^2\). Now, from Eq. 2 \(a\), the albumin diffusion coefficient necessary to explain the measured facilitated \(\text{CO}_2\) flux can be calculated:

\[
D_{\text{Alb}} = \frac{56 \cdot 10^{-12} \cdot 224 \cdot 10^{-4}}{13 \cdot 10^{-4}} = 9.6 \cdot 10^{-8} \text{ cm}^2 \text{s}^{-1}.
\]

Comparison with Experimental Data on \(D_{\text{Alb}}\) Keller et al. (1971) reported values of the albumin diffusion coefficient in highly concentrated bovine serum albumin solutions. They found in 30 g/100 ml solutions values of \(10 \cdot 10^{-8}\) (extrapolated from tracer diffusion experiments) and \(15 \cdot 10^{-8} \text{ cm}^2 \text{s}^{-1}\) (from mutual diffusion experiments). These values are in reasonable agreement with that calculated from the facilitated diffusion of \(\text{CO}_2\) (9.6 \cdot 10^{-8} \text{ cm}^2 \text{s}^{-1}).
We may conclude that, according to the above approximate treatment, the proposed mechanism of facilitated CO₂ diffusion (bicarbonate diffusion and simultaneous proton transport by translational diffusion of albumin) accounts quantitatively for the experimental data. The agreement of the measured and calculated values of the diffusion coefficient of albumin suggests that no other mechanism besides translational diffusion of albumin, e.g. rotary diffusion of the protein, is responsible for the H⁺ transport in facilitated CO₂ diffusion. In this respect albumin solutions seem to differ from hemoglobin solutions, where an additional mechanism of H⁺ transport was postulated (Gros and Moll, 1972).

This conclusion is valid for albumin solutions which have a pH = 7.2 at Pco₂ = 40 torr. However, similar calculations for the experiments with albumin solutions of pH = 5.9 show that equally in this pH range no additional mechanism has to be postulated to explain the measured rate of facilitated diffusion.

**Negligible Role of Carbamate Diffusion**

In the presence of CO₂ albumin forms carbamate and may function this way as a CO₂ carrier. This mechanism of facilitated CO₂ diffusion requires, like bicarbonate diffusion, a simultaneous transport of protons since per mole of carbamate formed 1–2 mol of H⁺ dissociate (Rossi-Bernardi and Roughton, 1967). As will be shown below, the concentration gradient of carbamate under the present conditions is too small as to sustain any significant carbamate diffusion.

To estimate carbamate concentrations the equations and constants describing the carbamate in serum protein solution given by Stadie and O'Brien (1937) were used. From the boundary Pco₂ and pH values in the case of the albumin solution of pH = 7.2 at Pco₂ = 40 torr (see p. 366) the boundary carbamate concentrations are calculated to be 2.3 · 10⁻⁴ mol/liter at the side of high Pco₂ and 2.6 · 10⁻⁴ mol/liter (i.e. higher!) at the side of low Pco₂.

If the carbamate reaction rate does not limit the process, the facilitated CO₂ flux resulting from the carbamate concentration difference, \( \Delta [\text{carbamate}] \), can be calculated from the following formula:

\[
F_{\text{fac}} = D_{\text{Alb}} \cdot \frac{\Delta [\text{carbamate}]}{d}.
\]

With \( D_{\text{Alb}} = 1 \cdot 10^{-7} \text{ cm}^2\text{s}^{-1} \) and \( d = 224 \cdot 10^{-4} \text{ cm} \) this flux turns out to be \(-0.13 \cdot 10^{-12} \text{ mol cm}^{-2}\text{s}^{-1} \), i.e. only 0.0001 of the measured facilitated flux. It takes place in the direction opposite to the net flux of CO₂.

Similar calculations for the albumin solutions of pH 5.9 and pH 8.0 (at Pco₂ = 40 torr) yield in both cases contributions of carbamate diffusion to the measured facilitated fluxes of less than 1%. Thus, we may conclude that
carbamate diffusion as a mechanism of facilitated CO₂ diffusion in albumin solutions can be neglected in a wide range of pH.

Role of Carbonate Diffusion

Under the present conditions carbonate, besides protein, may act as a buffer (forming bicarbonate). Thus, it may act as a proton carrier, too, and sustain facilitated CO₂ diffusion.

Indeed, Ward and Robb (1967) showed that facilitated CO₂ diffusion occurs in bicarbonate/carbonate solutions when a CO₂ partial pressure gradient is established. They pointed out that it is based on simultaneous, countercurrent fluxes of HCO₃⁻ and CO₃²⁻, whose relation is given by:

$$F_{\text{HCO}_3^-} = -2F_{\text{CO}_3^{2-}}.$$  \hspace{1cm} (6)

Half of the total bicarbonate flux should be considered as a proton flux, the protons being carried by carbonate. Thus, fluxes of bicarbonate and protons of equal size and direction are provided. A flux of unloaded proton carrier, CO₃²⁻, takes place in the opposite direction, having the same absolute value as the proton flux. Therefore, the facilitated CO₂ flux, $$F_{\text{fac}}$$, occurring by this mechanism can be calculated from the CO₃²⁻ flux according to the following equation:

$$F_{\text{fac}} = -F_{\text{CO}_3^{2-}}.$$  \hspace{1cm} (7)

Neglecting the effect of the diffusion potential the carbonate flux can be calculated from the CO₃²⁻ concentration difference across the layer, Δ[CO₃²⁻], and the diffusion coefficient of carbonate, DCO₃²⁻:

$$F_{\text{CO}_3^{2-}} = D_{\text{CO}_3^{2-}} \frac{\Delta[\text{CO}_3^{2-}]}{d}.$$  \hspace{1cm} (8)

On the basis of these considerations the possible role of carbonate diffusion in facilitated CO₂ diffusion in albumin solutions shall be evaluated in the following.

The boundary CO₃²⁻ concentrations can be calculated from the boundary H⁺ and HCO₃⁻ concentrations according to the mass action law. For this purpose the dissociation constant $$K_2$$ is calculated from the thermodynamic constant $$K_{2a}$$ (see Landolt-Börnstein, 1960) and the effect of the ionic strength (see Hastings and Sendroy, 1925) to be 1.1·10⁻¹⁰ mol/liter for the albumin solution of pH = 7.2 and 1.3·10⁻¹⁰ mol/liter for the solution of pH = 8.0.

In the solutions, which had a pH = 7.2 at $$P_{\text{CO}_2} = 40$$ torr, the carbonate concentration gradient appears to be too small as to sustain any significant carbonate diffusion: From the boundary pH values (7.45/7.84) and HCO₃⁻ concentrations (6.66/6.35·10⁻³ mol/liter) the boundary carbonate concen-
Facilitated Diffusion of CO₂ Across Albumin Solutions  

Concentrations are calculated to be 2.1 \( \times \) 10⁻⁵ and 4.8 \( \times \) 10⁻⁵ mol/liter. Inserting these values, \( d = 224 \times 10⁻⁴ \) cm, and \( D_{CO₂}⁻⁻ = 1.9 \times 10⁻⁶ \) cm²s⁻¹ (see Appendix) into Eq. 8 a carbonate flux of \(-2.3 \times 10⁻¹² \) mol cm⁻²s⁻¹ is obtained. This is only 4% of the measured facilitated CO₂ flux. Therefore carbonate diffusion as a mechanism of facilitated diffusion of CO₂ may be neglected in the albumin solutions which had a pH of 7.2 at \( P_{CO₂} = 40 \) torr. The same conclusion is reached for the solution of pH = 5.9.

However, the situation is quite different in the solutions which had a pH = 8.0 at \( P_{CO₂} = 40 \) torr. The following calculations show that in this case the major part of the measured facilitated CO₂ flux is based on CO₃⁻⁻ diffusion:

The total flux of CO₂ under these conditions was 194 \( \times \) 10⁻¹² mol cm⁻²s⁻¹, the flux of free CO₂ was 30 \( \times \) 10⁻¹² mol cm⁻²s⁻¹, as can be derived from the diffusion coefficients (48 \( \times \) 10⁻⁶ and 7.4 \( \times \) 10⁻⁶ cm²s⁻¹, see Tables I, II), the thickness of the layer (\( d = 197 \times 10⁻⁴ \) cm) and the boundary CO₂ partial pressures (7.13 and 5.09 torr) using Eq. 1. The rate of facilitated diffusion, therefore, was 164 \( \times \) 10⁻¹² mol cm⁻²s⁻¹.

Using the latter value the boundary concentrations of bicarbonate are calculated according to the principles described on pp. 356-66 to be 83.8 \( \times \) 10⁻³ and 82.4 \( \times \) 10⁻³ mol/liter. Inserting these values and the above boundary CO₂ partial pressures into the Henderson-Hasselbalch equation (\( pK₁ = 6.07 \), as determined from the CO₂ binding curve of this solution) the boundary pH values turn out to be 8.55 and 8.69.

From these data the facilitated CO₂ flux based on albumin diffusion can be calculated. From Eq. 3 the concentration difference of albumin buffered protons, \( \Delta[H⁺]ₐb \), is estimated to be 7.95 \( \times \) 10⁻³ mol/liter (using \( \beta = 12.9 \), as determined from the CO₂ binding curve of the pertinent solution, and \( [Alb] = 4.4 \times 10⁻³ \) mol/liter). With \( d = 197 \times 10⁻⁴ \) cm and \( D_{Alb} = 1 \times 10⁻⁷ \) cm²s⁻¹ (see pp. 366-67), the albumin facilitated CO₂ flux is calculated from Eq. 2 to be 40 \( \times \) 10⁻¹² mol cm⁻²s⁻¹. This is about 25% of the total measured facilitated CO₂ flux of 164 \( \times \) 10⁻¹² mol cm⁻²s⁻¹.

Can the diffusion of CO₃⁻⁻ account for the remainder of the measured facilitated flux (124 \( \times \) 10⁻¹² mol cm⁻²s⁻¹)? From the boundary HCO₃⁻ concentrations and pH values given above the CO₃⁻⁻ boundary concentrations are calculated to be 3.9 \( \times \) 10⁻³ and 5.2 \( \times \) 10⁻³ mol/liter. Now, from Eqs. 8 and 7 the facilitated CO₂ flux based on carbonate diffusion may be evaluated. With \( D_{CO₃}⁻⁻ = 1.8 \times 10⁻⁶ \) cm²s⁻¹ (see Appendix) it amounts to 119 \( \times \) 10⁻¹² mol cm⁻²s⁻¹. This value agrees reasonably well with 124 \( \times \) 10⁻¹² mol cm⁻²s⁻¹, the facilitated CO₂ flux not explained by albumin diffusion.

Thus, we may conclude that the rate of facilitated CO₂ diffusion measured in albumin solutions of pH 8.0 at \( P_{CO₂} = 40 \) torr can be fully explained on the basis of a carbonate and albumin facilitated H⁺ diffusion. Under these conditions about one-fourth of the total facilitated CO₂ flux is based on albumin diffusion, three-fourths on the diffusion of carbonate.
Diffusion coefficients of ions can be calculated from their equivalent conductivities using an equation first derived by Nernst (1888). However, corrections have to be made for the effects of ionic strength and protein concentration.

The dependence of the diffusivity of CO₂ on the protein concentration has been shown to be simply explainable on the basis of the geometry of the water space in the protein solution (Gros and Moll, 1971). As a first approximation it seems reasonable to assume that the protein affects the diffusion of other small molecules in a similar way. The diffusion coefficient of CO₂ in a 30 g/100 ml albumin solution (see Table I) is 44% of its value in water (see Table III). Since no experimental data are available, we assume that the same reduction holds for the diffusivities of HCO₃⁻ and CO₃²⁻.

**Evaluation of the Diffusion Coefficient of Bicarbonate**

From the equivalent conductivity of bicarbonate (Landolt-Börnstein, 1960) the diffusion coefficient at ionic strength μ = 0 and T = 25°C is calculated to be $11.7 \times 10^{-6}$ cm²s⁻¹. The ionic strength of the albumin solution of pH = 7.2 was μ = 0.13, it was μ = 0.18 in the albumin solution of pH = 8.0. Since the theory of Onsager is not applicable in this range, experimental data on the relation between ionic strength and conductivity of NaHCO₃ (Landolt-Börnstein, 1960) were used to estimate the reduction of the HCO₃⁻ conductivity/diffusivity by the above ionic strength. Accordingly, the diffusion coefficient of HCO₃⁻ is reduced to 77% for μ = 0.13, to 73% for μ = 0.18. Thus, correcting for the effects of ionic strength and protein concentration, the HCO₃⁻ diffusion coefficient is calculated to be $0.44 - 0.77 \times 11.7 \times 10^{-6} = 4.0 \times 10^{-6}$ cm²s⁻¹ in the albumin solution of pH 7.2, and $0.44 - 0.73 \times 11.7 \times 10^{-6} = 3.8 \times 10^{-6}$ cm²s⁻¹ in the albumin solution of pH 8.0.

**Evaluation of the Diffusion Coefficient of Carbonate**

The diffusion coefficient of CO₃²⁻ is calculated from the equivalent conductivity of CO₃²⁻ (Landolt-Börnstein, 1960) to be $9.5 \times 10^{-6}$ cm²s⁻¹ at μ = 0 and T = 25°C. In order to get a rough estimation of the effect of ionic strength, experimental data on the relation between ionic strength and equivalent conductivity of some 2-2-valent salts such as MgSO₄, MnSO₄, and CuSO₄ (Landolt-Börnstein, 1960) were used. From these data a reduction of the conductivity to 42–47% is expected for μ = 0.13, a reduction to 39–44% for μ = 0.18. Hence, the CO₃²⁻ diffusion coefficient is estimated to be $0.44 - 0.45 \times 9.5 \times 10^{-6} = 1.9 \times 10^{-6}$ cm²s⁻¹ in the albumin solution of pH = 7.2, $0.44 - 0.42 \times 9.5 \times 10^{-6} = 1.8 \times 10^{-6}$ cm²s⁻¹ in the albumin solution of pH = 8.0.

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