In 1915 Marie Krogh first described the single-breath diffusing capacity of the lung for carbon monoxide (DL_{CO}^{SB}) , proving that passive diffusion alone could explain gas transport across the air-blood barrier and putting to rest the once popular belief that the alveolar epithelium functioned like a gland, actively secreting oxygen (O_2) into capillary blood. Today, the test is recognized as a unique screening tool to examine the integrity of the air-blood barrier. In the United States, the average pulmonary function laboratory performs >800 DL_{CO}^{SB} tests per year. The "diffusing capacity" is not the best choice of term since it is a conductance (the inverse of resistance) rather than a "capacity," and CO uptake involves not only passive diffusion but also chemical binding with hemoglobin (Hb). Outside North America it is called the transfer factor.

**PHYSIOLOGIC FACTORS AFFECTING DL_{CO}^{SB}**

**COMPONENTS OF DL_{CO}^{SB}**

The pathway for CO from air to blood (Figures 57-1 and 57-2) classically consists of two resistances in series. The first is 1/DM, or the “extra-Hb resistance,” due to the diffusing capacity of the alveolar membrane (DM). This resistance is the result of passive diffusion through an astonishingly thin minimal tissue barrier (<0.6 μm), consisting of the alveolar epithelial type I cell, a fused basement membrane, and a capillary endothelial cell. Additionally, there is an intracapillary component consisting of an “unstirred” plasma layer surrounding the red cell, the red cell membrane, and cytoplasm.

The second resistance (1/VC), or the “intra-Hb resistance,” is owing to the CO conductance within the red cells in the pulmonary capillary blood. The rate (mL/min/mm Hg) of chemical combination of CO with Hb (θ), per mL of whole blood, measured in vitro using venous blood at a standard Hb concentration, estimates the finite reaction rate of CO binding with Hb. Knowing the total volume of capillary blood exposed to alveolar air (VC), the overall conductance of the blood for CO transfer (θVC) can be calculated (see Figures 57-1 and 57-2). Hence,

\[
1/\text{DL}_{CO} = 1/\text{DM} + 1/(\theta \text{VC})
\]  

(57-2)

The extra-Hb resistance (1/DM) is independent of alveolar [O_2], whereas the intra-Hb resistance (1/θVC) changes with alveolar [O_2] because O_2 competes with CO for the same Hb binding sites, causing θ to decrease with increasing [O_2]. DM and VC are estimated from at least two measurements of DL_{CO}^{SB} and θ at different alveolar [O_2]. A plot of 1/DL_{CO}^{SB} (y axis) versus 1/θ (x axis) yields a straight line where the y intercept is 1/DM and the slope is 1/VC. The measurement of VC depends critically on accurate measurements of θ, which must be indirectly estimated using in vitro mixing chambers. Furthermore, θ depends directly on the capillary hematocrit (Hct), which is assumed to be the same as systemic venous values but is actually substantially lower in...
the pulmonary capillaries than in the systemic circulation. In normal subjects $D_M$ is very large, so most of the diffusive resistance is a result of the chemical combination of CO with Hb (see Figure 57-1). Diseases that damage the alveolar-capillary membrane likely reduce both $D_M$ and $V_C$ because the alveolar epithelium and pulmonary capillaries are anatomically in such close proximity. Measuring the components of $D_{LCO}^{SB}$ ($D_M$ and $V_C$) usually adds little additional, useful clinical information, particularly because of inaccuracies in estimating $\theta$.

Although ignored in the classic analysis, part of the CO pathway involves gas-phase transport, by both convection and gas diffusion, from the more proximal airways to the more distal gas-exchanging bronchioles and alveoli (see Figure 57-2). The mixing of a gas rich in CO and a tracer gas, helium (He), is neither instantaneous nor complete during inhalation and short periods of breath holding ($t_{BH}$). Time-dependent gradients in CO and He concentration ([CO] and [He], respectively) develop in the lung periphery, distal to terminal bronchioles. This lowers the [CO] at the air-blood interface and, by reducing the effective driving pressure for CO, lowers $D_{LCO}^{SB}$. The simultaneously created, time-dependent gradients for He result in worsening of ventilation inhomogeneity. This incomplete gas-phase mixing creates a third “gas mixing” conductance, called $G_{\text{Mix}},$ which is normally so large that it does not affect the pathway for CO. However, when gas-phase mixing worsens, $G_{\text{Mix}}$ decreases. In this case, the pathway for CO becomes

$$\frac{1}{D_{LCO}} = \frac{1}{G_{\text{Mix}}} + \frac{1}{D_M} + \frac{1}{(\theta V_C)} \quad (57-3)$$

The increasing regional $V_C$ and $D_M$ approach zero. These factors determine the degree of pulmonary capillary recruitment in response to physiologic stimuli and in lung diseases.

**Hemoglobin**

$D_{LCO}^{SB}$ increases with increasing Hb concentration ([Hb]) and decreases with decreasing [Hb] because $\theta$ is directly proportional to [Hb]. Although adjustment of $D_{LCO}^{SB}$ to a standard [Hb] (146 g/L for men and 134 g/L for women) is recommended, the precise effect of changes in Hb on $D_{LCO}^{SB}$ is controversial. A commonly recommended theoretical adjustment, which assumes that $D_M/V_C$ is 0.7 (or 230 in SI units), underestimates the effect of anemia on $D_{LCO}^{SB}$. In patients recovering from severe anemia $D_{LCO}^{SB}$

**Pulmonary Capillary Recruitment**

$D_{LCO}^{SB}$ is not maximal in normal, seated subjects at rest because the pulmonary capillary bed is not fully recruited, that is, open or distended. Capillaries behave like millions of highly collapsible tubes connected in parallel and stacked in sheets one above the other. Gravitational forces create a gradient in pulmonary perfusion and, hence, also in diffusion that is greater in the base than the apex of the lung. There are three zones of perfusion that are not only influenced by pulmonary artery pressure ($P_a$) and pulmonary venous pressure ($P_v$). In zone III, at the bottom of the lung where $P_a$ and $P_v$ exceed $P_A$, capillaries are mostly recruited and distended. With distance down this zone, capillaries become more distended, thus increasing regional $V_C$. In zone II in the midlung region, where $P_a$ is higher than $P_v$, but $P_A$ exceeds $P_v$, a waterfall or sluice effect develops whereby capillary blood flow depends on the difference between $P_A$ and $P_a$. For capillaries situated gravitationally lower down this zone, there is progressively greater recruitment or distention, thus increasing regional $V_C$ and $D_M$. In zone I at the lung apex, where $P_A$ exceeds both $P_a$ and $P_v$, there is minimal blood flow, so the capillaries are nearly empty, and regional $V_C$ and $D_M$ approach zero. These factors determine the degree of pulmonary capillary recruitment in response to physiologic stimuli and in lung diseases.

**Clinical Respiratory Physiology**
increases 1.4 mL/min/mm Hg for every 10 g/L increase in 
[Hb].\textsuperscript{13} Empiric observations in anemic patients reveal that 
\( \text{DL}_{\text{CO}} \) decreases 7% for every 10 g/L decrease in [Hb],\textsuperscript{14} but the effect in a general (nonanemic) population is only about 2% per 10 g/L.\textsuperscript{16}

**CARBOXYHEMOGLOBIN**

Carboxyhemoglobin (COHb) is present in small concentrations (\textlesssim 1%) in nonsmokers but increases with repeated \( \text{DL}_{\text{CO}} \) testing\textsuperscript{17} and also increases up to 10% or higher in chronic smokers. Failure to adjust \( \text{DL}_{\text{CO}} \) for increasing COHb causes two separate effects. First a portion of the Hb becomes bound by CO and, hence, is no longer available for further CO uptake, thus reducing the effective [Hb]. This is called the “anemia effect.” Empirically increasing \( \text{DL}_{\text{CO}} \) by 1% for every 1% increase in COHb\textsuperscript{18} approximates the adjustment for this effect. Second \( \text{DL}_{\text{CO}} \) is still underestimated unless adjustment is also made for the ambient alveolar [CO], or “back-pressure,” in the lung prior to each single breath. This is assumed to be zero but is actually increased in smokers.\textsuperscript{17} When ignored, the decay of CO during \( t_{\text{BH}} \) is underestimated and, hence, \( \text{DL}_{\text{CO}} \) is reduced. For a COHb of 10%, \( \text{DL}_{\text{CO}} \) is still spuriously decreased by approximately 6 to 10% if adjusted for COHb but not for CO back-pressure.\textsuperscript{17} Abstaining from smoking for 24 hours prior\textsuperscript{14} eliminates any COHb and CO back-pressure, but this is not practical in heavy smokers.

**ALVEOLAR VOLUME**

If the normal lung were a collection of spheres and if \( \text{DL}_{\text{CO}} \) were directly proportional to the surface area of the spheres, then \( \text{DL}_{\text{CO}} \) would vary with alveolar volume (\( VA \)). In reality, capillary surface area, and hence \( \text{DL}_{\text{CO}} \), is preserved with decreasing \( VA \) because the alveolar-capillary membrane tends to fold, rather than shrink, as lung volume decreases. Partitioning of the longitudinal distribution of pulmonary vascular resistance reveals that the resistance of the “alveolar segment” decreases with decreasing \( VA \) from total lung capacity (TLC) to functional residual capacity (FRC)\textsuperscript{19} because pulmonary capillaries widen, thus increasing \( VC \) with decreasing \( VA \). Hence, decreasing \( VA \) from TLC to 50% of inspiratory capacity (IC) decreases \( \text{DL}_{\text{CO}} \) minimally.\textsuperscript{17} \( \text{DL}_{\text{CO}} \) is also affected by volume history because alveolar surface tension is lower on deflation versus inflation. Alveolar capillaries therefore bulge more into the alveolus on deflation than on inflation at the same absolute \( VA \)\textsuperscript{20} so \( \text{DL}_{\text{CO}} \) is higher when the same absolute \( VA \) is achieved by deflation from TLC versus inflation from FRC.\textsuperscript{21} A deep breath immediately prior to testing therefore increases \( \text{DL}_{\text{CO}} \) by at least 10%.\textsuperscript{22}

**CONVENTIONAL MEASUREMENTS OF \text{DL}_{\text{CO}}**

**HISTORICAL DEVELOPMENT OF CONVENTIONAL \text{DL}_{\text{CO}} TESTING**

Krogh had subjects inhale a vital capacity (VC) breath of CO-rich gas, at which time two alveolar samples were collected.\textsuperscript{1} The first was collected during an immediate exhalation to about 1/2 VC, to measure the [CO] (\( FA_{\text{CO}10} \)) at the beginning of breath holding (\( t_{\text{BH}} \)). After breath holding (\( t_{\text{BH}} \)) for 6 seconds, the second sample was collected, to measure the [CO] (\( FA_{\text{CO}11} \)) at the end of breath holding (\( t_{1} \)). \( VA \) was determined separately using the dilution of hydrogen. Assuming exponential CO decay, the “Krogh equation” accurately describes diffusion of CO into the blood for \( t_{\text{BH}} \) during apnea:

\[
\text{DL}_{\text{CO}} = VA \cdot \ln(FA_{\text{CO}10} / FA_{\text{CO}11}) / t_{\text{BH}} / (P_B - 47) \tag{57-4}
\]

\( P_B \) is the ambient, barometric pressure in dry gas. \( VA \) is TCL–dead space (\( V_D \)). The units, standard temperature, and pressure, dry (STPD), are mL/min/mm Hg. To convert to SI units (mmol/min/kPa), divide the value by 2.986.

Krogh’s \( \text{DL}_{\text{CO}} \) method was ignored until 1957, when Ogilvie\textsuperscript{23} proposed eliminating the first alveolar sample by incorporating an inert gas, He, in the test gas. Ogilvie’s\textsuperscript{23} standardized \( \text{DL}_{\text{CO}} \) test, still in use today (Figure 57-3), had subjects rapidly inhale test gas containing 0.3% CO, 10% He, and 21% \( O_2 \), balance \( N_2 \) from residual volume (RV) to TLC. After an arbitrary \( t_{\text{BH}} \) of 10 seconds, subjects exhaled to RV. After exhaling sufficient gas to clear \( V_D \), a single alveolar sample was collected to measure the alveolar [CO] (\( FA_{\text{CO}10} \)) and [He] (\( FA_{\text{He}11} \)) (see Figure 57-3). Instead of directly measuring the [CO] at time zero (\( FA_{\text{CO}10} \)), Ogilvie’s major modification\textsuperscript{23} was to use the simultaneously measured dilution of He to estimate the [CO] at the onset of breath holding, where

\[
FA_{\text{CO}10} = F_1 \cdot FA_{\text{He}11} / F_1 \tag{57-5}
\]

The [CO] and [He] in the inspired gas are \( F_1 \) and \( F_2 \), respectively. Ogilvie collected all of the alveolar gas, except the first liter (to clear \( V_D \)), and measured \( t_{\text{BH}} \) from onset of inhalation (\( t_0 \)) to the beginning of sample collection (\( t_{1} \)) (see Figure 57-3).\textsuperscript{23} \( \text{DL}_{\text{CO}} \) was

\[
\text{DL}_{\text{CO}} = VA \cdot \ln(F_1 FA_{\text{CO}10} / FA_{\text{CO}11}) / (t_1 - t_0) / (P_B - 47) \tag{57-6}
\]

In 1961, Jones and Meade\textsuperscript{24} recognized that errors were caused by applying the Krogh breath-holding equation (see Equation 57-3), valid only for the portion of \( t_{\text{BH}} \) during apnea, to Ogilvie’s method.\textsuperscript{23} Errors could be minimized by measuring an “adjusted” \( t_{\text{BH}} \), from 3/10 of the time of inhalation to the time for one-half of the alveolar sample collection, and by collecting a small (85 mL) alveolar gas sample (see Figure 57-3).\textsuperscript{24}

**CONVENTIONAL \text{DL}_{\text{CO}} \text{ METHOD IN CURRENT PRACTICE**

Current recommendations (see Figure 57-3) for a standardized method\textsuperscript{14,25} use modifications of Ogilvie’s method to calculate \( \text{DL}_{\text{CO}} \) (see Equation 57-6). Ogilvie used an independent method to measure \( VA \), but today \( VA \) (converted to STPD) is measured from the single breath dilution of He, as proposed by McGrath and Thompson,\textsuperscript{26} where

\[
VA = VC \cdot F_1 \text{He} / F_A \text{He} - V_D \tag{57-7}
\]

The Jones and Meade\textsuperscript{27} method (see Figure 57-3) of measuring the adjusted \( t_{\text{BH}} \) is preferred,\textsuperscript{24} but alternate methods remain in common use. Many lung function testing systems
still allow washout of a fixed volume (0.75–1.0 L) during the initial phase of exhalation, assuming that \( V_D \) is smaller than this value. Thereafter, a fixed alveolar sample (0.5–1.0 L) is collected to measure alveolar [CO] and [He]. More recently, use of rapidly responding analyzers for both CO and a tracer gas (methane) improves testing by more accurately defining the dead space washout of CO, so that the timing of the alveolar sample is appropriate (see Figure 57-3, sample A).

**PITFALLS IN CONVENTIONAL DL\(_{CO}^{SB} \)**

**COMPUTATIONAL ERRORS**

Since the Krogh equation is valid only when inhalation and exhalation are infinitely fast, conventional methods are accurate only when single breath approaches this ideal. Deviations from the ideal cause computational errors in DL\(_{CO}^{SB} \) because the Krogh equation does not accurately describe the changes in alveolar [CO] during either inhalation or exhalation, even assuming a homogeneous lung model. This is an important pitfall because only about 70% of the adjusted tBH actually occurs during apnea at TLC, even under optimal conditions. At least 10% of the adjusted tBH occurs during inhalation, whereas at least an additional 20% usually occurs during exhalation (see Figure 57-3). Rapid exhalation is not mandated because of limitations in testing equipment and patient cooperation or capability.\(^{14,25}\) Decreasing the exhaled flow rate increases DL\(_{CO}^{SB} \)\(^{23,24,28,29}\) because a greater proportion of CO uptake occurs at lung volumes below TLC when CO is disappearing from the lung, not only by diffusion into the blood but also by exhalation. When the inhaled flow rate decreases, DL\(_{CO}^{SB} \) decreases because the adjusted tBH is represented mostly by inhalation, during which time CO has had insufficient time to reach the air-blood barrier.\(^{29}\) Decreasing tBH markedly reduces the proportion of time that actually occurs during apnea, and, hence, the Krogh equation is no longer mathematically accurate. Its use has variable effects on DL\(_{CO}^{SB} \), depending on the measurement of the adjusted tBH, the amount of time spent during inhalation and exhalation, and the details of alveolar sample collection. DL\(_{CO}^{SB} \) increases with decreasing tBH using Ogilvie's method,\(^{23}\) whereas Jones and Meade's method, using small (85 mL) alveolar samples, is less affected by decreasing tBH in normal subjects.\(^{27}\)

The size and timing of the alveolar sample collection cause further variability. Collecting the sample too early will include \( V_D \) in the alveolar gas, causing DL\(_{CO}^{SB} \) to be grossly underestimated because CO-rich dead space gas contaminates the alveolar sample (see Figure 57-3, sample B).\(^{30}\) DL\(_{CO}^{SB} \) is slightly overestimated if \( V_D \) is not subtracted from \( V_A \). In the conventional DL\(_{CO}^{SB} \) method a fixed washout volume is used (0.75–1.0 L),\(^{14}\) which assumes that this volume is adequate to wash out \( V_D \). However, the washout volume actually exceeds 1.0 L in 26% of cases when measured using a rapidly responding analyzer.\(^{31}\) Furthermore, DL\(_{CO}^{SB} \) is progressively overestimated the later in exhalation that an alveolar sample of fixed size is collected because a greater proportion of the adjusted tBH occurs during exhalation (see Figure 57-3).\(^{28,29}\) When the entire alveolar sample is collected, DL\(_{CO}^{SB} \) is overestimated\(^{32}\) because Ogilvie's adjusted tBH (see Figure 57-3), which stops at the onset of sample collection,\(^{23}\) ignores CO diffusion into the blood during sample collection.
LUNG VOLUME
Measuring $DL_{CO}^{SB}/V_A$, the so-called Krogh's constant, does not compensate for the effect of changing $V_A$ because this ratio is not constant as lung volume decreases. With decreasing $V_A$, $DL_{CO}^{SB}$ decreases far less than $V_A$, so $DL_{CO}^{SB}/V_A$ actually increases, and the increase is much greater when the single breath is immediately preceded by a deep breath or sigh.\(^{31}\) For maneuvers preceded by a deep breath, $DL_{CO}^{SB}/V_A$ increases by 15% when IC decreases from 100 to 50%.\(^{31}\) The practice of using $DL_{CO}^{SB}/V_A$ to “correct” $DL_{CO}^{SB}$ for variations in lung volume is therefore not recommended.

INERT GAS DILUTION
The principle of inert gas dilution\(^{23,26}\) assumes homogeneous mixing of inspired gas with alveolar gas. When the distribution of ventilation is nonuniform, computational errors occur in measuring $V_A$ (see Equation 57-7) and $F_A\text{CO}_2$ (see Equation 57-5) because the phase III slope for the tracer gas (He), rather than being horizontal, is negative (Figure 57-4) and the slope is steeper with shorter $t_{BH}$, with smaller inspired volumes, and in patients with airway disease. This causes $F_A\text{He}_{t_{1}}$ to be higher both when an alveolar sample is collected early versus late in exhalation (see Figure 57-3, sample A vs C) and when a smaller versus larger alveolar gas sample is collected after a fixed washout volume. Whenever $F_A\text{He}_{t_{1}}$ increases, $V_A$ decreases (see Equation 57-7) and $F_A\text{CO}_2$ increases (see Equation 57-5).\(^{31}\) In patients with airflow obstruction, $V_A$ is underestimated by 7 to 9% when measured from a 1.0 L alveolar sample and by 9 to 13% when measured from a 0.2 L alveolar sample.\(^{9}\) The two errors (measuring $V_A$ and $F_A\text{CO}_2$) have opposite effects on $DL_{CO}^{SB}$, but if $F_A\text{CO}_2$ (see Equations 57-4 and 57-6) were the same in the calculation, the net effect is an increase in $DL_{CO}^{SB}$ because the CO decay term is logarithmic (see Equation 57-6). In practice, the overall effect is complicated since the $[CO]$ in the alveolar gas sample ($F_A\text{CO}_2$) also varies with the size and timing of sample collected (see Figure 57-3).

ALVEOLAR PRESSURE
$P_A$, not routinely monitored, is assumed to be atmospheric $P_B$ (see Equation 57-6). When the actual mean $P_A$ differs from $P_B$, computational errors occur (about 5–10%), which spuriously increase $DL_{CO}^{SB}$ when $P_A$ is higher than $P_B$ (Valsalva's maneuver) and decrease $DL_{CO}^{SB}$ when $P_A$ is lower than $P_B$ (Muller's maneuver). However, the dominant effect of changes in $P_A$ on $DL_{CO}^{SB}$ is in the opposite direction and is caused by a direct effect on the pulmonary capillaries. A Muller maneuver creates subatmospheric $P_A$, which increases $DL_{CO}^{SB}$ by increasing pulmonary capillary recruitment. $DL_{CO}^{SB}$ (corrected for changes in $P_A$) increases by 10 to 20% if $P_A$ is reduced only during slow constant $V_C$ inhalation\(^{34,35}\) and by 6% if subjects actively inspire against a closed glottis or valve near TLC during breath holding.\(^{36}\) Alternatively, increasingly positive $P_A$ (Valsalva's maneuver) during breath holding decreases cardiac output\(^{37}\) and $V_C$,\(^{36}\) thus decreasing $DL_{CO}^{SB}$ by about 20%.\(^{36}\) Highly positive $P_A$ during rapid, forced exhalation also lowers $DL_{CO}^{SB}$, particularly with increasing airflow obstruction.\(^{38}\)

REPRODUCIBILITY
Large variability in the conventional $DL_{CO}^{SB}$ exists, despite rigid standardization,\(^{14,25}\) which, in practice, is not achieved in one-third of healthy subjects,\(^{39}\) and success is even less likely in patients with lung disease. Predicted values for $DL_{CO}^{SB}$ vary markedly and need to be chosen to match results from a locally studied healthy population. The limits for “normality” should be based on the 95% confidence limits, which result in a wide range for normality (about 70–130% predicted). The source of this wide variability is threefold. First, there is biologic variability resulting from the effects of changes in Hb, $P_A$, cardiac output, COHb, and CO back-pressure, as well as the effects of the circadian rhythm and variations during the normal menstrual cycle in women. $DL_{CO}^{SB}$ decreases by 9% by the third day of menses compared with the week preceding menses.\(^{70–130%}\) The source of this wide variability is threefold. First, there is biologic variability resulting from the effects of changes in Hb, $P_A$, cardiac output, COHb, and CO back-pressure, as well as the effects of the circadian rhythm and variations during the normal menstrual cycle in women. $DL_{CO}^{SB}$ decreases by 9% by the third day of menses compared with the week preceding menses.\(^{70–130%}\) Second, there is variability related to how the single breath is performed, that is, variations in the inhaled and exhaled flow rates, $V_A$, volume history, $t_{BH}$, and the speed, size,
and timing of alveolar sample collection. However, this type of variability is disease specific, that is, in patients with airflow obstruction the reduced flow rates prolong exhalation, whereas in patients with restrictive lung disease, $V_A$ is reduced. Third, there are technical factors related to calibration procedures, instrument linearity, measurement errors, algorithms for measuring $t_{BH}$, and $[O_2]$ in the test gas, which in Europe is 17% O$_2$ but in North America is 21% O$_2$.

Although DL$_{CO}$SB is routinely used, along with spirometry, to classify respiratory impairment and disability, the wide variability in DL$_{CO}$SB limits its usefulness in this regard.  

### VALUE OF CONVENTIONAL DL$_{CO}$SB TESTING IN CLINICAL PRACTICE

#### Obstructive Lung Diseases

A low DL$_{CO}$SB may help in establishing a diagnosis of emphysema since loss of overall alveolar surface area reduces DL$_{CO}$SB, and the decrease correlates with emphysema severity, detected by high-resolution computed tomography (HRCT). In the seated position, DL$_{CO}$SB is more sensitive to lower lobe and diffuse emphysema but does not reliably detect bullous emphysema in the lung apex. A very low DL$_{CO}$SB, along with a low forced expiratory volume in 1 second (FEV$_1$), predicts poor survival for chronic obstructive pulmonary disease (COPD) patients after lung volume reduction surgery. DL$_{CO}$SB testing plays a limited role in detecting emphysema in patients with severe airflow obstruction because the test may be low in patients with little emphysema (by HRCT) but can be normal in patients with a substantial degree of emphysema. The test is either normal or low in patients with cystic fibrosis (CF). It may help to separate asthma patients from COPD patients since DL$_{CO}$SB in asthma patients is either normal or may be increased as a result of either computational errors caused by the decreased exhaled flow or more negative inspiratory pressure during forced inhalation.

#### Interstitial Lung Disease

DL$_{CO}$SB is reduced early in the course of chronic interstitial lung diseases (ILD) when the chest radiograph may still be normal, presumably because the disease process both thickens the air-blood barrier and decreases the number of capillaries participating in gas diffusion. The decrease in DL$_{CO}$SB correlates with overall lung involvement by HRCT and appears more sensitive than changes in lung volume in screening patients at risk for pneumoconiosis. DL$_{CO}$SB decreases in acute ILD, such as Mycoplasma pneumonia, early lung transplant rejection, and bronchiolitis obliterans, and also decreases in chronic ILD, which occurs in scleroderma, rheumatoid arthritis, systemic lupus erythematosus, idiopathic pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, lymphangioleiomyomatosis, and ILD induced by drugs, such as nitrofurantoin, bleomycin, or amiodarone. DL$_{CO}$SB may be useful in the detecting and following the progression of drug-induced lung disease.

### Pulmonary Vascular Obstruction

DL$_{CO}$SB is decreased by pulmonary vascular obstruction (PVO), which occurs following lymphangiography, talc emboli in talc granulomatosis, recurrent leukocyte sequestration as a result of chronic hemodialysis, and pulmonary emboli (PE) of either thrombotic or tumor origin. Although the test has no current role in the investigation of suspected PE, a low value should raise the suspicion of this diagnosis. DL$_{CO}$SB is decreased more in PE, the greater the PVO, and is also decreased in patients with chronic unresolved PE and primary pulmonary hypertension. However, in the seated position at rest, the reduction in DL$_{CO}$SB may be masked by compensatory recruitment or distention of the pulmonary capillaries in lung zones spared from vascular occlusion. “Static pulmonary capillary blood,” distal to obstructed pulmonary vascular segments, could cause the diffusing capacity to decrease from the first to the fifth of five sequential single breaths, but this method of PE detection needs further validation.

### Cardiovascular Disease

When pulmonary vascular resistance (PVR) is normal, DL$_{CO}$SB changes according to the principles that govern pulmonary capillary recruitment. DL$_{CO}$SB increases when cardiac output or inflow pressure ($P_a$) increases (exercise, left-to-right shunt) or when outflow pressure ($P_v$) increases (mild congestive heart failure [CHF] or early mitral stenosis). However, DL$_{CO}$SB decreases when $P_a$ decreases (pulmonary stenosis, or systemic venodilation following administration of nitroglycerin). When mitral stenosis or CHF becomes chronic, pulmonary vascular remodeling increases PVR, leading to a decrease in DL$_{CO}$SB. The vascular remodeling appears fixed in CHF since the low DL$_{CO}$SB does not improve following heart transplantation.

#### “Isolated” Low DL$_{CO}$SB

When DL$_{CO}$SB is low, but lung function is otherwise normal, consider the following possibilities: anemia, focal emphysema, early interstitial lung disease, pulmonary vascular diseases, or undetected PE. Unlike factors include a decreased $P_a$ due to pulmonary stenosis or systemic venodilation, such as occurs with administration of a systemic vasodilator. Rule out testing errors, including contamination of the alveolar sample with $V_D$, slowing of inhaled flow, excessively long $t_{BH}$, undetected Valsalva’s maneuver, underestimation of $V_A$, or increases in COHb or CO back-pressure due to smoking or repeated DL$_{CO}$SB testing.

#### “Isolated” High DL$_{CO}$SB

When lung function is otherwise normal, but DL$_{CO}$SB is increased, consider polycythemia; anxiety or exercise immediately preceding testing; “static” alveolar red cells, which occurs in patients with pulmonary hemorrhagic syndromes; increased body mass index, seen in obstructive sleep apnea; mild asthma, left-to-right shunts; or transiently increased $P_a$ (mild left heart failure or left atrial myxoma). Rule out testing errors including decreased exhaled flow rate; shortened $t_{BH}$; collection of the alveolar sample late in exhalation; undetected Muller’s maneuver; overestimation; and timing of alveolar sample collection. However, this type of variability is disease specific, that is, in patients with airflow obstruction the reduced flow rates prolong exhalation, whereas in patients with restrictive lung disease, $V_A$ is reduced. Third, there are technical factors related to calibration procedures, instrument linearity, measurement errors, algorithms for measuring $t_{BH}$, and $[O_2]$ in the test gas, which in Europe is 17% O$_2$ but in North America is 21% O$_2$.

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of $F_A CO_{al}$ by underestimating the tracer gas dilution; or overestimation of $V_A$.

**Stress Testing to Recruit $V_C$**

Most airway narrowing is patchy, so regional ventilation in more affected lung zones decreases, causing regional hypoxic pulmonary vasoconstriction (HPV), which diverts blood flow to more normal lung zones with relatively preserved regional ventilation. In the seated position at rest, compensatory capillary recruitment increases regional $V_C$ in relatively normal lung zones and, hence, limits the reduction in $DL_{CO-SB}$. However, HPV decreases the potential for further capillary recruitment, so the increase in $DL_{CO-SB}$, in response to interventions that normally fully recruit pulmonary capillaries, is reduced or abolished. Interventions that recruit $V_c$ include the 15° head-down position,34 exercise,23 and negative inspiratory pressure.35 In patients with CF, the increase in $DL_{CO-SB}$ on assuming the 15° head-down position or in response to high negative inspiratory pressure is reduced or abolished. Interventions that normally fully recruit pulmonary capillaries, so the increase in $DL_{CO-SB}$, in response to interventions that normally fully recruit pulmonary capillaries, is reduced or abolished. Interventions that recruit $V_c$ include the 15° head-down position, exercise, and negative inspiratory pressure. In patients with scleroderma, sarcoidosis, CF, and insulin-dependent diabetes, the supine $DL_{CO-SB}$ may therefore be more sensitive in the early detection of changes in the pulmonary vasculature, but few normal regressions for supine $DL_{CO-SB}$ exist.70

**Alternative $DL_{CO-SB}$ Testing Methods**

**Steady-State $DL_{CO}$**

The flow or uptake of CO is measured during steady-state tidal breathing and divided by the driving pressure, measured from the mean alveolar [CO] ($P_{A CO}$).71 The method, not in common use today, is usually measured during exercise because substantial errors in $P_{A CO}$ occur during resting tidal breathing.

**Rebreathing $DL_{CO}$**

During rapid rebreathing, the decrease in [CO] from a rebreathing bag, measured using a rapidly responding CO analyzer, is assumed to reflect the uptake of CO from the lungs. The test, used mostly in normal subjects during exercise,23 requires considerable subject cooperation, and the high airflow rate and lack of $t_{BH}$ actually impair diffusive gas mixing in the lung periphery (see Figure 57-2).73

**“Intrabreath” $DL_{CO}$ or $DL_{CO}$ (Exhaled)**

This method24,38 assumes that the decrease in CO, measured only during constant flow, slow exhalation using rapidly responding analyzers,74–76 represents the diffusion of CO across the air-blood barrier. However, the decrease in CO (Figure 57-5) during exhalation is not only due to ongoing diffusion of CO into the blood but is also influenced both by transient, $t_{BH}$-dependent gradients in [CO] within peripheral gas-exchanging units (see Figure 57-2)77 and by differences in [CO] among larger parallel lung units (due to regional differences in specific ventilation), which empty sequentially.78 Therefore, ventilation inhomogeneity alone changes the slope for CO, and this effect is superimposed on true change in [CO] during exhalation owing to diffusion of CO into the blood.77 The strategy of “correcting” the observed CO slope for the simultaneously measured slope of He ($F_A CO_{al} (corrected) = F_A CO_{al} · F_A He_{et} / F_A He_{al}$) does not satisfactorily eliminate the effects of ventilation inhomogeneity on the slope of the CO decay curve because ongoing CO diffusion into the blood causes the regional ratios

![FIGURE 57-5 Three-equation method ($DL_{CO-SB-3EQ}$).](image-url)

The single breath consists of slow (0.5 L/s) VC inhalation of test gas, variable breath holding at TLC (5 s in the example), and slow (0.5 L/s) exhalation to RV. Since the factors that determine the change in [CO] in the alveolar space are actually different in the three different phases of the single breath, three separate mass balance equations are used. During inhalation CO enters the alveolar space through the mouth as it simultaneously leaves the alveolar space by diffusion. CO leaves the alveolar space only by diffusion during breath holding (Krogh equation). During exhalation, CO simultaneously leaves the alveolar space by both exhalation and by diffusion. The three equations (upper graph) are solved using an iteration technique that finds the single value of $DL_{CO-SB-3EQ}$ that best predicts the observed amount of CO exhaled. Digital recording eliminates the need for physically collecting a gas sample because any “virtual” gas sample can be constructed. The entire alveolar gas sample provides the best estimate of $DL_{CO-SB}$ of the average diffusion characteristics for the entire lung. To calculate an index of diffusion nonuniformity, $D_i$ (see Figure 57-6), $DL_{CO-SB-3EQ}$ is calculated from each of four equal alveolar samples (Sample 1, 2, 3, 4), expressed as a percentage of the $DL_{CO-SB-3EQ}$ from the entire alveolar gas sample.
of alveolar $[CO]/[He]$ to become dramatically different over time, particularly when diffusion inhomogeneity coexists. The DLCO$_{(\text{exhaled})}$ method is therefore not suitable for measuring lung diffusion in the presence of increased ventilation inhomogeneity.

**Diffusing Capacity Using Nitric Oxide**

Nitric oxide (NO) binds tightly with Hb with an affinity that is 400 times greater than that of CO. Diffusing capacity using nitric oxide (DLCO$_{\text{NO}}$) can be measured simultaneously with DLCO$_{\text{SB}}$. It is fourfold larger than DLCO$_{\text{SB}}$, independent of $[O_2]$, and measures primarily $D_M$ in normal subjects. However, in patients with lung disease, NO may alter its binding with Hb, making DLCO$_{\text{NO}}$ less accurate.

**Three-Equation Single-Breath Diffusing Capacity**

The three-equation single-breath diffusing capacity (DLCO$_{\text{SB-3EQ}}$) method, which is a significant advance over conventional methods, takes advantage of rapidly responding analyzers to continuously measure airflow rates, volume, CO, and a tracer gas, such as He or methane, throughout the single breath. By digitally recording all the data, the need to physically collect an alveolar gas sample is eliminated. A “virtual” sample of any size can be constructed and analyzed for either CO or He for any time period (see Figure 57-5). Rather than using the single Krogh equation, three separate mass balance equations, each valid for the respective phases of inhalation, breath holding, and exhalation, are used to accurately describe the instantaneous changes in CO in the alveolar space throughout the entire single breath (see Figure 57-5). The equations are solved using a standard iteration technique, which matches the predicted alveolar [CO] to that measured in “virtual” sample, consisting of all the exhaled alveolar gas (see Figure 57-5).

Successive iterations (usually <12) converge to a single DLCO$_{\text{SB-3EQ}}$, which predicts the measured alveolar [CO] to within a tolerance of 0.1%. The method assumes a homogeneous lung with a constant DLCO$_{\text{SB}}$, a single Fowler VC, measured for each breath (see Figure 57-4); a single homogeneously mixed alveolar space; and the same instantaneous changes in flow rate and $V_A$, observed in the actual maneuver (see Figure 57-5). To measure $V_A$ more accurately [He] is monitored continuously, and a mass balance equation is used to describe the behavior of He throughout the entire single breath (see Figure 57-4). Subjects perform a slow deep breath of room air from FRC to TLC, with 5 seconds of tBH, immediately before the DLCO$_{\text{SB}}$ test to eliminate variations in DLCO$_{\text{SB}}$ due to previous volume history. The mean alveolar [CO] during the exhalation phase of the deep breath is used to adjust DLCO$_{\text{SB-3EQ}}$ for CO back-pressure and for COHb. Since DLCO$_{\text{SB-3EQ}}$ is accurate for a homogeneous lung, despite changes in the way the single breath is performed or the way the alveolar sample is collected, measurements can be obtained not only during large VC single breaths but also during submaximal “near-tidal” breaths with slow flow rates. Since it is not necessary to strictly standardize $t_{BH}$, as is the case using the conventional method, DLCO$_{\text{SB-3EQ}}$ is more precise and accurate in both normal subjects and patients with lung disease and is independent of $t_{BH}$ in normal subjects.

DLCO$_{\text{SB-3EQ}}$ could potentially decrease with decreasing $t_{BH}$ if worsening ventilation inhomogeneity increases $1/G_{MIX}$. In patients with significant airflow obstruction and ventilation inhomogeneity (COPD, asthma, and emphysema), DLCO$_{\text{SB-3EQ}}$, measured from the entire alveolar sample, actually decreased with decreasing $t_{BH}$ during slow VC breaths, suggesting that the pathway for CO was significantly affected by an increase in $1/G_{MIX}$ (see Figure 57-2) during short periods of $t_{BH}$. When submaximal single breaths are performed near the tidal volume range, $t_{BH}$-dependent inhomogeneity in gas mixing in the lung periphery increases dramatically, even in normal subjects, thus potentially increasing $1/G_{MIX}$. However, no effect of decreasing $t_{BH}$ was found in either normal subject nonsmokers or smokers with normal forced exhaled flow rates when DLCO$_{\text{SB-3EQ}}$ was measured from the entire alveolar sample during slow inhalation-exhalation “near-tidal” breaths, performed from FRC to one-half IC with exhalation to RV. In contrast, $t_{BH}$-dependent ventilation inhomogeneity did affect DLCO$_{\text{SB-3EQ}}$ in normal subjects during near-tidal breaths when DLCO$_{\text{SB-3EQ}}$ was measured.
separately from each of four equal alveolar samples, revealing temporal changes in DL\textsubscript{CO}\text{SB-3EQ} from early versus late samples.\textsuperscript{82} DL\textsubscript{CO}\text{SB-3EQ} was reduced in the first alveolar sample, early in exhalation, but increased for alveolar samples later in exhalation (Figure 57-6).\textsuperscript{82} An index of diffusion nonuniformity (D\textsubscript{I}) quantified how each of the four normalized DL\textsubscript{CO}\text{SB-3EQ} values deviated from DL\textsubscript{CO}\text{SB-3EQ}, measured from the entire alveolar sample and was measured using the root mean square difference.\textsuperscript{10,80} In normal, nonsmokers D\textsubscript{I} was exquisitely sensitive to t\textsubscript{BH}-dependent ventilation inhomogeneity in the lung periphery since D\textsubscript{I} was highest for single breaths without t\textsubscript{BH} and decreased dramatically with as little as 6 seconds of t\textsubscript{BH} (see Figure 57-6).\textsuperscript{82}

For “near-tidal” breaths without t\textsubscript{BH}, the normalized phase III He slope was also increased in smokers, even although both the forced exhaled flows and the phase III slope of the standard, vital capacity and single-breath nitro-

gene exchange (DI) quantified how each of the four normalized DL\textsubscript{CO}\text{SB-3EQ} values deviated from DL\textsubscript{CO}\text{SB-3EQ}, measured from the entire alveolar sample and was measured using the root mean square difference.\textsuperscript{10,80} In normal, nonsmokers D\textsubscript{I} was exquisitely sensitive to t\textsubscript{BH}-dependent ventilation inhomogeneity in the lung periphery since D\textsubscript{I} was highest for single breaths without t\textsubscript{BH} and decreased dramatically with as little as 6 seconds of t\textsubscript{BH} (see Figure 57-6).\textsuperscript{82}

For “near-tidal” breaths without t\textsubscript{BH}, the normalized phase III He slope was also increased in smokers, even although both the forced exhaled flows and the phase III slope of the standard, vital capacity and single-breath nitrogen washout were normal.\textsuperscript{83} In this same group of smokers, D\textsubscript{I} was markedly increased for near-tidal breaths without t\textsubscript{BH} (see Figure 57-6) and correlated with both age and pack-years of smoking.\textsuperscript{10} D\textsubscript{I} decreased with increasing t\textsubscript{BH} in both smokers and nonsmokers, but the change was much greater in smokers. With increasing t\textsubscript{BH} the change in D\textsubscript{I} also correlated with the improvement of the phase III He slope.\textsuperscript{10} The change in D\textsubscript{I} with t\textsubscript{BH} therefore quantifies an effect of an increase in I/G\textsubscript{MIX} for single breaths within the tidal volume range and, may, along with changes in the phase III He slope, be useful in detecting smoke-induced inflammation in small airways.\textsuperscript{10,83}

**SUMMARY**

The conventional DL\textsubscript{CO}\text{SB} is a clinically useful, noninvasive tool to assess the integrity of the air-blood barrier but lacks sensitivity and specificity because it is affected by a number of physiologic variables and because, even for a homogeneous lung, the single, Krogh breath-holding equation causes maneuver-related computational errors that are disease specific. DL\textsubscript{CO}\text{SB-3EQ} avoids the computational errors of the conventional DL\textsubscript{CO}\text{SB} method because the mass balance equations accurately describe the behavior of both CO and He throughout a single breath. DL\textsubscript{CO}\text{SB-3EQ} is equally valid for maneuvers with varying flow rates, V\textsubscript{A}, and t\textsubscript{BH}. The advantages of slow inhalation–exhalation single breaths are that the distribution of an inert tracer gas can be measured simultaneously, the effects of wide swings in P\textsubscript{A} are minimized, and the maneuvers with short t\textsubscript{BH} can be performed equally well by both normal subjects and patients alike. The best estimate of DL\textsubscript{CO}\text{SB-3EQ} for the whole lung is obtained using the entire alveolar gas sample. When I/G\textsubscript{MIX} increases, the pathway for CO is altered, causing changes, with t\textsubscript{BH}, both in DL\textsubscript{CO}\text{SB-3EQ} measured from the entire alveolar sample in patients with airflow obstruction and in D\textsubscript{I} in smokers with normal lung function.

**REFERENCES**


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