A Method for Estimating Contact Time of Red Blood Cells through Lung Capillary from O₂ and CO₂ Concentrations in Rebreathing Air in Man

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Abstract The gas exchange ratio (R) obtained from O₂ and CO₂ concentrations measured in rebreathing air usually shows a linear relation to the P_{CO_2} . By referring to this relation and the R which equals the Haldane effect coefficient and zero, the true- and oxygenated-venous P_{CO_2} are obtained in addition to the alveolar P_{CO_2} . When these P_{CO_2} are evaluated, the R - P_{CO_2} line can also be computed theoretically from a numerical solution on the overall O₂ and CO₂ diffusions in the red blood cell. By comparing both the experimental and theoretical R - P_{CO_2} lines with each other, we derived a contact time equation. Since the linear approximation of the R - P_{CO_2} relation gave rise to an error in the contact time (t_c), first a factor to correct the linearity of the R - P_{CO_2} line was derived. Next, using these parameter values, t_c was quantitatively determined from experimental data obtained during rebreathing in 5 normal subjects and it was compared with that estimated from the pulmonary diffusing capacity for CO in the same rebreathing experiment. When the extracellular HCO₃[−] dehydration rate was taken to be 0.1 s in time constant, the t_c , being ca. 0.7 and 0.4 s at rest and during exercise, respectively, showed good agreement with those obtained from the diffusing capacity for CO.

Key words: rebreathing, arterial-venous O₂ difference, cardiac output, contact time, CO₂ diffusion.

From the numerical solution on simultaneous O₂ and CO₂ diffusions in the red blood cell (RBC) (MOCHIZUKI and KAGAWA, 1986), the reaction (diffusion) rate factor of CO₂ with RBC ($F_c(\text{CO}_2)$; s^{−1}·Torr^{−1}) was calculated as:

$$F_c(\text{CO}_2) = \Delta C_{\text{CO}_2}(t_c) / (\overline{\Delta P_{\text{CO}_2}} \cdot t_c), \quad (1)$$

where $\overline{\Delta P_{\text{CO}_2}}$ and $\Delta C_{\text{CO}_2}(t_c)$ are the time average of the P_{CO_2} gradient across the RBC

boundary and the CO_2 diffusion quantity during the contact time (t_c), respectively. Since the $F_c(\text{CO}_2)$ is in a narrow range of 2.2×10^{-2} to $2.6 \times 10^{-2} \text{ s}^{-1} \cdot \text{Torr}^{-1}$ in a contact time range of 0.3 to 1.0 s, we attempted to derive the t_c by modifying Eq. (1) (SHIMASAKI and MOCHIZUKI, 1985).

In the numerical solution mentioned above, the extracellular HCO_3^- dehydration reaction is assumed to be catalyzed by carbonic anhydrase in the capillary wall with a time constant of 1.5 s according to KLOCKE (1978). The $F_c(\text{CO}_2)$ depends, to some extent, on the reaction rate of extracellular dehydration. The contact time of Eq. (1), when it is estimated from the O_2 and CO_2 concentrations during rebreathing by using the $F_c(\text{CO}_2)$, is influenced by the extracellular HCO_3^- dehydration reaction. Therefore, we estimated the time constant of the dehydration reaction by comparing the t_c obtained from the analysis of CO_2 diffusion with that evaluated from the pulmonary diffusing capacity for CO (UCHIDA *et al.*, 1986). A factor to correct the relation between the R and P_{CO_2} in rebreathing air was also determined.

DERIVATION OF CONTACT TIME EQUATION

The CO_2 diffusion out of the RBC in the lung occurs as a result of the P_{CO_2} gradient between the RBC and alveolar air, and the Haldane effect. Thus, the venous-arterial CO_2 content difference $((v-a)C_{\text{CO}_2})$ can be subdivided into two components, one due to the P_{CO_2} gradient $((v-a)C_{\text{CO}_2}(P))$, and the other due to the Haldane effect $((v-a)C_{\text{CO}_2}(H))$:

$$(v-a)C_{\text{CO}_2} = (v-a)C_{\text{CO}_2}(P) + (v-a)C_{\text{CO}_2}(H), \quad (\text{vol}\%) . \quad (2)$$

When venous blood is oxygenated without changing the CO_2 content, the P_{CO_2} in blood increases due to the Haldane effect. The P_{CO_2} before and after the oxygenation have hitherto been referred to as true-venous ($\text{tr}P\bar{V}_{\text{CO}_2}$) and oxygenated-venous P_{CO_2} ($\text{ox}P\bar{V}_{\text{CO}_2}$), respectively (CHRISTIANSEN *et al.*, 1914). The gas exchange ratio obtained from both the changes in O_2 and CO_2 concentrations in rebreathing air is usually linearly related to the P_{CO_2} thereof. Let the gas exchange ratio at the $\text{tr}P\bar{V}_{\text{CO}_2}$ and any alveolar P_{CO_2} (P_{ACO_2}) during the rebreathing process be HEC (Haldane effect coefficient) and RS, respectively. Denoting the arterial-venous O_2 content difference at the P_{ACO_2} by $(a-v)C_{\text{O}_2}$ (vol%), the $(v-a)C_{\text{CO}_2}(P)$ in Eq. (2) is given by

$$(v-a)C_{\text{CO}_2}(P) = (RS - \text{HEC}) \cdot (a-v)C_{\text{O}_2} . \quad (3)$$

Due to the Bohr effect, $(a-v)C_{\text{O}_2}$ is influenced by the P_{ACO_2} . The effect usually decreases as the P_{ACO_2} approaches the $\text{tr}P\bar{V}_{\text{CO}_2}$ during the rebreathing, since the intracellular pH (pH_c) decreases with increasing P_{ACO_2} . KAGAWA and MOCHIZUKI (1987) designated the $(a-v)C_{\text{O}_2}$ at the P_{ACO_2} , where the pH_c in arterial blood equals that in venous blood, as the standard $(a-v)C_{\text{O}_2}$ ($(a-v)C_{\text{O}_2}^*$). Furthermore, they developed a method to obtain the $(a-v)C_{\text{O}_2}^*$ by a modification of the method originated by MOCHIZUKI *et al.* (1984). Let the middle between $\text{tr}P\bar{V}_{\text{CO}_2}$ and $\text{ox}P\bar{V}_{\text{CO}_2}$

be P_{m_2} and the slope of the CO_2 dissociation curve at P_{m_2} be $\alpha'(P_{m_2})$ ($\text{vol}\% \cdot \text{Torr}^{-1}$). Moreover, let the slope of the $R\text{-}P_{\text{CO}_2}$ line obtained from gas analysis of rebreathing air be θ . MOCHIZUKI *et al.* (1984) estimated the $(a\text{-}v)C_{\text{O}_2}$ by dividing the $\alpha'(P_{m_2})$ by θ . Since the $(a\text{-}v)C_{\text{O}_2}$ thus obtained, however, was not accurate enough, KAGAWA and MOCHIZUKI (1987) derived a correcting factor ($F(H)$) from the numerical solution of O_2 and CO_2 diffusions in the RBC and attempted to estimate the $(a\text{-}v)C_{\text{O}_2}^*$:

$$(a\text{-}v)C_{\text{O}_2}^* = F(H) \cdot \alpha'(P_{m_2}) / \theta. \quad (4)$$

$F(H)$ was given by a linear function of the $(a\text{-}v)C_{\text{O}_2}^*$ as:

For $(a\text{-}v)C_{\text{O}_2}^* \leq 6.8 \text{ vol}\%$:

$$F(H) = 1.005 - 0.67 \times 10^{-3} \{ (a\text{-}v)C_{\text{O}_2}^* - 4.9 \}, \quad (5a)$$

and for $(a\text{-}v)C_{\text{O}_2}^* > 6.8 \text{ vol}\%$:

$$F(H) = 1 - 0.96 \times 10^{-2} \{ (a\text{-}v)C_{\text{O}_2}^* - 7.2 \}. \quad (5b)$$

The P_{CO_2} -dependency of $(a\text{-}v)C_{\text{O}_2}$ was further described with a ratio of $(a\text{-}v)C_{\text{O}_2} / (a\text{-}v)C_{\text{O}_2}^*$ ($F(avC_{\text{O}_2})$) as given by

$$\begin{aligned} F(avC_{\text{O}_2}) &= (a\text{-}v)C_{\text{O}_2} / (a\text{-}v)C_{\text{O}_2}^* \\ &= \left(\frac{2.25}{(a\text{-}v)C_{\text{O}_2}^*} - 0.09 \right) \frac{\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2} + 1.2}{100} + 0.99. \end{aligned} \quad (6)$$

Thus, from Eqs. (3), (4), and (6), the $(v\text{-}a)C_{\text{CO}_2}(P)$ is given by

$$(v\text{-}a)C_{\text{CO}_2}(P) = (RS - \text{HEC}) \cdot F(avC_{\text{O}_2}) \cdot F(H) \cdot \alpha'(P_{m_2}) / \theta. \quad (7)$$

On the other hand, from the $R\text{-}P_{\text{CO}_2}$ line the $(RS - \text{HEC})$ of the above equation is expressed as:

$$RS - \text{HEC} = \theta \cdot (\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2}). \quad (8)$$

Setting $(RS - \text{HEC})$ of Eq. (8) into Eq. (7), the $(v\text{-}a)C_{\text{CO}_2}(P)$ is further simplified as follows:

$$(v\text{-}a)C_{\text{CO}_2}(P) = F(avC_{\text{O}_2}) \cdot F(H) \cdot \alpha'(P_{m_2}) \cdot (\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2}). \quad (9)$$

The CO_2 diffusion quantity of Eq. (1), $\Delta C_{\text{CO}_2}(t_c)$, does not include the change in the amount of CO_2 dissolved in the plasma. Therefore, the latter quantity must be subtracted from the $(v\text{-}a)C_{\text{CO}_2}(P)$ for obtaining the $\Delta C_{\text{CO}_2}(t_c)$. The change in dissolved CO_2 is calculated from $\alpha_p' \cdot (\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2})$, where α_p' is the CO_2 solubility in plasma compartment in terms of $\text{vol}\% \cdot \text{Torr}^{-1}$. Thus, the $\Delta C_{\text{CO}_2}(t_c)$ of Eq. (1) is given by

$$\Delta C_{\text{CO}_2}(t_c) = (v\text{-}a)C_{\text{CO}_2}(P) - \alpha_p' \cdot (\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2}). \quad (10)$$

By inserting $(v\text{-}a)C_{\text{CO}_2}(P)$ of Eq. (9) into Eq. (10), the $\Delta C_{\text{CO}_2}(t_c)$ is given by

$$\Delta C_{\text{CO}_2}(t_c) = \{ F(avC_{\text{O}_2}) \cdot F(H) \cdot \alpha'(P_{m_2}) - \alpha_p' \} \cdot (\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2}). \quad (11)$$

The P_{CO_2} difference between venous blood and alveolar air which was used to compute $\overline{\Delta P_{\text{CO}_2}}$ and $F_c(\text{CO}_2)$ of Eq. (1) is not exactly linear against the R . However, the R -value obtained from the rebreathing experiment is approximated by a linear line against the P_{ACO_2} . Thus, the "experimental" $(\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2})$ defined by the given (RS-HEC) on the measured R - P_{CO_2} line is not always equal to the "theoretical" one which gives the same (RS-HEC) through the numerical solution. Therefore, to distinguish the former $(\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2})$ from the latter, we put an asterisk on the notation of the theoretical P_{CO_2} difference as $(\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2})^*$, and attempted to eliminate both the differences by using the following ratio:

$$F(\Delta P) = (\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2}) / (\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2})^* . \quad (12a)$$

On the other hand, let the ratio of $(\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2})^*$ to the $\overline{\Delta P_{\text{CO}_2}}$ be L , that is,

$$L = (\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2})^* / \overline{\Delta P_{\text{CO}_2}} . \quad (12b)$$

Then, from Eqs. (12a) and (12b), the L -ratio is expressed as

$$L = (\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2}) / (F(\Delta P) \cdot \overline{\Delta P_{\text{CO}_2}}) . \quad (12c)$$

By setting $(\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2})$ of Eq. (12c) into Eq. (11), the $\Delta C_{\text{CO}_2}(t_c)$ is written as

$$\Delta C_{\text{CO}_2}(t_c) = L \cdot F(\Delta P) \{ F(\text{av}C_{\text{O}_2}) \cdot F(H) \cdot \alpha'(P_{m_2}) - \alpha_p' \} \cdot \overline{\Delta P_{\text{CO}_2}} . \quad (13)$$

From both the equations of (1) and (13), the contact time is given by

$$t_c = F(\Delta P) \{ F(\text{av}C_{\text{O}_2}) \cdot F(H) \cdot \alpha'(P_{m_2}) - \alpha_p' \} / (F_c(\text{CO}_2)/L) . \quad (14)$$

Denoting the middle between $\text{tr}P\bar{V}_{\text{CO}_2}$ and P_{ACO_2} by P_{m_1} , the $F_c(\text{CO}_2)/L$ is approximated by a hyperbolic function of t_c , within a t_c range of 0.3 to 1.0 s:

$$F_c(\text{CO}_2)/L = C(P_{m_1}) / \{ t_c + D(P_{m_1}) \} , \quad (15)$$

where $C(P_{m_1})$ and $D(P_{m_1})$ are quadratic functions of P_{m_1} , though they depend upon the time constant of the direct extracellular HCO_3^- dehydration reaction. By setting P_{m_1} into the equations of $C(P_{m_1})$ and $D(P_{m_1})$, these values are easily evaluated (Eq. (18)). Thus, from both the equations of (14) and (15), the t_c equation is simplified as

$$t_c = D(P_{m_1}) \cdot E / \{ C(P_{m_1}) - E \} , \quad (16a)$$

where

$$E = F(\Delta P) \{ F(\text{av}C_{\text{O}_2}) \cdot F(H) \cdot \alpha'(P_{m_2}) - \alpha_p' \} . \quad (16b)$$

When the $\text{tr}P\bar{V}_{\text{CO}_2}$ and $\text{ox}P\bar{V}_{\text{CO}_2}$ are obtained through a rebreathing experiment, P_{m_2} and $\alpha'(P_{m_2})$ are readily calculated. Because the P_{ACO_2} during rebreathing is arbitrarily decided according to the initial conditions used in the computation of $F_c(\text{CO}_2)$ and L , t_c can be computed with ease as described below.

EVALUATION OF $F_c(\text{CO}_2)/L$ OF Eq. (15)

For evaluating the $F_c(\text{CO}_2)$ down the P_{CO_2} gradient across the RBC membrane, we computed the outward CO_2 diffusion by changing the extracellular P_{CO_2} from the $\text{tr}P\bar{V}_{\text{CO}_2}$ to P_{ACO_2} . The boundary and initial conditions were determined by referring to the rebreathing experiment at rest in normoxia (SHIBUYA *et al.*, 1987). Between the $\text{tr}P\bar{V}_{\text{CO}_2}$ and P_{ACO_2} there was a consistent relation:

$$P_{\text{ACO}_2} = 0.936 \cdot \text{tr}P\bar{V}_{\text{CO}_2} - 6.03, \quad (\text{Torr}), \quad (17)$$

where the correlation coefficient was 0.93. Setting an experimental $\text{tr}P\bar{V}_{\text{CO}_2}$ into Eq. (17), the conjugated P_{ACO_2} is evaluated. Thus, we computed the changes in intracellular P_{CO_2} and C_{CO_2} in capillary blood by varying the $\text{tr}P\bar{V}_{\text{CO}_2}$ successively within a physiological range. Figure 1 shows an example of the change in intracellular P_{CO_2} , ΔP_{CO_2} , and that in C_{CO_2} , ΔC_{CO_2} , in which the change in extracellular dissolved CO_2 is not included. The $\text{tr}P\bar{V}_{\text{CO}_2}$ and P_{ACO_2} were taken to be 50 and 40.8 Torr, respectively, and P_{O_2} was kept at 100 Torr for calculating $(v-a)C_{\text{CO}_2}(P)$. The time constant of the direct extracellular HCO_3^- dehydration reaction was assumed to be 0.1 s, as reported by BIDANI *et al.* (1983). The time average of the P_{CO_2} gradient, $\overline{\Delta P_{\text{CO}_2}}$ of Eq. (1), was computed by dividing the shaded integrated area of ΔP_{CO_2} by the contact time, t_c . The reaction (or diffusion) rate factor, $F_c(\text{CO}_2)$ was obtained by dividing the ΔC_{CO_2} at t_c , ($\Delta C_{\text{CO}_2}(t_c)$), by the $\overline{\Delta P_{\text{CO}_2}}$.

The $F_c(\text{CO}_2)$ depended on the $\text{tr}P\bar{V}_{\text{CO}_2}$, P_{ACO_2} , and the time constant of extracellular HCO_3^- dehydration reaction as well as the contact time. The influence of P_{ACO_2} , however, was eliminated by using the standardized relation between the

Fig. 1. A schematic illustration of changes in CO_2 content (ΔC_{CO_2}) and the P_{CO_2} gradient across the RBC boundary (ΔP_{CO_2}) used for explaining how to derive the $F_c(\text{CO}_2)$. $\Delta C_{\text{CO}_2}(t_c)$ is the change in C_{CO_2} at the contact time (t_c) and $\overline{\Delta P_{\text{CO}_2}}$ is the time average of ΔP_{CO_2} during the t_c .

Fig. 2. Reaction rate factor of CO_2 with the oxygenated RBC ($F_c(\text{CO}_2)$) plotted against t_c , computed from such curves as shown in Fig. 1. The P_{O_2} was kept at 100 Torr, and the extracellular P_{CO_2} was altered as described on the curves.

Fig. 3. The t_c -dependency of L -ratio obtained from the same ΔP_{CO_2} curves as used in the calculation of $F_c(\text{CO}_2)$ shown in Fig. 2.

$\text{tr}P\bar{V}_{\text{CO}_2}$ and P_{ACO_2} of Eq. (17). Figure 2 shows the $F_c(\text{CO}_2)$ which were calculated at three different $\text{tr}P\bar{V}_{\text{CO}_2}$ of 40, 50, and 60 Torr. The $F_c(\text{CO}_2)$ decreases exponentially as the contact time is prolonged and linearly as the $\text{tr}P\bar{V}_{\text{CO}_2}$ is increased. The time

Fig. 4. Relation between the $F_c(\text{CO}_2)/L$ and t_c . The $F_c(\text{CO}_2)/L$ was calculated by dividing $F_c(\text{CO}_2)$ of Fig. 2 by the L shown in Fig. 3.

Fig. 5. The P_{m1} -dependency of the factors, $C(P_{m1})$ and $D(P_{m1})$, used for the hyperbolic expression of $F_c(\text{CO}_2)/L$ (Eq. 15).

constant of the extracellular dehydration reaction was 0.1 s.

The L -ratio, on the other hand, linearly increases with the increase in the contact time. The slope of L against t_c is also increased with increasing $\text{tr}P\bar{V}_{\text{CO}_2}$, as shown in Fig. 3. Thus, the t_c -dependency of $F_c(\text{CO}_2)/L$ is relatively higher than that of $F_c(\text{CO}_2)$ alone, as shown in Fig. 4. The $F_c(\text{CO}_2)/L$ was numerically expressed by Eq. (15), where $C(P_{m_1})$ ($\text{vol}\% \cdot \text{Torr}^{-1}$) and $D(P_{m_1})$ (s^{-1}) were given as: For $P_{m_1} \leq 50$ Torr:

$$C(P_{m_1}) = 1.0483 - 0.2115 \times 10^{-1} \cdot P_{m_1} + 0.162 \times 10^{-3} \cdot P_{m_1}^2, \quad (18a)$$

$$D(P_{m_1}) = 0.2487 - 0.5247 \times 10^{-2} \cdot P_{m_1} + 0.395 \times 10^{-4} \cdot P_{m_1}^2, \quad (18b)$$

and for $P_{m_1} > 50$ Torr:

$$C(P_{m_1}) = 1.0775 - 0.2222 \times 10^{-1} \cdot P_{m_1} + 0.172 \times 10^{-3} \cdot P_{m_1}^2, \quad (18c)$$

$$D(P_{m_1}) = 0.231 - 0.452 \times 10^{-2} \cdot P_{m_1} + 0.32 \times 10^{-4} \cdot P_{m_1}^2. \quad (18d)$$

Figure 5 shows the $C(P_{m_1})$ of Eq. (18a) and $D(P_{m_1})$ of Eq. (18b). Both the C and D values decrease as the P_{m_1} increases.

The dependency of $F_c(\text{CO}_2)$ on the reaction rate of extracellular HCO_3^- dehydration seemed to be much more complicated than that on the P_{m_1} . When the time constant of the dehydration is longer than 0.4 s, the $F_c(\text{CO}_2)$ increases together with the t_c . However, when the time constant is shorter than 0.4 s, the $F_c(\text{CO}_2)$

Fig. 6. The $F_c(\text{CO}_2)$ of the oxygenated RBC computed by varying the time constant of extracellular dehydration reaction along the contact time. The extracellular P_{CO_2} was changed from 50 to 40.8 Torr, whereas the P_{O_2} was invariably maintained at 100 Torr.

Fig. 7. The relation between the $C(P_{m1})$ and $D(P_{m1})$ computed from $F_c(\text{CO}_2)$ of Fig. 6 by using the relation of Eq. (15) and the time constant of the extracellular HCO_3^- dehydration reaction.

decreases as the t_c increases: The shorter the time constant, the higher the decrease rate of the $F_c(\text{CO}_2)$ versus the contact time. Figure 6 shows the $F_c(\text{CO}_2)$ at 6 different time constants of 0.1 to 0.6 s, where the $\text{tr}P\bar{V}_{\text{CO}_2}$ and P_{ACO_2} were 50 and 40.8 Torr, respectively. The P_{O_2} thereof was invariably taken to be 100 Torr. In contrast to the $F_c(\text{CO}_2)$, the L -ratio was almost independent of the reaction rate of extracellular dehydration. The dependency of $F_c(\text{CO}_2)/L$ on the time constant was less than that on the t_c . All the $F_c(\text{CO}_2)/L$ could be expressed by an equation similar to Eq. (18), whose coefficients, $C(P_{m1})$ and $D(P_{m1})$, are illustrated in Fig. 7. At the time constant of 0.1 s, $C(P_{m1})$ and $D(P_{m1})$ are $0.422 \text{ vol}\% \cdot \text{Torr}^{-1}$ and 0.092 s^{-1} , respectively, which are obtained by inserting $P_{m1} = 45.38$ into Eqs. (18a) and (18b).

EVALUATION OF $F(\Delta P)$ AND CONTACT TIME

The $F(\Delta P)$ of Eq. (12a) is used to correct the linear experimental R - P_{CO_2} line. As shown in the preceding paper (KAGAWA and MOCHIZUKI, 1987, Fig. 8), the theoretical R - P_{CO_2} line deviates upwards as the P_{ACO_2} is decreased against the constant $\text{tr}P\bar{V}_{\text{CO}_2}$. Therefore, the $F(\Delta P)$ which equals the ratio of $(\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2})$ to $(\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2})^*$ should be greater than the unity. Moreover, when the diffusion across the capillary wall is taken into account, $(\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2})$ should exceed $(\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2})^*$. From the above two reasons it is conceivable that $F(\Delta P)$ becomes greater than the unity and that it approaches the unity as P_{ACO_2}

Fig. 8. The P_{m1} dependency of the $C(P_{m1})$ (A) and $D(P_{m1})$ (B) obtained by varying both the $trP\bar{V}_{CO_2}$ and the difference between $trP\bar{V}_{CO_2}$ and P_{ACO_2} . The curves of I, II, III, and IV were obtained from the relations between $trP\bar{V}_{CO_2}$ and P_{ACO_2} given by Eqs. (17), (19a)–(19c), respectively.

approaches $trP\bar{V}_{CO_2}$. In addition, the contact time should not be affected by the change in P_{ACO_2} during the course of rebreathing. Taking the above limitations into account, $F(\Delta P)$ was determined by checking whether it satisfied the following criteria: (1) $F(\Delta P) \geq 1$, (2) $F(\Delta P)$ approaches unity as the P_{ACO_2} approaches $trP\bar{V}_{CO_2}$, and (3) $F(\Delta P)$ gives the constant t_c value independent of the P_{ACO_2} . Finally, we compared the t_c obtained from P_{m1} and P_{m2} with that obtained from the pulmonary diffusing capacity for CO ($D_L(\text{CO})$), and confirmed the reliability of the $F(\Delta P)$ value as well as that of the other parameter values.

A practical procedure of computing $F(\Delta P)$ consists of two steps: The first step is the derivation of $F_c(\text{CO}_2)/L$ at four different P_{ACO_2} and the second is the determination of the $F(\Delta P)$, in which the independency of the t_c of the above four different P_{ACO_2} was confirmed. The details of the computation are as follows:

1) Determination of $C(P_{m1})$ and $D(P_{m1})$: Because the $F_c(\text{CO}_2)/L$ depends, though slightly, on the P_{ACO_2} , it was computed under the three relations between

PA_{CO_2} and $trP\bar{V}_{CO_2}$ in addition to the relation of Eq. (17) as follows:

$$PA_{CO_2} = 0.951 \cdot trP\bar{V}_{CO_2} - 4.52, \quad (19a)$$

$$PA_{CO_2} = 0.968 \cdot trP\bar{V}_{CO_2} - 3.01, \quad (19b)$$

$$PA_{CO_2} = 0.984 \cdot trP\bar{V}_{CO_2} - 1.51. \quad (19c)$$

All the $Fc(CO_2)/L$ computed with the above three relations were given by the hyperbolic equations of the t_c similar to Eq. (15), though the $C(P_{m_1})$ and $D(P_{m_1})$ were slightly displaced from one another, as shown in Fig. 8A and B. The lines I, II, III, and IV represent the $C(P_{m_1})$ and $D(P_{m_1})$ of Eqs. (17), (19a)–(19c), respectively. Both the $C(P_{m_1})$ and $D(P_{m_1})$ decrease though only slightly as the P_{m_1} increases.

UCHIDA *et al.* (1986) measured the $DL(CO)$ ($ml \cdot min^{-1} \cdot Torr^{-1}$) and the cardiac output ($\dot{Q}$; $ml \cdot min^{-1}$), by means of a rebreathing technique (MOCHIZUKI *et al.*, 1984). Furthermore, they estimated the t_c by dividing the $DL(CO)$ by a product of the $\dot{Q}$, the fractional hematocrit and the rate factor of CO replacement reaction for O_2 from oxygenated red blood cell ($Fc(CO)$; $s^{-1} \cdot Torr^{-1}$). Thus, from the same $R-P_{CO_2}$ line we attempted to obtain P_{m_1} and P_{m_2} to obtain the t_c value.

First, the $trP\bar{V}_{CO_2}$ was determined by the following equation for HEC (KAGAWA and MOCHIZUKI, 1987):

$$HEC = 0.294 - 0.57 \times 10^{-2} \cdot (a-v)C_{O_2}^*. \quad (20)$$

where $(a-v)C_{O_2}^*$ was obtained from the following standardized relation between $(a-v)C_{O_2}^*$ and $\dot{V}_{O_2}$ (SHIBUYA *et al.*, 1987):

$$(a-v)C_{O_2}^* = 9.5 \cdot \dot{V}_{O_2}^{0.35}. \quad (21)$$

Setting the measured $trP\bar{V}_{CO_2}$ into Eqs. (17) and (19a)–(19c), we calculated the four different PA_{CO_2} and P_{m_1} . Then, $C(P_{m_1})$ and $D(P_{m_1})$ were calculated from the respective equations such as Eqs. (18a)–(18d) and as illustrated in Fig. 8. Next, by extrapolating the $R-P_{CO_2}$ line to the abscissa, the $oxP\bar{V}_{CO_2}$ was evaluated to calculate the P_{m_2} . From the P_{m_2} , the slope of the CO_2 dissociation curve, $\alpha'(P_{m_2})$, was decided, by setting the P_{m_2} into the following equation (TAZAWA *et al.*, 1983):

$$\alpha'(P_{m_2}) = 3.962 \cdot P_{m_2}^{-0.5857}, \quad (vol\% \cdot Torr^{-1}). \quad (22)$$

2) Determination of $F(\Delta P)$: After the determination of $C(P_{m_1})$, $D(P_{m_1})$, and $\alpha'(P_{m_2})$, the t_c was computed by inserting them together with the $F(H)$ of Eqs. (5a) or (5b) and $F(avC_{O_2})$ of Eq. (6) into Eqs. (16a) and (16b). The $F(\Delta P)$ was determined by a trial-and-error method so that the criteria mentioned before were satisfied.

When the time constant of extracellular HCO_3^- dehydration reaction was taken to be 0.1 s, and the $F(\Delta P)$ was given by the following linear equation of $(trP\bar{V}_{CO_2} - PA_{CO_2})$, the t_c was fairly independent of the PA_{CO_2} and agreed with the t_c value obtained from the $DL(CO)$:

$$F(\Delta P) = 1 + 0.442 \times 10^{-2} \cdot (trP\bar{V}_{CO_2} - PA_{CO_2}). \quad (23)$$

Fig. 9. The t_c calculated from experimental P_{m_1} and P_{m_2} referring to the $C(P_{m_1})$ and $D(P_{m_1})$ illustrated in Fig. 8. The shaded area represents the mean $\pm$ S.D. of the t_c obtained from the diffusing capacity for CO which was measured together with the P_{m_1} and P_{m_2} in the same subject (UCHIDA *et al.*, 1986). The upper and lower plots were obtained at rest and during exercise, respectively. The plotted points and arrows are the mean and S.D. of the t_c obtained in the present study.

Fig. 10. Relation between two t_c values obtained from the P_{m_1} and P_{m_2} and those computed from the diffusing capacity for CO. The correlation coefficient was 0.91, and the regression line coincided fairly well with the identity line. The P_{ACO_2} was obtained from Eq. (17) referring to the experimental $trP\bar{v}_{CO_2}$.

Table 1. Contact times obtained from the pulmonary diffusing capacity for CO ($t_c(\text{CO})$) and from the P_{m_1} and P_{m_2} ($t_c(\text{CO}_2)$), and the difference between two t_c values, $\Delta t_c = t_c(\text{CO}) - t_c(\text{CO}_2)$.

a. At rest

Subj.	<i>n</i>	$t_c(\text{CO})$ (s)	$t_c(\text{CO}_2)$ (s)	Δt_c (s)
AS	5	0.701 ± 0.073	0.745 ± 0.038	-0.044 ± 0.082
IS	5	0.687 ± 0.058	0.710 ± 0.055	-0.023 ± 0.054
KI	4	0.713 ± 0.036	0.653 ± 0.021	0.061 ± 0.045
KN	6	0.681 ± 0.054	0.689 ± 0.026	-0.008 ± 0.046
KU	6	0.708 ± 0.063	0.728 ± 0.015	-0.020 ± 0.073
Mean $\pm$ S.D.		0.698 ± 0.014	0.705 ± 0.036	0.007 ± 0.040

b. Exercise

Subj.	<i>n</i>	$t_c(\text{CO})$ (s)	$t_c(\text{CO}_2)$ (s)	Δt_c (s)
AS	6	0.354 ± 0.035	0.404 ± 0.021	-0.050 ± 0.041
IS	7	0.387 ± 0.038	0.464 ± 0.018	-0.077 ± 0.054
KI	6	0.446 ± 0.038	0.378 ± 0.037	0.069 ± 0.063
KN	8	0.395 ± 0.052	0.389 ± 0.036	0.006 ± 0.059
KU	8	0.409 ± 0.045	0.415 ± 0.061	-0.006 ± 0.044
Mean $\pm$ S.D.		0.398 ± 0.049	0.410 ± 0.034	-0.012 ± 0.056

Figure 9 shows the t_c at rest and during exercise plotted against ($\text{tr}P\bar{V}_{\text{CO}_2} - P_{\text{ACO}_2}$). The plotted points represent the mean and the S.D. The shaded area illustrates the S.D. around the mean t_c obtained from the $DL(\text{CO})$ in individual subjects (UCHIDA *et al.*, 1986). Both the t_c values coincided well with each other, regardless of which equation of Eqs. (17) and (19a)–(19c) was chosen for the relation between $\text{tr}P\bar{V}_{\text{CO}_2}$ and P_{ACO_2} , or the difference between $\text{tr}P\bar{V}_{\text{CO}_2}$ and P_{ACO_2} . In Fig. 10, the t_c values obtained from $C(P_{m_1})$ and $D(P_{m_1})$ of Eqs. (18a)–(18d) are plotted against the t_c obtained simultaneously from the $DL(\text{CO})$. The correlation coefficient was 0.91 and the regression line coincided fairly well with the identity line. Both the t_c values and the difference between them (Δt_c) are summarized with respect to the individual subjects in Table 1. In a sitting position at rest the t_c was about 0.7 s, and during exercise of about $1.3 \text{ l(STPD)} \cdot \text{min}^{-1}$, it was around 0.4 s. The difference in t_c between the two methods has an S.D. of about 0.05 s, which suggests the reliability of analyzing the CO_2 diffusion process from the RBC.

Figure 11 shows the mean and S.D. of the t_c computed by changing the time

Fig. 11. The dependency of the t_c upon the time constant of extracellular HCO_3^- dehydration reaction. The $C(P_{m1})$ and $D(P_{m1})$ were computed from the experimental $\text{tr}\bar{V}_{\text{CO}_2}$ by using the relation between $\text{tr}\bar{P}_{\text{CO}_2}$ and P_{ACO_2} of Eq. (17).

constant of extracellular HCO_3^- dehydration reaction from 0.1 to 0.6 s. Good agreement was observed when the time constant was shorter than 0.2 s.

DISCUSSION

Although the presence of carbonic anhydrase in the lung capillary was confirmed by EFFROS *et al.* (1978) and KLOCKE (1978), the role of the enzyme on the CO_2 release in the lung has not been clarified quantitatively as yet. Figure 12 shows the changes in CO_2 content in blood computed by reducing the extracellular P_{CO_2} from 50 to 40.8 Torr at four different time constants of extracellular dehydration of 15, 1.5, 0.4, and 0.1 s. The solid line was obtained by increasing the extracellular P_{O_2} from 40 to 100 Torr, while the broken line was computed by keeping the extracellular P_{O_2} at 100 Torr. That is, the Haldane effect is included in the former curves, but not in the latter. In any event, the amount of CO_2 liberated out of the capillary blood plasma due to carbonic anhydrase in the capillary wall is estimated to be 0.3 to 0.4 vol%, as given by the difference in C_{CO_2} between two time constants of 15 and 0.1 s. In the practical computation of the CO_2 diffusion (MOCHIZUKI and KAGAWA, 1986), however, the change in extracellular P_{CO_2} was disregarded, assuming that the dehydration reaction is fairly slow compared with the CO_2 diffusion rate across the alveolar membrane. Although the change in extracellular P_{CO_2} was neglected, the amount of CO_2 released out of the plasma by the extracellular dehydration reaction was implied in the computed diffusion quantity out of the RBC shown in Fig. 2. That is, in the computed $F_c(\text{CO}_2)$ of Eqs. (1) and (15), the extracellular dehydration of HCO_3^- has been taken into account. Strictly

Fig. 12. The change in blood C_{CO_2} when the extracellular P_{CO_2} was decreased from 50 to 40.8 Torr at four different time constants of extracellular dehydration reaction. In the examples having oxygenation, the $P\bar{V}_{O_2}$ and $P_{A_{O_2}}$ were 39.4 and 100 Torr, and in the other examples with no oxygenation both the $P\bar{V}_{O_2}$ and $P_{A_{O_2}}$ were 100 Torr.

speaking, therefore, $F_c(CO_2)$ is not the real rate factor of the diffusion in the RBC. However, for the same reason that $F_c(CO_2)$ includes the extracellular HCO_3^- dehydration rate, the time constant of carbonic anhydrase in the capillary blood plasma could be evaluated.

The theoretical evidence that the time constant of carbonic anhydrase in the capillary wall is less than 0.2 s, as shown in Fig. 11, is one of the most important findings in this study. In our previous study (MOCHIZUKI and KAGAWA, 1986), we assumed that the time constant of extracellular HCO_3^- dehydration was 1.5 s according to the experimental data of KLOCKE (1978). At that time, the requirement that t_c should be independent of the P_{ACO_2} during the rebreathing, was not taken into account. To meet the above requirement, the correcting factor, $F(\Delta P)$, was determined as given by Eq. (23). In addition, to satisfy the requirement that the t_c must coincide with that estimated from the $DL(CO)$, the time constant of extracellular dehydration of less than 0.2 s was decided. BIDANI *et al.* (1983) measured the reaction rate of the extracellular HCO_3^- dehydration in isolated rat lungs, and obtained a rate similar to ours which suggests the validity of our

theoretical data.

When the CO_2 output caused by changing P_{CO_2} from venous to alveolar level ($\dot{V}_{\text{CO}_2}(P)$) is estimated by using the HEC of Eq. (20), the $\dot{V}_{\text{CO}_2}(P)$ may be described from Eq. (1) as

$$\begin{aligned}\dot{V}_{\text{CO}_2}(P) &= \dot{Q} \cdot (v-a) C_{\text{CO}_2}(P), \\ &= \dot{Q} \{ Fc(\text{CO}_2) \cdot t_c \cdot \overline{\Delta P_{\text{CO}_2}} + \alpha_p' \cdot (\text{tr} P \bar{v}_{\text{CO}_2} - P_{\text{ACO}_2}) \} .\end{aligned}\quad (24)$$

By inserting the L -ratio of Eq. (12c) into the above equation and eliminating $(\text{tr} P \bar{v}_{\text{CO}_2} - P_{\text{ACO}_2})$, the $\dot{V}_{\text{CO}_2}(P)$ is rewritten as

$$\dot{V}_{\text{CO}_2}(P) = \dot{Q} \{ Fc(\text{CO}_2) \cdot t_c + \alpha_p' \cdot F(\Delta P) \cdot L \} \cdot \overline{\Delta P_{\text{CO}_2}} .\quad (25)$$

Disregarding the resistance of CO_2 across the alveolar membrane and defining the pulmonary diffusing capacity for CO_2 ($DL(\text{CO}_2)$) as the quotient of $\dot{V}_{\text{CO}_2}(P)$ divided by $\overline{\Delta P_{\text{CO}_2}}$, analogously to the treatment of the diffusing capacity for O_2 and CO (GIBSON *et al.*, 1955), the $DL(\text{CO}_2)$ is given by

$$DL(\text{CO}_2) = \dot{Q} \cdot L \cdot t_c \{ Fc(\text{CO}_2)/L + \alpha_p' \cdot F(\Delta P)/t_c \} .\quad (26)$$

ROUGHTON and FORSTER (1957) introduced the $\theta(\text{CO})$ value ($\text{min}^{-1} \cdot \text{Torr}^{-1}$) as a reaction rate factor of CO with the oxygenated blood and evaluated the red cell component of $DL(\text{CO})$ by multiplying $\theta(\text{CO})$ by the capillary blood volume. According to their definition, $\theta(\text{CO}_2)$ may be given by the following equation:

$$\theta(\text{CO}_2) = 60 \cdot L \{ Fc(\text{CO}_2)/L + \alpha_p' \cdot F(\Delta P)/t_c \} .\quad (27)$$

Figure 13 shows the $\theta(\text{CO}_2)$ value obtained from the $Fc(\text{CO}_2)/L$ shown in Fig. 4. It

Fig. 13. The t_c -dependency of $\theta(\text{CO}_2)$ computed from Eq. (27) by using the $C(P_{m1})$, $D(P_{m1})$, and $F(\Delta P)$ of Eqs. (18a)–(18d) and (23), respectively.

increases as the t_c is shortened, suggesting that the $DL(CO_2)$ shows the same increasing tendency against t_c . When the following experimental conditions are given: $trP\bar{V}_{CO_2} = 50$ Torr, $P_{ACO_2} = 40.8$ Torr, and the capillary blood volume = 90 ml, the $DL(CO_2)$ is estimated to be $155 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1}$ for the t_c of 0.7 s. HYDE *et al.* (1968) and PIIPER *et al.* (1980) measured the $DL(CO_2)$ by using $^{13}CO_2$ in human lungs. The $DL(CO_2)$ at rest measured by HYDE *et al.* (1968) was higher than $200 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1}$ and that obtained by PIIPER *et al.* (1980) was, on an average, $180 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1}$. According to PIIPER *et al.* (1980), the slope of the CO_2 dissociation curve of $^{13}CO_2$ was $1.18 \text{ vol}\% \cdot \text{Torr}^{-1}$, or ca. 2.5 times as great as that of the normal dissociation curve. In their calculation, the change in blood $^{13}CO_2$ tension was ignored during the contact time. Moreover, the diffusion of $^{13}CO_2$ was directed from the plasma into the RBC, as contrasted with the normal outward direction. Taking the above differences into account, the difference in $DL(CO_2)$ between 180 and $155 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1}$ seems to be permissible.

During the outward CO_2 diffusion, the inward HCO_3^- shift always occurs, raising the intracellular P_{CO_2} (NIIZEKI *et al.*, 1984; SHIMOUCI *et al.*, 1984; KAGAWA and MOCHIZUKI, 1984; MOCHIZUKI *et al.*, 1985). According to the experimental data on the CO_2 diffusion in RBC suspension (PIIPER, 1964; CONSTANTINE *et al.*, 1965; NIIZEKI *et al.*, 1983), the permeability of CO_2 across the RBC membrane is not so great as to be disregarded. Hence, insofar as the inward HCO_3^- shift proceeds, the intracellular P_{CO_2} should be higher than the extracellular P_{CO_2} . In the pulmonary gas exchange, most of CO_2 molecules diffuse out of the RBC into alveolar air. Thus, the diffusing capacity for CO_2 may be better approximated by dividing the total CO_2 diffusion quantity by the P_{CO_2} gradient between the RBC and alveolar air, as shown by Eq. (26).

In the calculation of the $R-P_{CO_2}$ line shown in Fig. 8 of the preceding paper (KAGAWA and MOCHIZUKI, 1987), the resistance of the alveolar membrane to the CO_2 diffusion was ignored. Provided that the CO_2 transfer in the lung is, more or less, limited by the diffusion across the capillary wall, the difference between P_{m_1} and P_{m_2} in Eqs. (16a) and (16b) will increase just as during exercise, causing the underestimation of the t_c value. Thus, to make the two t_c values obtained from Eqs. (16a) and (16b) and $DL(CO)$ fit to each other, it becomes necessary to solve the differential equations of CO_2 diffusion repeatedly, reducing the transfer coefficient of CO_2 , until the optimum $F_c(CO_2)/L$ is yielded. As seen in Table 1 and Figs. 9 and 10, no significant difference was observed between both the t_c values obtained from $DL(CO)$ and the present method. In addition, the $F(\Delta P)$ is fairly close to the unity, as shown in Eq. (23). Thus, it may be safe to say that the alveolar membrane is much more permeable to CO_2 than the RBC membrane at least in the normal subject. HYDE *et al.* (1968) and PIIPER *et al.* (1980) reported that the diffusion of CO_2 is limited, to some extent, by the alveolar membrane. On the other hand, SCHUSTER (1985) measured the CO_2 diffusivity across the alveolar membrane by using ^{18}O -labeled carbon dioxide in human subjects. As the results, he clarified that the membrane component of the $DL(CO_2)$, being greater than $862 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1}$,

was 4–5 times as great as the $DL(\text{CO})$ measured by PIIPER *et al.* (1980), supporting our experimental and theoretical evidences.

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