

# Editorial

## The single-breath carbon monoxide transfer test 25 years on: a reappraisal

### 1—Physiological considerations

The breath-holding or single-breath technique for the measurement of the *diffusing capacity*, or *transfer factor*, of the lung for carbon monoxide—which I shall symbolise as DL or DLCO—in man is now 25 years old<sup>1</sup> and this provides a moment of nostalgia and perspective, for which I am grateful.

The technique consists of inspiring rapidly and maximally from near-residual volume a gas mixture consisting of a low percentage of carbon monoxide (about 0.4%) and an inert gas such as helium (10%) in a balance of oxygen (usually 20%) and nitrogen. This inspiration is held for 10 seconds and breath is then rapidly expired. An alveolar sample is collected and analysed for carbon monoxide and helium. DL, defined as the volume of carbon monoxide transferred into the blood per minute per mm Hg of partial pressure of carbon monoxide (Pco), is computed from the relation

$$DL = \frac{\text{alveolar volume nat log}}{(P_B - 47) \Delta t} \times \frac{[\text{CO}] \text{ inspired } [\text{He}] \text{ alveolar}}{[\text{CO}] \text{ alveolar } [\text{He}] \text{ inspired}} \quad (1)$$

Alveolar volume is in ml STPD;  $\Delta t$  is the time of breath holding in seconds;  $P_B$  is barometric pressure, and 47 is the vapour pressure of normal saline, both in mm Hg. The concentrations of gases are represented by square brackets and are in compatible units.

M Krogh devised an earlier method for the measurement of DL 68 years ago using two alveolar samples.<sup>2</sup> The major innovation of the single-breath method was the introduction of an inert tracer gas in the inspired mixture, permitting calculation of the concentration of carbon monoxide in the expired alveolar sample before any carbon monoxide had been absorbed by the blood and eliminating the need for more than one alveolar gas sample. Clearly the single-breath DLCO technique did not represent a giant step beyond the work of the Kroghs.

While we<sup>3</sup> had been able to calculate reasonable values for DL in normal subjects from a semi-

logarithmic plot of the concentration of expired alveolar carbon monoxide against time of breath-holding in repeated experiments, I expected that the variability in DL measured by single samples would be so great as to render the data of minimal value for clinical work. My colleagues went ahead despite my views, fortunately. There was a crisis when we found that after the subject had run rapidly up four flights of stairs DL was the same as at rest. Our senior adviser commented that since it was well known that the pulmonary diffusing capacity increased on exercise<sup>2,4</sup> the single-breath method was clearly in error and we should seek another field of research. Happily we discovered that the single-breath DL value did increase during exercise, but fell rapidly after the exercise stopped. With the clumsy experimental set-up we were using at that time we simply could not obtain a measurement rapidly enough.

There is disagreement about the best term (and the best symbol) for what I have indicated by DL, which is in fact best defined by the above equation. This ratio was designated the *diffusing capacity of the lung* by a group of American respiratory physiologists<sup>5</sup> in 1950 in an effort to reduce the confusion existing then (most of this group are active today and I urge that any complaint should be directed to them). The term is far from perfect; *capacity* implies an unintended maximal limit and *diffusion* implies that the exchange occurs by diffusion alone, which is incorrect. Another term, *transfer factor*,<sup>6</sup> symbolised by TL, was introduced in 1965—an improvement with less specification of the exchange process. Other exasperatingly similar terms have been used for DLCO, among which are *diffusion constant*,<sup>7</sup> *diffusion coefficient*<sup>8</sup> of the lung, and *diffusion factor* of the lung.<sup>9</sup> The choice of D to symbolise diffusing capacity does not have a profound origin. The small L was introduced by Roughton and myself<sup>10</sup> to separate the overall transfer properties of the lung-capillary system (DL) from that of the membrane alone (DM) and from those of the red cells in the pulmonary alveolar capillaries ( $\theta V_c$ , where  $\theta$  is the rate of combination of carbon monoxide with intracellular haemoglobin in 1 ml of normal whole blood expressed in ml CO min<sup>-1</sup> mm Hg<sup>-1</sup> and  $V_c$  is the volume of the capillary bed in

Address for reprint requests: Dr Robert E Forster, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA.

ml). This is presumably the reason why the term transfer factor was suggested. I cannot but applaud efforts to make our terminology more logical and consistent, but "diffusing capacity" seems well established in the New World, and I wonder whether the energy required to cause its wide replacement will be justified. I consider, however, that this is a question for the users.

It was originally assumed by physiologists two decades ago that the rate-limiting process in the exchange of carbon monoxide in lungs was diffusion across the pulmonary membrane, lying between perfectly mixed alveolar gas and perfectly mixed blood in which intracellular haemoglobin reacts instantaneously with ligand. The measurement of breath-holding DL at different levels of alveolar Po<sub>2</sub> and analysis of the data by equation 2 below has provided estimates of capillary blood volume and diffusing capacity of the membrane and of the relative resistance to exchange in the blood and in the membrane. Our present view is that the alveolar capillaries expose nearly naked red cells to alveolar gas, that the chemical reaction and simultaneous diffusion within the erythrocyte is a major rate-limiting process even in normal subjects breathing air, and that the diffusing capacity of the membrane (DM) is often so great as to be difficult to measure precisely.

It is not entirely clear what DM and Vc represent physically in the lung, although the basics of the model described by equation 2 have stood up well.

$$\frac{1}{D_L} = \frac{1}{D_M} + \frac{1}{\theta V_c} \quad (2)$$

Cutting through the equations and manipulations,  $1/V_c$  is that part of the carbon monoxide transfer resistance that changes with alveolar Po<sub>2</sub>, and  $1/D_M$  is the transfer resistance that is left over.  $1/D_M$  includes the diffusion resistance of the pulmonary membrane, but may also include the resistance of a stagnant layer of plasma. Intuition suggests that any mechanical changes in the capillary bed that alter Vc might also alter the surface area of the membrane in a parallel manner in normal subjects. Experimentally during exercise this is certainly the case.<sup>4</sup> Burns and Shepard<sup>11</sup> attempted to measure diffusion resistance of the pulmonary membrane alone in an isolated dog lung perfused with blood containing dithionite, which can react extremely rapidly with oxygen. Their object was to eliminate any oxygen gradients in the blood so that the overall diffusing capacity for oxygen ( $D_{LO_2}$ ) would equal membrane diffusing capacity ( $D_{MO_2}$ ). Their results imply that DM for oxygen and for carbon monoxide is at least an order of magnitude larger than we have believed from measurements of DLCO at different alveolar Po<sub>2</sub> (technique of Roughton and Forster<sup>19</sup>). Even

the estimate of DM made by Burns and Shepard is less than the true one because their experimental technique could reduce but not eliminate oxygen diffusion gradients within the capillary blood. With an infinite velocity for the reaction of oxygen and dithionite there would still have been diffusion gradients within the capillary.

The original pathological concept of the reduction of DL by disease was that it thickened the alveolar membrane, decreasing DM. It appears from the published reports that DM and Vc generally both decrease in disease and perhaps this is what would be expected. Anaemia<sup>12,13</sup> clearly can affect the diffusing capacity of the erythrocytes in the alveolar capillaries without altering DM appreciably, but it is  $\theta$  that is changed, not Vc. (Vc I define as the capillary blood volume independent of the packed cell volume.) It is likely that thickening of the capillary wall that would lower DM would also reduce the lumen—that is, reduce Vc. This problem of the independence of DM and Vc remains unsettled.

The values of  $\theta$  available<sup>10</sup> for the calculation of DM and Vc are based on a limited series of measurements made in vitro with two different types of rapid-mixing apparatus, stop-flow and continuous-flow, 25 years ago on subjects in Britain and the United States, and corrected theoretically to reduce the Pco from about 75 mm Hg (10 kPa) in vitro to physiological in vivo levels of about 1 mm Hg (0.13 kPa) and for possible diffusion resistance of the red cell membrane. We are at present expanding these data with improved techniques under more consistent conditions and fortunately are finding results similar to those of 1957.

In calculating DM and Vc from measurements of DL only data obtained at levels of alveolar Po<sub>2</sub> greater than about 150 mm Hg (20 kPa) should be used. At lower alveolar Po<sub>2</sub> it is not safe to assume a constant Po<sub>2</sub> inside the erythrocyte equal to alveolar Po<sub>2</sub> along the capillary, a basic assumption in the selection of in vitro estimates of  $\theta$  for insertion into equation 2. Although the data obtained by violators of this rule appear reasonable, the magnitude of the error introduced is hard to determine.

In 1957 we were aware of several common important variables that could change DL in normal subjects; others have been added since. The clinician should be aware of these influences if he is to control them or at least take account of them in interpreting measurements of DL. A list of these factors, which is not exhaustive, is given in table 1.

It is the unfortunate reality that in the presence of regional non-uniformity of DL/VA and of inspired volume/alveolar volume, we can measure the total effective transfer characteristics of the lung but cannot, within wide limits, determine the true Vc and

Table 1 Factors that may influence measured  $D_L$ 

Factor	Effects
<i>Factors that perturb computation of <math>D_L</math></i>	
↑Breath-holding time	$D_L$ unchanged in normal; often ↓ $D_L$ in chronic lung disease
Collect alveolar sample later in expirate	↓ $D_L$ <sup>14</sup>
↓Alveolar $P_{CO}$	$D_L$ unchanged in normals <sup>15 16</sup>
<i>Factors that change total <math>D_L</math></i>	
↑Body size	↑ $D_L$ as measured by height, weight, surface area, even alveolar (lung) volume, and metabolic rate <sup>1 2 6</sup>
↑Haemodynamic forces	↑ $D_L$ <sup>4</sup>
↑Pulmonary blood flow (exercise; position—lying > sitting > standing; Müller manoeuvre)	
↑Capillary transmural pressure (base > apex lung)	
↑Alveolar volume	↑ $D_M$ <sup>17-21</sup> (1) Direct effect via $D_M$ or $V_{CL}$ or both (2) Alteration in uniformity of distribution of gas or distribution of $D_L/V_A$ or both
↑Alveolar $P_{O_2}$	↓ $D_L$ from ↓ $\theta$ <sup>3</sup>
↑Alveolar $P_{CO_2}$	↑ $D_L$ from ↑ $\theta$ presumably <sup>22</sup>
↓Temperature	↓ $D_L$ predicted from theory
↑Age	↓ $D_L$ <sup>2</sup>

$D_M$ . The total  $V_c$  and total  $D_M$  of the lung are the sums of the individual capillary volumes and membrane-diffusing capacities of all alveoli. Two types of non-uniformity are particularly pertinent to the measurement of  $D_L$ —uneven distribution of inspired gas to the gas already in the alveoli and  $D_L/V_A$  (table 2). The first depends upon mechanical changes in lung volume with inspiration, the second upon the volume and surface area of the alveolar capillary bed in relation to existing alveolar volume. There is no obvious reason why these two types of non-uniformity should be related to each other, except over extreme ranges.

Table 2 Non-uniformities in the lungs of normal subjects

	Apical alveoli	Basal alveoli
Receive inspirate	Early	Late
Deliver expirate	Late	Early
Helium dilution ratio:		
Expired alveolar [He]	Low	High
inspired [He]		
Alveolar capillaries	Collapsed	Distended
$D_L/V_A$	Low	High

No aspect of the single-breath method has caused so much discussion and confusion as the measurement of alveolar volume. In concept,  $D_L$  is independent of alveolar volume—that is, it is a measure of the ease with which gases move from alveolar gas through the pulmonary membrane to the haemoglobin molecule within the erythrocyte. If the transport properties of the alveolar membrane and the erythrocytes remain constant, changing alveolar volume will not change  $D_L$ . Why then is alveolar volume included in the equation for the computation of  $D_L$ ? The answer is that it is there to calculate the flux of carbon monoxide across the alveolar membrane, which in this method equals the instantaneous rate of change of alveolar  $[CO] \times$  (alveolar volume). In the rebreathing method the flux equals the instan-

taneous rate of change of alveolar  $[CO] \times$  (alveolar + rebreathing bag volume). (Would anyone expect the size of the rebreathing bag to affect  $D_L$ ?) In the steady-state method the CO flux equals the rate of CO inspired minus the rate of CO expired: alveolar volume does not enter the computation at all.

Changing alveolar volume, however, can actually change measured  $D_L$  either by perturbing the calculation of  $D_L$ , without necessarily altering  $D_M$  or  $V_c$ , or by factors that actually change  $D_M$  or  $V_c$  or both (table 1). Experimentally  $D_L$  increases with increasing alveolar volume in a single individual,<sup>17-21</sup> primarily because of an increase in  $D_M$ .<sup>19</sup> M Krogh found that  $D_L$  increased proportionally to increases in  $V_A$ , so that  $D_L/V_A$  remained constant,<sup>2</sup> making it a seductive choice as an index of transfer properties of the lung. Unfortunately more recent studies,<sup>17-21</sup> while they corroborate the increase in  $D_L$  with increasing  $V_A$ , find the proportional increase in  $D_L$  is considerably less than the proportional increase in  $V_A$ , so that  $D_L/V_A$  does not remain constant but decreases with increasing  $V_A$ . I am not aware that anyone has found steady-state  $D_L$  to increase with increasing  $V_A$ .

Another measure that has been used to get round the difficulties of measuring  $V_A$  in severe non-uniformity of the lung is to calculate it from the helium dilution ratio. This procedure has been supported by the argument that the alveolar volume is calculated from the same gas sample as that from which the carbon monoxide disappearance is calculated. I have never seen the logical justification for this intuitive belief, however; rather I should compare it to a legal system where two wrongs make a right.

$$\frac{[\text{He}] \text{ alveolar } [\text{CO}] \text{ inspired}}{[\text{He}] \text{ inspired } [\text{CO}] \text{ alveolar}}$$

provides correctly, and independently of any non-uniformity, the ratio of the  $[CO]$  that was originally

in the collected sample to that after exchange with the capillary blood. With non-uniformity of  $DL/VA$  this ratio is a complicated and incalculable weighted average of the  $DL/VA$  of the contributing alveoli. In the presence of non-uniformity the alveolar volume calculated from the helium dilution ratio will depend on the distribution of inspired gas/alveolar volume, an entirely different form of non-uniformity from that of  $DL/VA$ . In extreme dysfunction this calculated  $V_A$  may be better than a correct estimate, such as one derived from the body plethysmograph, which would include alveolar volumes that contributed little to the expirate; but it would be a matter of luck. If this type of measurement is helpful clinically that is sufficient justification for its use, but we should be cautious about interpreting the underlying pathophysiological disorders from such a value of  $DL$ .

A third solution to the vexing problem of measuring  $V_A$  that many authors have used is to be satisfied with  $DL/VA$ . This requires only measurement of  $[He]$  and  $[CO]$  in the expired alveolar sample. Any conclusion about the state of the transfer properties of the lung, however, requires—implicitly if not explicitly—information about  $V_A$ .<sup>19</sup>

Once it was shown that  $DL/VA$  was not some kind of natural constant of the lung its physiological utility vanished. Again, if  $DL/VA$  is useful in the care of patients, that is sufficient for its adoption in those circumstances. My concern has been that there are pathological processes that can alter lung volume independently of  $DM$  and  $V_c$  which can make it misleading to use  $DL/VA$  as an index of the true state of the pulmonary capillary bed. For example, a patient with a restricted lung volume and reduced  $DM$  and  $V_c$  might be considered to have a normal pulmonary capillary bed because  $DL/VA$  was normal.

History has come full circle. The breath-holding DLCO measurement was developed by the Kroghs<sup>27</sup> to determine whether it was really necessary to assume secretion across the alveolar membrane to explain the increased uptake of oxygen during exercise, as argued by JS Haldane.<sup>24</sup> As we now know well,  $DL$  increases considerably during exercise, making the assumption of oxygen secretion unnecessary. It has recently been proposed that there is facilitated transport of carbon monoxide across the pulmonary membrane by a carrier protein.<sup>25</sup> The argument has been based primarily on the findings that  $DL$  is greater at a very low alveolar  $P_{CO}$  than it is at high levels, which has been interpreted as being the result of chemical saturation of the carrier. Several other studies, however, failed to find any change in  $DL$  over a range of alveolar  $[CO]$  from 1 to 10 000 ppm (0.0001–1%)<sup>15,16</sup> and it seems doubtful whether this provocative suggestion has

clinical significance.

As I admitted earlier, I originally doubted whether the single-breath DLCO method would provide data of sufficient reproducibility to be of clinical value. I am therefore especially delighted that the technique is in such wide use today.

ROBERT E FORSTER II

Department of Physiology  
University of Pennsylvania School of Medicine  
Philadelphia, Pennsylvania  
USA

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monary capillary blood. The same disease process can of course give rise to more than one of these three faults, sometimes with opposing effects. For example, in chronic obstructive lung disease, carbon monoxide transfer might be increased by recruitment of alveoli or capillaries enlarging the area of membrane available for diffusion, or by polycythaemia; on the other hand, it may be reduced by mismatching of air and blood or by destruction of alveoli and capillaries by emphysema. When separate measurements of membrane diffusion (Dm) and pulmonary capillary volume (Vc) are not available, the clinician must attempt to interpret the overall result of the carbon monoxide transfer test according to whether it is normal, low, high, or (in serial measurements) changing from one to the other (see table, p8). In every case it is important to take into account the lung volume at which the measurement was made and, if this is itself abnormally high or low, to calculate the carbon monoxide transfer per unit of lung volume (diffusion coefficient: Kco).

There are many formulae for the prediction of normal values, most of them based on age, sex, and height. The formula most widely used is that of Cotes.<sup>1</sup> There is, however, some disagreement between these formulae<sup>2</sup> and factors other than age, sex, and height may determine the value obtained. Apart from differences between laboratories in the performance and analysis of the breath-holding manoeuvre<sup>3</sup> and in the measurement of lung volume,<sup>4</sup> there are physiological and environmental variables such as posture,<sup>5</sup> weather,<sup>6</sup> diurnal fluctuations,<sup>7</sup> altitude,<sup>8</sup> levels of habitual activity,<sup>9</sup> and hormonal influences.<sup>10,11</sup> Ideally, each clinical laboratory should standardise its own technique, establish reference values for the population and environment from which its patients are drawn, and make regular serial measurements in “longstay” members of the hospital staff to ensure the continuing reliability of the method and equipment used.

The applications of the method to the investigation of lung disease can be considered under three headings: (1) the identification of an environmental hazard and its early detection in individual subjects; (2) monitoring the progress of lung disease in relation to the need for or the response to treatment; (3) differential diagnosis.

### Environmental hazards

A potential environmental hazard to the lungs may be identified in cross-sectional studies of an exposed group in comparison with a control group, while early detection of lung damage in individual subjects can be achieved by longitudinal studies. Carbon

## 2—Clinical considerations

The single-breath test for measuring carbon monoxide transfer is swift and painless for both patient and operator and, considering the great number of variables involved, it is surprisingly reproducible. The snags are the relatively high cost and complexity of the equipment, the need for the patient to be well enough to co-operate in breathing manoeuvres, and the intellectual discomfort of not knowing exactly what is being measured. Notwithstanding this last objection, the test does seem to have some empirical value and now—after 25 years' experience—its role in clinical practice can be more clearly defined.

Abnormalities of carbon monoxide transfer may be due to faults in matching at the air-blood interface, in the diffusing membrane itself, or in the pul-

Address for reprint requests: Dr Colin Ogilvie, Royal Liverpool Hospital, Liverpool L7 8XP.