Red cell distortion and conceptual basis of diffusing capacity estimates: finite element analysis

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Red cell distortion and conceptual basis of diffusing capacity estimates: finite element analysis. J. Appl. Physiol. 83(4): 1397–1404, 1997.—To understand the effects of dynamic shape distortion of red blood cells (RBCs) as it develops under high-flow conditions on the standard physiological and morphometric methods of estimating pulmonary diffusing capacity, we computed the uptake of CO across a two-dimensional geometric capillary model containing a variable number of equally spaced RBCs. RBCs are circular or parachute shaped, with the same perimeter length. Total CO diffusing capacity (DLCO) and membrane diffusing capacity (DmCO) were calculated by a finite element method. DLCO calculated at two levels of alveolar PO2 were used to estimate DmCO by the Roughton-Forster (RF) technique. The same capillary model was subjected to morphometric analysis by the random linear intercept method to obtain morphometric estimates of DmCO. Results show that shape distortion of RBCs significantly reduces capillary diffusive gas uptake. Shape distortion exaggerates the conceptual errors inherent in the RF technique (J. Appl. Physiol. 79: 1039–1047, 1995); errors are exaggerated at a high capillary hematocrit. Shape distortion also introduces additional error in morphometric estimates of DmCO caused by a biased sampling distribution of random linear intercepts; errors are exaggerated at a low capillary hematocrit.

Roughton-Forster technique; morphometry; pulmonary diffusing capacity; membrane diffusing capacity; random linear intercept; capillary model

However, under dynamic flow conditions, RBCs become distorted and assume a variety of asymmetric shapes, including parachute-like shapes (15). Such deformation of the RBC reduces shear stress and flow resistance (2, 3) but can have deleterious effects on diffusive gas exchange (17). Shape distortions might also exaggerate the conceptual errors inherent in the RF and morphometric techniques of estimating DLCO, although the magnitude of such effects has never been examined. We have utilized the geometric model and analytic approach described previously (10) to examine the effect of shape change of RBCs on the diffusive uptake of CO estimated by different methods.

METHODS

Geometric model. The capillary model consists of a cross section (1 µm thick) through the long axis of a pulmonary capillary segment. Different numbers of RBCs are equally spaced within the capillary and are circular, as described previously (10), or parachute shaped with the same perimeter length as the circular RBCs (Fig. 1). The parachute shape of RBCs was digitized from illustrations by Skalak and Brannmark (15) and Wang and Popel (17). We assume an infinite reservoir of CO in the alveolar air space. The RBCs represent infinite sinks for CO [CO partial pressure (PCO) within RBCs = 0]. The RBC component of CO uptake (1/θCO) is modeled as a resistance to CO diffusion across a thin RBC membrane; the resistance is varied in accordance with the assumed PAO2 (in Torr) to accurately mimic the values of θCO measured by Holand (9) in dog RBCs at 39°C

\[
\frac{1}{\theta_{CO}} = 0.929 + 0.0042\text{PO}_2
\]  

Dimensions and constants employed (6, 9, 12) are listed in Table 1.

FEM. We assume that the flux of CO is due entirely to tension gradients of CO driving CO diffusion into RBCs and that PCO gradients reach steady state immediately. Diffusive transport is described by the partial differential equation

\[
|\alpha D_{CO} \nabla^2 PCO| = 0
\]  

where \(\alpha\) is Bunsen solubility coefficient in lung tissue, \(D_{CO}\) is diffusion coefficient, and \(\nabla\) is gradient operator (= \(i\alpha \frac{\partial \phi}{\partial x} + j \alpha \frac{\partial \phi}{\partial y} + k \frac{\partial \phi}{\partial z}\)). The boundary conditions are \(PCO = 1\text{Torr}\) in the alveolar phase 5 µm above the air-tissue interface and \(PCO = 0\text{Torr}\) at the inner membrane surface of the RBCs. Because RBCs are equally spaced and symmetric with respect to the longitudinal axis of the capillary segment, we
need only examine one typical unit consisting of one-half of an RBC and its surrounding membrane-plasma barrier and air (Fig. 1). This unit is divided into 1,264 connecting quadrilateral elements and 1,200 nodal points, each with its own respective diffusion properties in air, tissue, and plasma (Fig. 2). Through this discretization process, Eq. 2 is transformed into 1,100 simultaneous algebraic equations (excluding boundary constraints), from which the PO2 at each nodal point can be solved as described previously (10). The matrix equation has the form

\[
[D][P] = [\text{flux}]
\]

or

\[
[\text{diffusive properties}][P] = [\text{CO flux}]
\]

Once the distribution of PO2 is determined, the diffusive flux of CO for each element is computed as

\[
\text{CO flux} = a D_{\text{CO}} \frac{\partial P_{\text{CO}}}{\partial n}
\]

where \(a P_{\text{CO}}/\partial n\) denotes PO2 gradients evaluated along the normal direction from a constant P CO surface. The total CO flow, equivalent to DL CO of each typical region, is obtained by summing the flow along the boundary surface of the air-tissue barrier for all the elements

\[
D_{\text{LCO(FEM)}} = \sum \text{flow} = \sum \frac{\text{flux} \cdot \Delta \text{area}}{P_{\text{ACO}}}
\]

where \(P_{\text{ACO}}\) is the mean alveolar PO2 at the air-tissue interface. The DL CO of the entire capillary segment is obtained by multiplying DL CO of a typical unit by the number of units in the geometric model. DM CO is computed by the FEM [DM CO(FEM)] as follows

\[
D_{\text{MCO(FEM)}} = \frac{\text{total CO flow}}{(P_{\text{ACO}} - \text{mean } P_{\text{CO}} \text{ over outer surface of RBC membrane})}
\]

A commercial software package (ANSYS, Swanson Analysis System) running on a DECstation 5000 computer was employed for this analysis. We computed DL CO using different numbers of equally spaced RBCs per capillary segment (i.e., different capillary hematocrit) and at 80 and 560 Torr PAO2. Analysis was carried out for as many RBCs as could be packed into a 100-µm capillary without overlapping adjacent RBCs, i.e., 13 circular and 17 parachute-shaped RBCs. Parachute-shaped cells can be packed closer without overlap between cells.

Comparison with RF method. DL CO(FEM) calculated at 80 and 560 Torr PAO2 was introduced into the RF equation (13)

\[
\frac{1}{D_{\text{LCO}}} = \frac{1}{D_{\text{MCO}}} + \frac{1}{\theta_{\text{CO}} V_c}
\]

where \(\theta_{\text{CO}}\) is the specific rate of CO uptake by RBC and binding with hemoglobin (in ml CO·ml blood\(^{-1}\)·min\(^{-1}\)·Torr\(^{-1}\)) and \(V_c\) is the total pulmonary capillary blood volume (in ml). Because \(V_c\) and the number of capillary RBCs are equivalent quantities as long as RBC volume and capillary hematocrit are known, we modified Eq. 7 as follows

\[
\frac{1}{D_{\text{LCO}}} = \frac{1}{D_{\text{MCO}}} + \frac{1}{\theta_{\text{CO}}(\text{no. of RBCs})}
\]

The DM CO and number of RBCs recovered by Eq. 8 [DM CO(FEM)] were compared with the anatomically defined number of RBCs and DM CO determined by FEM.

Comparison with morphometric method. The geometric capillary model was subjected to standard morphometric analysis (18). Alveolar-capillary surface area and number of RBCs of the anatomic model is known. Morphometric DM CO [DM CO(morphometry)] was estimated using the modified method of

<table>
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<tr>
<th>Table 1. Dimensions and constants of capillary model</th>
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<tr>
<td>Length of capillary segment</td>
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<td>Alveolar septal thickness</td>
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<td>Thickness of tissue barrier</td>
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<td>Internal capillary diameter</td>
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<td>Alveolar PO2</td>
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<td>Diffusion coefficient for CO</td>
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<td>Tissue and plasma</td>
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<td>(\theta_{\text{CO}})</td>
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<td>560 Torr</td>
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\(RBC\), red blood cell; \(\theta_{\text{CO}}\), specific rate of CO uptake by RBC and binding with hemoglobin; \(a\), Bunsen solubility coefficient in lung tissue; \(D_{\text{CO}}\), diffusion coefficient for CO. *Values measured 39°C. †Assuming \(5.1 \times 10^8\) RBCs/ml blood.
Weibel et al. (19) and compared with \( \text{DMCO(FEM)} \). A grid was randomly laid over the capillary model; the distance of all intercepts of the test line with the barrier \( l \), from the epithelial surface to the nearest RBC membrane, was measured with a logarithmic ruler. Intercepts that do not cross both epithelial and RBC surfaces were not measured. Orientation of the grid was varied, and the measurements were repeated until at least 60 intercept lengths had been measured. The harmonic mean intercept length through the tissue-plasma barrier \( l_{hb} \) is given by the mean of all reciprocal intercept lengths:

\[
l_{hb} = \frac{1}{\text{magnification}} \left( \frac{1}{\sum_{i=1}^{m} n_i} \right)
\]

where \( n \) is the number of linear intercepts of length \( l \). Because the test lines intercept the epithelium at random angles, in the standard morphometric method a factor of \( \frac{2}{3} \), derived from stereological principles, was introduced into Eq. 9 to correct for the mean intercept angle and to estimate the harmonic mean thickness of the tissue-plasma barrier \( \tau_{hb} \) in a direction perpendicular to the epithelial surface (7, 20)

\[
\tau_{hb} = \frac{2}{3} \cdot l_{hb}
\]

\( \text{DMCO} \) was then calculated as follows

\[
\text{DMCO(morphometry)} = \alpha \cdot D_{CO} \cdot \frac{S_A + S_c}{2 \cdot \tau_{hb}}
\]

where \( S_A \) and \( S_c \) are alveolar and capillary surface areas, respectively, and the diffusion and solubility coefficients of CO are taken from Table 1. In a previous analysis we showed that \( \text{DMCO} \) obtained using the quantity \( \tau_{hb} \) in Eq. 11 greatly underestimates true diffusive resistance of the barrier. Analysis of the flux of CO suggests that application of the statistical factor \( \frac{2}{3} \) is inappropriate; i.e., the randomly oriented \( l_{hb} \) is a better index of mean path length of molecular diffusion than the \( \tau_{hb} \) oriented perpendicular to the epithelial surface. Hence, in the present study we also calculated \( \text{DMCO(morphometry)} \) using \( l_{hb} \)

\[
\text{DMCO(morphometry)} = \alpha \cdot D_{CO} \cdot \frac{S_A + S_c}{2 \cdot l_{hb}}
\]

RESULTS

CO flux. The pattern of CO flux over the RBC surface is shown for one-half of an RBC in Fig. 3. The magnitude of flux is represented by the length of the vector. The distribution of flux is inhomogeneous, being more concentrated over the trailing tails of the parachute-shaped cell than over the leading surface. CO flux is low across a large portion of the RBC membrane along the infolded trailing surface. The inhomogeneity of flux distribution is more pronounced when spacing between RBCs is small and when \( \text{PAO}_2 \) is low. Results obtained from circular RBCs have been published previously (10) and are not shown here.

Diffusing capacity estimated by FEM. Figure 4 shows total \( \text{DMCO} \) for the capillary as well as \( \text{DMCO} \) per RBC estimated by FEM at two levels of \( \text{PAO}_2 \). This analysis again shows that conductance of the tissue-plasma membrane for CO decreases as \( \text{PAO}_2 \) increases; thus, for a given number of parachute-shaped RBCs in the capillary, estimated \( \text{DMCO} \) is lower at a higher \( \text{PAO}_2 \). This is because \( \text{PO}_2 \) alters the distribution of local \( \text{PCO} \) gradients. At a low \( \text{PAO}_2 \), \( \text{PCO} \) gradients and, hence, CO uptake over the surface of each RBC become more uniform (i.e., mean diffusion path shifts to a longer length); hence, resistance of the membrane component...
increases. DMCO per RBC remains almost constant as the number of RBCs per capillary increases up to about six RBCs per capillary; beyond this point DMCO per RBC progressively declines as the number of RBCs increases. This decline occurs because adjacent cells are sufficiently close that they compete for CO flux across the same intermediate endothelial surface between cells. Hence, above six RBCs per capillary, the increase in total DMCO due to increased number of RBCs is counterbalanced by a fall in DMCO per RBC. Beyond ~15 RBCs per capillary, total DMCO per 100-µm capillary approaches a plateau. A similar pattern is seen for DLCO estimated by FEM (13% lower at 1 RBC per capillary and 6% lower at 13 RBCs per capillary).

Fig. 4. Relationship of total membrane diffusing capacity for CO estimated by FEM [DMCO(FEM)] (top) and DMCO(FEM) per RBC (bottom) to number of parachute-shaped RBCs calculated at 80 and 560 Torr alveolar PO2 (PAO2).

Fig. 5. Comparison of total DMCO(FEM) (top) and DMCO(FEM) per RBC (bottom) for circular and parachute-shaped RBCs.

For a given number of RBCs in the capillary model, DMCO(FEM) per 100-µm capillary and DMCO(FEM) per RBC are lower for parachute-shaped than for circular RBCs (Fig. 5); the difference diminishes as the number of RBCs increases (17% lower at 1 RBC per capillary and 8% lower at 13 RBCs per capillary). A similar pattern is seen in DLCO estimated by FEM (13% lower at 1 RBC per capillary and 6% lower at 13 RBCs per capillary).

Diffusing capacity estimated by morphometric method. Figure 6 shows the changes in mean linear diffusion path between the epithelial surface and the RBC membrane (lhb); for a given number of capillary RBCs, lhb is significantly longer for parachute-shaped than for circular RBCs. Comparison of DMCO per 100-µm capillary estimated by different methods is shown in Fig. 7 for circular and parachute-shaped RBCs. When the harmonic barrier thickness (τhb) is used to estimate the path length for diffusion (Eq. 11), morphometric estimates are grossly elevated compared with corresponding estimates by FEM for both RBC shapes. Differences between FEM and morphometric estimates diminish as the number of capillary RBCs increases. Morphometric estimates range from 352% (2 cells) to 52% (12 cells) higher than corresponding estimates by FEM for circular RBCs and from 418% (2 cells) to 57% (16 cells)
higher for parachute-shaped RBCs. As the number of capillary RBCs increases, morphometric overestimation of $D_{MCO}$ diminishes more rapidly for parachute-shaped than for circular cells. At 10 cells per 100-µm capillary, overestimation of $D_{MCO}$ is similar for circular and parachute-shaped cells. Above 10 cells per 100-µm capillary, overestimation of $D_{MCO}$ is slightly greater for circular cells. When values at the same number of RBCs per 100-µm capillary are compared, morphometric estimates of $D_{MCO}$ are 5% (2 cells) and 16% (12 cells) lower for parachute-shaped than for circular cells. Similarly, morphometric estimates of $D_{LCO}$ are 2% (2 cell) to 13% (12 cells) lower for parachute-shaped than for circular cells.

Figure 8 shows the ratio of morphometric $D_{MCO}$ estimated using $l_{hb}$ (Eq. 12) to $D_{MCO}$ estimated by FEM. We previously showed that $l_{hb}$ more accurately reflects the molecular diffusion distance than does $t_{hb}$ (10); Eq. 12 yields significantly lower estimates of $D_{MCO}$ and $D_{LCO}$ than Eq. 11, i.e., smaller differences than estimates by FEM, particularly at low numbers of capillary RBCs. In fact, above 10 parachute-shaped RBCs per capillary, $D_{MCO}$ (morphometry) calculated using $l_{hb}$ is slightly (5–10%) below corresponding $D_{MCO}$ estimated by FEM. This slight underestimation disappears at 16 parachute-shaped cells per 100-µm capillary when the cells are almost maximally packed.

Diffusing capacity estimated by RF method. Deviations of $D_{MCO\text{(RF)}}$ from $D_{MCO\text{(FEM)}}$ are modest (Fig. 9). At a...
low hematocrit (≤6 RBCs), DM_{CO(RF)} for circular RBCs is 2% higher than corresponding DM_{CO(FEM)}, whereas DM_{CO(RF)} for parachute cells is 5% higher than DM_{CO(FEM)}. As capillary hematocrit increases, errors in DM_{CO(RF)} increase progressively for both RBC shapes to reach ~9–13% above corresponding DM_{CO(FEM)}.

DISCUSSION

The importance of capillary hematocrit in determining capillary resistance to CO diffusion has again been demonstrated, as in our previous analysis using circular RBCs. The present analysis also reveals that shape distortion of RBCs, as it develops under high-flow conditions, significantly reduces diffusive uptake of CO in the lung capillaries. In addition, shape distortion of RBCs exaggerates the overestimation of DM_{CO} caused by conceptual simplifications inherent in the RF technique. Shape distortion also exerts complex effects on the errors inherent in the morphometric technique of estimating DM_{CO}. These effects are modulated by spacing between adjacent RBCs and are discussed below.

Hematocrit and RBC distribution. By the classic concept of diffusive gas transfer in the alveoli, the rate of gas uptake is dependent on the diffusivity of the gas in tissue and plasma, the alveolar-capillary surface area, and the diffusion distance across the alveolar-capillary-plasma barrier. This concept does not formally consider the particulate nature of RBCs. Packaging hemoglobin within discrete RBCs retains the respiratory pigment within the vascular space and avoids the undesirable effects of hemoglobin on vascular tone. On the other hand, it leads to an inherently nonuniform distribution of hemoglobin, i.e., a mismatch of gas exchange surfaces between the RBC and the capillary endothelium. The distribution of RBCs within capillaries is a complex function of interactions among quantity, size, and deformability of RBCs, local flow dynamics, and physical properties of the capillary network. The flow and distribution of RBCs are also affected by margination and sequestration of leukocytes in capillaries (11). That static and dynamic properties of RBCs can alter diffusive gas exchange is shown by various recent reports. Geiser and Betticher (5) reported in isolated perfused rabbit lung that pulmonary diffusing capacity for O_2 (DLO_2) was lower when the lung was perfused with RBC suspensions than with hemoglobin solutions. Federspiel (4) modeled RBCs as spheres flowing in single file through a cylindrical capillary surrounded by a uniform annulus of alveolar tissue and reported a reduction in membrane diffusing capacity for O_2 with increasing RBC spacing (or decreasing hematocrit) greater than the associated reduction in RBC diffusing capacity. Vock and Weibel (16) showed in rabbit lungs that massive hemorrhage led to a significantly reduced DLO_2 estimated by morphometric methods. Similar effects of hematocrit on diffusive gas uptake have been reported in skeletal muscles (8). We previously examined the uptake of CO (DLCO) in a single pulmonary segment containing various numbers of circular RBCs and found changes induced by hematocrit similar to those reported by Federspiel for O_2. In addition, this kind of analysis allows us to dissect the sources of conceptual errors inherent in the physiological and morphometric methods of estimating diffusing capacity (10).

Deformation of RBCs. Effects of RBC deformation on gas transport have been modeled in a single capillary by Wang and Popel (17), who reported that a change from circular to parachute-shaped RBCs decreases O_2 flux by 26%; this shape effect is inversely related to the RBC residence time within the capillary. Betticher et al. (2) demonstrated in isolated rabbit lungs that reduced RBC deformability reduces DLO_2. They attribute this effect to the resistance offered by a thicker unstirred layer of plasma outside the RBC membrane; thickness of the unstirred layer is enhanced around undeformed RBCs flowing at low velocities and diminished by the increased mixing associated with deformation of RBCs at high flow velocities. Sarelius (14) points out an alternative explanation for the observation of Betticher et al.: i.e., reduced deformability of RBCs is associated with less uniformity of resistance to RBC flow in the capillaries, leading to nonuniform regional hematocrits. Our present analysis is consistent with the finding of Wang and Popel (17) that the shape distortion of the RBC that occurs under high-flow conditions can significantly impair the DLCO across the capillary. This theoretical impairment is due to a greater inhomogeneity in the distribution of CO flux over the surface of each RBC, but such a deleterious effect may be offset by simultaneous improvements in hydrodynamics of the deformed cells, which might lead to greater homogeneity in the distribution of capillary hematocrits.

Errors in physiological estimate of diffusing capacity. Our previous analysis shows that, within the geometric capillary model containing circular RBCs, DM_{CO(RF)} estimates are modestly higher than DM_{CO(FEM)} estimates at hematocrits at or above physiological level. This overestimation occurs because the RF technique...
assumes \( \text{DMCO} \) to be constant regardless of \( \text{PAO}_2 \). Finite element analysis has shown that, in fact, \( \text{DMCO} \) estimated as CO flux decreases as \( \text{PAO}_2 \) increases, because distribution of \( \text{PCO} \) over the RBC surface becomes more uniform, and as a consequence the distribution of molecular diffusion paths shifts toward longer lengths. Reducing \( \text{PAO}_2 \) (i.e., increasing \( \theta_{\text{CO}} \) in Eq. 7) increases CO flux into the RBC, and at the higher rates of flux CO uptake by RBCs preferentially shifts to areas of the RBC surface nearer the alveolar-capillary surface, thereby reducing mean diffusion distance. The resulting error in the RF technique, caused by assuming a constant \( \text{DMCO} \) as \( \theta_{\text{CO}} \) changes, is further exaggerated by shape distortion of RBCs, because for a given number of capillary RBCs, the effect of \( \text{PAO}_2 \) on flux distribution is greater for parachute-shaped than for circular RBCs (Fig. 3) (10). The magnitude of overestimation for parachute-shaped cells increases to 13% at the highest number of RBCs that can be packed into a capillary segment without overlap.

Errors in morphometric estimate of diffusing capacity. On the other hand, previous analysis shows that estimates of \( \text{DMCO} \) by Weibel's morphometric technique are grossly elevated with respect to estimates by FEM when the number of capillary RBCs is low, but differences progressively diminish as the number of capillary RBCs increases. Much of the discrepancy between morphometric and FEM estimates could be attributed to an error in the stereological construct, which imposes an arbitrary factor of \( \frac{2}{3} \) to correct for the angle between the mean random linear intercept from the epithelium to the RBC membrane and the normal to the epithelial surface. When this arbitrary factor was omitted, agreement between these two methods becomes much closer in the physiological range of hematocrits (10). Overestimation of \( \text{DMCO} \) by morphometry is moderately exaggerated by shape distortion of the RBCs when the number of capillary RBCs per 100-µm capillary is <10 (Fig. 8). At >10 RBCs per 100-µm capillary, morphometry (using \( h_b \) rather than \( h_a \) to estimate mean barrier distance) actually underestimates true \( \text{DMCO} \) by up to 10%. This seemingly paradoxical pattern can be explained by several observations that lead to opposing effects that counterbalance one another as the number of capillary RBCs increases.

1) The morphometric technique measures the distance of randomly oriented linear diffusion paths from the epithelial surface to the RBC surface, whereas FEM reveals that local \( \text{PCO} \) gradients constrain CO flux by diffusion to markedly curvilinear paths over much of the RBC surface. This curvilinearity is more pronounced for parachute-shaped than for circular RBCs and also more marked when RBCs are far apart than when they are close together. Thus approximation of diffusion distance using random linear intercepts as employed in the morphometric method yields an underestimation of true diffusion distance and an overestimation of \( \text{DMCO} \). As more RBCs are packed into the capillary, the mean diffusion path becomes shorter and more nearly linear; thus errors in \( \text{DMCO} \) due to measured values of mean linear path length (\( l_{pb} \)) progressively diminish. 2) The morphometric method utilizes the entire available alveolar-capillary surface area in the calculation of \( \text{DMCO} \) regardless of the number of capillary RBCs. However, FEM analysis demonstrates that most of the CO flux occurs across only a small portion of the tissue membrane close to an RBC. As the number of capillary RBCs increases, the distribution of CO flux along the alveolar-capillary surface becomes more uniform; i.e., the effective alveolar-capillary surface available for diffusive gas exchange increases and approaches that estimated by morphometry. Thus morphometry grossly overestimates \( \text{DMCO} \) at a low hematocrit, and errors diminish progressively as capillary hematocrit increases. 3) The error in morphometry caused by underestimation of molecular diffusion distance due to linear approximation of a curvilinear diffusion path is counterbalanced in parachute-shaped cells by another error arising from a biased sampling distribution over the RBC surface. This source of error is related to RBC geometry and the probability that some portions of the infolded perimeter of the parachute-shaped cell are preferentially sampled by a randomly oriented line, particularly as RBC spacing diminishes (Fig. 8). The probability of sampling any given point along the infolded perimeter of a parachute-shaped RBC by a randomly oriented line through a given point on the epithelial surface (point a) varies from a finite value (regions 1 and 3) to zero (region 2), even though these regions subtend the same angle. Because of the concentration gradient of CO and the axial symmetry of the capillary segment, according to FEM most of the CO flux across point a will reach region 3 of the RBC, whereas a random linear intercept from point a to region 1 in fact violates physical laws by running against the local \( \text{PCO} \) gradient. Therefore, by the random linear intercept method, a significant portion of the infolded RBC perimeter closest to point a is undersampled, whereas the regions farthest from point a are

![Fig. 10. Sampling bias introduced by morphometric method when applied to an asymmetric RBC shape. Through a given point along upper epithelial surface (point a), random linear intercept method oversamples region 1 of infold surface of RBC perimeter and undersamples region 3, even though both regions subtend the same angle. Region 2 is not sampled from point a, because it cannot be intercepted by a straight line. Biased sampling occurs because, according to physical laws governing \( \text{PCO} \) gradient distribution, CO flux in region 1 originates from across lower epithelial surface; a random line from point a to region 1 runs against local \( \text{PCO} \) gradient. Effect of biased sampling becomes more significant as spacing between RBCs decreases, only to disappear at maximum possible hematocrit, when RBCs are so tightly packed and lateral RBC surfaces become completely hidden from epithelial membrane.](http://jappl.physiology.org/content/120/3/322/F10)
oversampled. The net result of this sampling bias is an overestimation of mean diffusion path length over the infolded surface of the RBC, leading to an underestimate of \(D_{\text{MCO}}\). As the number of capillary RBCs increases, this sampling bias increases. However, beyond a certain closeness of RBC packing (16 cells per capillary), this bias disappears, because lateral surfaces of adjacent RBCs become relatively hidden and inaccessible to linear sampling from the epithelial surface. Hence, the apparent mean barrier thickness again decreases (Fig. 6) and morphometric \(D_{\text{MCO}}\) abruptly increases (Fig. 7). This sampling bias arises for parachute-shaped but not circular RBCs, because parachute-shaped cells lack full rotational symmetry. We would expect a similar sampling bias to occur in other asymmetric shapes assumed by RBCs.

Limitations of FEM. As pointed out previously (10), our model is not meant to reproduce reality but, rather, to provide a uniform framework and an independent analytic technique that could be utilized to explore the conceptual basis of our understanding of the pulmonary diffusion process and to reconcile differences between current physiological and morphometric methods of estimating pulmonary diffusing capacity. This stylized capillary model is two-dimensional and static; no motion of RBCs is implied. The selection of cell shapes is necessarily arbitrary, since RBCs, in fact, can assume numerous irregular shapes during capillary transit. However, the circular and parachute shapes are representative of a symmetric and an asymmetric configuration, respectively. Furthermore, the parachute is a shape seen in perfused capillaries under direct observation. The boundary conditions are also arbitrary, but variations would not have altered our general conclusions. The primary variable examined in this study is \(D_{\text{MCO}}\) and the potential sources of error in its estimation by the RF and the morphometric methods; we assumed that in vitro measurements of \(\theta_{\text{CO}}\) at different levels of \(P_{\text{O}_2}\) are correct. For the sake of simplicity, the reaction kinetics of \(O_2\) displacement by CO are not explicitly included in the model. We have employed the same values of \(\theta_{\text{CO}}\) for the FEM, morphometric, and RF estimations of \(D_{\text{MCO}}\); the conclusions drawn are independent of the accuracy of the relationship between \(1/\theta_{\text{CO}}\) and \(P_{\text{O}_2}\) and of the reaction kinetics inside the RBCs.

We conclude from finite element analysis that shape distortion of the RBCs as develops under high-flow conditions alters the distribution of CO flux across the RBC surface and reduces the diffusive uptake of CO. Distortion of RBCs exaggerates conceptual errors in the RF and the morphometric technique of estimating diffusing capacity via different mechanisms. Errors in the RF technique arise from the same source regardless of RBC shape and are most sensitive to changes in RBC spacing in the physiological range of hematocrits. The various sources of error in the morphometric technique exert opposing effects on the estimate of \(D_{\text{MCO}}\); their net effect is most sensitive to changes in RBC spacing when the capillary hematocrit is low. In vivo, the unfavorable effect of RBC shape distortion on diffusive gas uptake may be mitigated by its favorable effect on hydrodynamics and the distribution of capillary RBC flow.

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