Historical review: the carbon monoxide diffusing capacity ($D_{L_{CO}}$) and its membrane (D_M) and red cell ($\Theta \cdot V_c$) components

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Abstract

The single breath carbon monoxide diffusing capacity ($D_{L_{CO}}$ sb), also called the transfer factor ($T_{L_{CO}}$), was introduced by Marie and August Krogh in two papers (Krogh and Krogh, Skand. Arch. Physiol. 23, 236–247, 1909; Krogh, J. Physiol., Lond. 49, 271–296, 1915). Physiologically, their measurements showed that sufficient oxygen (by extrapolation from CO) diffused passively from gas to blood without the need to postulate oxygen secretion, a popular theory at the time. Their $D_{L_{CO}}$ sb technique was neglected until the advent of the infra-red CO meter in the 1950s. Ogilvie et al., J. Clin. Invest., 1957, 36, 1–17 published a standardized technique for a 'modified Krogh' single breath $D_{L_{CO}}$, which eventually became the method of choice in pulmonary function laboratories. The Roughton–Forster equation (J. Appl. Physiol., 1957 11, 290–302) was an important step conceptually; it partitioned alveolar–capillary diffusion of oxygen (O_2) and carbon monoxide (CO) into a membrane component (D_M) and a red cell component ($\theta \cdot V_c$) where θ is the $D_{L_{CO}}$ (or $D_{L_{O_2}}$) per ml of blood (measured in vitro), and V_c is the pulmonary capillary volume. This equation was based on the kinetics of O_2 and CO with haemoglobin (Hb) in solution and with whole blood Hartridge and Roughton, Nature 1923, 111, 325–326; Proc. R. Soc. Lond. Ser. A 1923, 104, 376–394; (Proc. R. Soc. Lond. Ser. B 1923, 94, 336–367; Proc. R. Soc. Lond. Ser. A, 1923, 104, 395–430; J. Physiol., Lond. 1927, 62, 232–242; Roughton, Proc. R. Soc. Lond. Ser. B 1932, 111, 1–36) and on the relationship between alveolar P_{O_2} and $1/D_{L_{CO}}$. Subsequently, the relationship between $D_{L_{O_2}}$ (Lilienthal et al., Am. J. Physiol. 147, 199–216, 1946) and $D_{L_{CO}}$ was defined. More recently, the measurement of the nitric oxide diffusing capacity ($D_{L_{NO}}$) has been introduced. For $D_{L_{O_2}}$ and $D_{L_{NO}}$ the membrane component (as $1/D_M$) is an important part of the overall diffusion (transfer) resistance. For the $D_{L_{CO}}$, $1/\theta \cdot V_c$ probably plays the greater role as the rate limiting step. A crucial question, the effect of unstirred plasma layers on the 'true' value of θ_{CO} in vivo, has not been resolved, but this does not detract from the clinical role of the $D_{L_{CO}}$ sb ($T_{L_{CO}}$) as an essential test of lung function.

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1. Introduction

The single breath test of carbon monoxide (CO) uptake has a long history—from its birth (Krogh and Krogh, 1909) to the first publication describing a standardized technique for the diffusing capacity measurement (DL_{CO}) (Ogilvie et al., 1957). The DL_{CO} was devised originally as a physiological tool to test the notion (now abandoned) that the lung, like the swim bladder of some deep-sea fish, could secrete oxygen against the normal tension gradient provided by inspired air.[1]

The DL_{CO}[2] was introduced as a *clinical* test by Marie Krogh (1915), but the measurement never caught on because methods of measuring carbon monoxide were so cumbersome. In the 1950s, with the introduction of the infra-red CO meter (developed in Germany, in World War II) interest in the DL_{CO} revived, and several different methods for measuring DL_{CO} in patients with pulmonary diseases were in use—various steady state methods, the single breath and rebreathing techniques. For various reasons (see later), the single breath technique is the one in general use today.

2. 1891–1915: early measurements

2.1. Oxygen secretion and the origins of the DL_{CO} measurement

It still comes as a surprise that one hundred years ago, the most distinguished respiratory physiologists of the day, Christian Bohr (1855–1911) and John Scott Haldane (1860–1936), believed that the lung functioned like a gland and secreted oxygen. It is possible that there was resistance at that time to the notion that the lung was a non-responsive gas exchange membrane.

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[1] Now known not to be a secretory process in the normal sense, but to be dependent on lactic acid production by the epithelium and counter-current blood flow in the rete mirabile (Scheid et al., 1990).

[2] DL_{CO} was the original term, and it is still used widely in North America but TL_{CO} (transfer factor) is used throughout Europe and the UK. Both terms are used here.

Bohr (1891) found alveolar oxygen tension (PA_{O_2}) to be as much as 30 mmHg *less* than arterial P_{O_2} (Pa_{O_2}). Haldane and Lorrain Smith (1896) measured Pa_{O_2} by an indirect method involving measurements of PA_{CO} and HbCO after achieving equilibrium with inhaled CO, and found it to be 185–200 mmHg breathing *air* (PI_{O_2} 150 mmHg). Of course, at that time, chemical analysis of the small amounts of oxygen and CO dissolved in blood was very difficult. August Krogh (1874–1949), a Nobel Prize winner, started his research career as an assistant in Bohr's laboratory in the University of Copenhagen. He improved Bohr's aerotonometer method for measuring Pa_{O_2}, and showed that it was always less than PA_{O_2}. His seven 'small devils', a series of papers in volume 25 of the *Skandinavische Archiv für Physiologie* in 1909, effectively demolished the Bohr–Haldane Oxygen Secretion theory. In the introductory section of one of these papers Krogh (1909) wrote touchingly "*I shall be obliged ….. to combat the views of my teacher, Professor Bohr, in certain essential points …… I wish here not only to acknowledge the debt of gratitude which I personally owe to him, but to emphasize the fact, patent to everybody, ….. that the real progress made during the last 20 years in the knowledge of the processes in the lungs, is mainly due to his labours*".

Another nail in the coffin of the Oxygen Secretion theory was Krogh's paper in the same series, with his wife Marie (1874–1943) as coauthor, entitled '*On the rate of diffusion of carbonic oxide into the lungs of man*' (Krogh and Krogh, 1909). Paradoxically, it was Bohr himself (1909) who suggested the method by which his oxygen secretion theory was disproved, the principle being that the alveolar (A)–capillary (c) diffusion pressure (the $PA–P\bar{c}$ difference, where $P\bar{c}$ is mean capillary pressure) can be estimated quite easily with inhalations of low concentrations of CO because $P\bar{c}$ for CO will be negligible because of the very high affinity of haemoglobin for carbon monoxide.

August and Marie Krogh's purpose and quantitative approach is clear from their opening paragraph (Krogh and Krogh, 1909) " ….. *To see whether the conditions of gas diffusion in the lung were such as to allow the necessary quantities*

to pass through at the tension differences which could be found. In this case, and in this one only, the secretion theory ought, in our opinion, to be abandoned". Leaving aside the details of their technique (see later), the Kroghs' found that at rest the normal human lung transferred 31 ml of CO min^{-1} $mmHg^{-1}$ CO tension gradient from alveolar gas to pulmonary capillary blood [$D_{L_{CO}} = 31$ ml min^{-1} $mmHg^{-1}$]. On the assumption that the resistance to transfer ($1/D_{L_{CO}}$) [$D_{L_{CO}}$ itself has the units of a conductance] lay entirely in the alveolar-capillary membranes, Krogh proposed that the diffusing capacity for oxygen ($D_{L_{O_2}}$) would be greater than the $D_{L_{CO}}$ by a factor equal to the ratio of their diffusivities in tissue (~ 1.23), i.e. 38 ml min^{-1} $mmHg^{-1}$. Multiplying this value by the alveolar–mean capillary (P_A–$P\bar{c}$) oxygen tension gradient on exercise (68 mmHg) (a calculation made possible by Bohr's recently published integration technique (Bohr, 1909)), the Kroghs calculated that an oxygen consumption of 2580 ml min^{-1} (38×68) could occur from passive diffusion alone, "... *certainly sufficient to cover the absorptions actually observed in almost all cases*".

Haldane, with some justification, pointed out that on the Pike's Peak expedition led by himself (Douglas et al., 1913), his colleague Douglas (1882–1963) at an altitude of 4300 m (457 mmHg) had achieved on exercise a $\dot{V}_{O_2}$ of 2195 ml min^{-1} at a $P_{A_{O_2}} \sim 60$ mmHg; the 'diffusion' hypothesis would mean that the mean gradient (P_A–$P\bar{c}$), using data of Krogh and Krogh (1909), had to be 2195/38, i.e. 58 mmHg, and this clearly was impossible since the mixed venous P_{O_2} would have to be < 2 mmHg (60–58). Marie Krogh responded to these criticisms in her 1915 paper. By then, she had shown a 20–40% increase in $D_{L_{CO}}$ on exercise, giving by calculation a $D_{L_{O_2}}$ of as much as 56 ml min^{-1} $mmHg^{-1}$ (Krogh, 1915). This would require a mean gradient for Douglas of only 39 mmHg; she re-estimated his $P_{A_{O_2}}$ as 69 mmHg, and by Bohr integration calculated that Douglas's $P_{a_{O_2}}$ and $P\bar{v}_{O_2}$ would have been 44 mmHg and 16 mmHg respectively, giving an [a–v]O_2 HbO_2 saturation difference of 55% ($S_{a_{O_2}} \sim$ 80%), which for a haemoglobin concentration of 24 g dl^{-1} gives a cardiac output of 16.6 L min^{-1}.

Her calculations were very reasonable in the context of recent work (Wagner et al., 1986). Once again, there was no necessity to postulate oxygen secretion.

2.2. $D_{L_{CO}}$ methodology 1910–1915

In the first paper, the Kroghs tried two different methods of measuring CO uptake (Krogh and Krogh, 1909). The first was a steady state technique, later revived by D.V. Bates in the 1950s (Bates et al., 1955), in which 0.1% CO in air was breathed for up to one minute and $D_{L_{CO}}$ calculated as $\dot{V}_{CO}/P_{A_{CO}}$ ($P\bar{c}_{CO}$ being ignored, see above). They discarded this method because they could not measure $P_{A_{CO}}$ accurately, a difficulty later discussed extensively in the 1950s. Their second method (a single breath technique) was, in many respects, not dissimilar from the $T_{L_{CO}}$ measurement in general use today (Fig. 1). After an exhalation to residual volume, the subject inspired a mixture of CO (1%) in air to total lung capacity, and after a short pause (< 2 sec) expired forcefully about 50% of their vital capacity (VC), the last portion of which was sampled for its CO concentration (sample A at $t = 0$, A_0). This was followed by further identical inspirations and expirations except that inspiratory–expiratory pause was increased in stages up to ~ 10 sec (samples B_1, B_2, B_3). Marie Krogh (1915) later modified the method so that the procedure became a single breath rather than a multi-breath one; after giving sample A_0, the breath was held for about 6–8 sec, and a further expiration of about half the VC was made and measured for CO as before (sample B_t) (see Fig. 1). The calculation was essentially the same as that used today:

$$D_{L_{CO}} = \log_e[CO_{A_0}/CO_{B_t}]/BHT \times [V_A/Pb*]$$

$$= [k_{CO} \times V_A]/Pb* \qquad (2)$$

where BHT is the breath-holding time, $\log_e[CO_{A_0}/CO_{B_t}]/BHT$ is the rate constant for alveolar CO uptake during the breath hold period (also designated k_{CO}), V_A is the absolute lung volume [STPD] (determined separately) during the breath-hold, and Pb* is barometric pressure minus the water
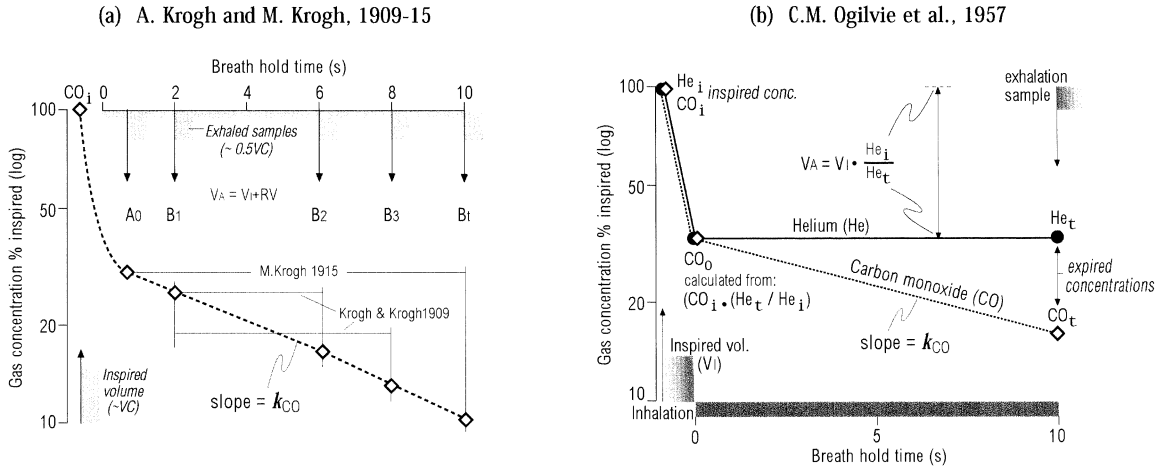
Single Breath DL_{CO} (TL_{CO}) manoeuvre

(a) A. Krogh and M. Krogh, 1909-15   (b) C.M. Ogilvie et al., 1957

Fig. 1. The DL_{CO} (TL_{CO}) manoeuvre as described by August and Marie Krogh (1909) and by Ogilvie et al. (1957). Log gas concentrations plotted against breath hold time in seconds. In Krogh and Krogh (1909) B_1, B_2 and B_3 were exhalations following separate identical inspirations. In Krogh (1915), A_0 and B_t were two successive exhalations following a single inspiration. In Ogilvie et al. (1957), there was only a single exhalation at 10 sec (He_t and Co_t), CO_0 being calculated from the dilution of an inert marker gas (helium).

vapour pressure (P_{H_2O}) at 37 °C. In the original method, the ratio $[CO_{A_0}/CO_{B_t}]$ was calculated separately for B_1, B_2, B_3 (they were usually very similar), and a mean value taken.

The main differences between the Marie Krogh (1915) breath hold technique and the 'modified' method (Ogilvie et al., 1957) in general use today are:

1) Breath holding in the 1915 paper occurred at a lower lung volume ($\sim$ end-expired volume), rather than at TLC.
2) There was no reference gas such as helium to make a 'gas mixing' or 'inhomogeneity' correction for sample A. Since the 1950s, sample A has been calculated from the inspired concentration of CO and the helium dilution ratio rather than measured from an exhalation (see Fig. 1).
3) The Kroghs' terminology was different. They called DL_{CO} ($\sim TL_{CO}$) the 'diffusion constant', and $\log_e[CO_{A_0}/CO_{B_t}]/BHT$ ($\sim k_{CO}$) was designated the 'permeability, k' (it is now referred to as the diffusing capacity (transfer

factor) per unit alveolar volume or transfer coefficient, DL_{CO}/VA or TL_{CO}/VA or K_{CO}).

The main findings by Krogh (1915) were:

i) DL_{CO} was greater in men than in women, and greater in adults than in children.
ii) DL_{CO}/VA was approximately the same in men and women.
iii) DL_{CO} increased by 18–40% with exercise.
iv) DL_{CO} decreased as lung volume decreased until the 'mid capacity' was reached ($\sim$ FRC$+0.5V_T$ [c.60%TLC]) after which there was no further change; above the mid capacity, k ($\sim DL_{CO}/VA$) was proportional to volume, i.e. constant.

Marie Krogh's technique came under criticism because sample A was probably not representative of mean alveolar concentrations (Forster et al., 1954b). This had been recognised by Haldane (1922). He wrote "*...it is quite impossible, as I have convinced myself with repeated experiments...., to secure an even distribution of a gas through the lung air by taking in a single deep breath. The first alveolar sample contains an undue proportion of the*

atrial air [respiratory bronchioles and alveolar ducts] *containing a higher initial percentage of CO, while the second sample comes exclusively from the alveoli of the air-sac system, in which the percentage of CO was never nearly so high as in the atria''*. Thus, M. Krogh's measurements would have overestimated the k and the $D_{L_{CO}}$. W.S. Fowler, a member of the Philadelphia group in the 1950s, realised that in Krogh (1915) samples A and B were "*part of a single exhalation separated by a period of breath-holding*" (Fowler, 1949). Following Haldane, Fowler had shown that the alveolar plateau for nitrogen on expiration, following an inspiration of 100% O_2, always had a positive slope versus volume or time. Ward Fowler suggested an important modification to the Krogh technique—the addition of helium as an inert reference gas to the inspired mixture so that sample A could be omitted, and calculated instead by gas dilution from the inspired CO concentration and the dilution ratio of helium (expired/inspired) sampled *after* the breath-holding period (Forster et al., 1954b) (see Fig. 1). This became known as the *modified Krogh single breath* $D_{L_{CO}}$. It was, in fact, the only modification of substance required to Krogh's 1915 description of the single breath technique—"*not a giant step*" was the comment from Forster (1983)!

In spite of this 'error' which should have lead to an overestimation of $D_{L_{CO}}$ and $D_{L_{CO}}/V_A$, Marie Krogh's results at rest (and on exercise) are very much in line with later work using an improved technique (see later, Table 3), suggesting that, in normal subjects, inhomogeneity of inspired gas (in terms of the distribution of $\dot{V}_I/V_A$) is relatively modest. Where she *was* in error was in her comments about the effects of lung volume on $D_{L_{CO}}$ and $D_{L_{CO}}/V_A$. It is now well established (see Stam et al., 1994, for example) that $D_{L_{CO}}/V_A$ *increases* in a linear fashion as the lung volume of the measurement is reduced from TLC, and that $D_{L_{CO}}$ continues to *decrease* from TLC throughout the whole volume range. The statements of M. Krogh about lung volume and $D_{L_{CO}}$ and k are much quoted and have lead to some confusion. In fact, she misinterpreted her own data probably because her observations were limited to just two

or three subjects and did not encompass the whole lung volume range.

3. 1923–1957: chemical reaction of O_2 and CO with blood

3.1. Reaction rates of O_2 and CO with haemoglobin solutions and red cells

Up to 1922, the time resolution for following the process of a chemical reaction, such as haemoglobin with oxygen, was of the order of minutes. But, in the following year, a Letter to Nature (Hartridge and Roughton, 1923a) reported "*the combination* (Hb with oxygen) *was a very rapid one, the reaction being complete in one hundredth part of a second ...*". This startling gain of almost 100 000 in time resolution, a major advance by any standard, which reduced the time frame to 0.001 sec, was achieved by means of a rapid reaction apparatus devised by Hamilton Hartridge (1886–1976) and F.J.W. Roughton (1899–1972) (Hartridge and Roughton, 1923b). Hartridge at the time, was a physiologist, Fellow of King's College, Cambridge, interested in the special senses (vision and hearing), and with a special gift for solving mechanical problems. Roughton, only 23 years old, had won a scholarship to Trinity College to read medicine, but gave up this career in favour of science because of repeated attacks of paroxysmal tachycardia (Gibson, 1973). Except for the spectroscope, the rapid reaction apparatus consisted of simple household and laboratory utensils! A dilute O_2-free Hb solution was rapidly brought together with tap water, equilibrated with air, in a special mixing chamber, the design of which was based on Hartridge's expertise in redesigning the carburettor of his own car (Rushton, 1977). Under a pressure of 50 mmHg, the solution was forced down a tube at velocities of 800 cm sec^{-1}, such that sequential observations made at 1 cm intervals down the length of the tube corresponded to time intervals of 0.00125 sec (1.25 ms). Mixing occurred within 1.0 ms (< 1.0 cm from the entry to the observation tube). The flow regime was turbulent. They referred to this as a 'continuous–flow rapid–reaction' apparatus. $HbO_2\%$

(or combinations of HbCO and HbO_2) was measured in a complex manner with a reversion spectroscope (see below and Fig. 2). The reaction of Hb with CO was also studied, without the necessity for mixing, by exposing an HbCO solution to an intensely bright light which dissociated the CO from Hb. The flowing solution entered a dark tube and the rate of recombination of Hb and CO was measured with the reversion spectroscope (Hartridge and Roughton, 1923c) from the transit time from the light source to the spectroscope. The dissociation of oxygen from HbO_2 (Hartridge and Roughton, 1923d) was studied by rapid mixing of HbO_2 solutions with sodium dithionite ($Na_2S_2O_4$), a powerful reducing agent, later used by Burns and Shepard (1979), in a study of $D_{L_{O_2}}$ and by others investigating the 'unstirred' plasma layer in the 1980s (see later).

The reversion spectroscope was another of Hartridge's ingenious contrivances, invented (Hartridge, 1912) in 1908–9 during his fourth year at Cambridge, when he was aged 23. In the visible green region of the spectrum, HbO_2 and HbCO have α bands at 577 and 570 nm, respectively. Hb or blood with a given HbO_2/HbCO mixture appears as a single ill-defined band in this 6.0 nm (60 Å) window. Hartridge arranged two slits and collimators symmetrically to the right and left, one higher than the other, so that the red to violet spectra ran counter to each other (i.e. 'reversed'). An auxiliary trough of pure HbCO was a reference point and the α bands were superimposed. When HbO_2/HbCO (or HbO_2/Hb) mixtures enter the observation tube, the bands moved in opposite directions, and the operator brought them together again by turns of calibrated micrometer screws. Thus, small shifts in a broad band were doubled in amplitude, while accuracy was increased by the overlap technique. The displacements were calibrated in terms of Hb and/or HbCO fractions.

Hartridge and Roughton's initial studies (1923b–d) of O_2 and CO kinetics were of lysed red cells (Hb solutions) but later (Hartridge and Roughton, 1927) they reported their results on dilute solutions of whole blood. Roughton (1932) commented on their findings "*In the corpuscle experiments the time scale had to be expressed in*

hundredths *of a second instead of in* thousandths *of a second as in the haemoglobin solution experiments*". Table 1 summarizes later data (confirmatory of the earlier studies) on the reaction velocities for human blood or Hb solutions.

The $t_{1/2}$ for these reactions varies from 2.0 ms for O_2 uptake in Hb solution to > 200 ms for CO uptake in hyperoxia. Notice in Table 1 the slower reaction rates in the intact red cell, with Hb/red cell ratios of 20 to 1.3. In red cells, the velocity constants are a complex function of simultaneous diffusion (inside and immediately outside the cell) and chemical reaction with Hb. The latter is common to both systems, so as the Hb/red cell velocity ratio approaches infinity, diffusion dominates as the rate limiting step in red cell kinetics. Conversely, a ratio of 1.0 implies that the reaction with Hb is the more important rate limiting step.

The velocity constants in Table 1 are 'apparent' because the simultaneously occurring back reaction (the dissociation of CO or O_2 from the Hb ligand) is neglected. It is a popular misconception[3] that the high affinity of the haemoglobin molecule for CO (ca. 200 times greater than O_2) is caused by its more rapid binding or association. In fact, its velocity of association is slower than that for oxygen (Table 1), but its dissociation constant is 1000 times slower than its association constant (or the dissociation constant for O_2), which explains the high affinity.

3.2. Measurement of O_2 and CO kinetics: methodological advances

The Hartridge–Roughton technique (Fig. 2) was robust but somewhat clumsy. The reversion spectroscope required skill and experience, and it took time; more objective and faster techniques

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[3] Roughton recalled suggesting to Hartridge in 1923 that it would be a good idea to measure the rate of uptake of CO with Hb, as they had done for oxygen. Hartridge replied, "We would be wasting our time; CO is so tightly bound that the reaction would proceed too fast for us to measure it". Roughton (the junior member) did not argue, but when Hartridge was away, he did the experiment and found the CO velocity constant was slower not faster than that for O_2! (R.A.B. Holland kindly contributed this reminiscence).

Table 1
Velocity constants ($mM^{-1} s^{-1}$) in dilute solutions of human Hb and red cell suspensions at 37 °C using the continuous flow rapid reaction apparatus (from Roughton, 1959)

Kinetic reaction	Hb solutions	Red cell suspensions	Hb/red cell ratio
			$\rightarrow \infty$ diffusion limitation
O_2+deoxyHb	1800	92	20
CO+deoxyHb	670	79	8.5
CO+low oxyHb	120	43	2.8
CO+high oxyHb	21	16	1.3
			$\rightarrow 1.0$ reaction limitatation

supplanted it. Millikan (1933) introduced a photovoltaic cell and galvanometer for the rapid recording of changes in light absorption; in an improved version, two photocells with oppositely responding filters compensated for extraneous factors such as lamp intensity or turbidity changes. Motor driven syringes replaced pressurized bottles. Spectrophotometers/photomultiplier tubes, and cathode ray oscilloscopes or A/D converters were introduced.

The continuous-flow apparatus was thirsty with reagents. Up to 10 L of Hb or red cell solutions (~ 100 ml blood, even when diluted to 1%) were required for 60 sec of flow to characterise one kinetic curve. Hence, most of the early work used sheep's blood. With Millikan's apparatus, 6–8 capillary tubes (mean diameter 0.2 mm) replaced the 6.8 mm bore observation tube (Fig. 2), and each point on a kinetic curve required flow for
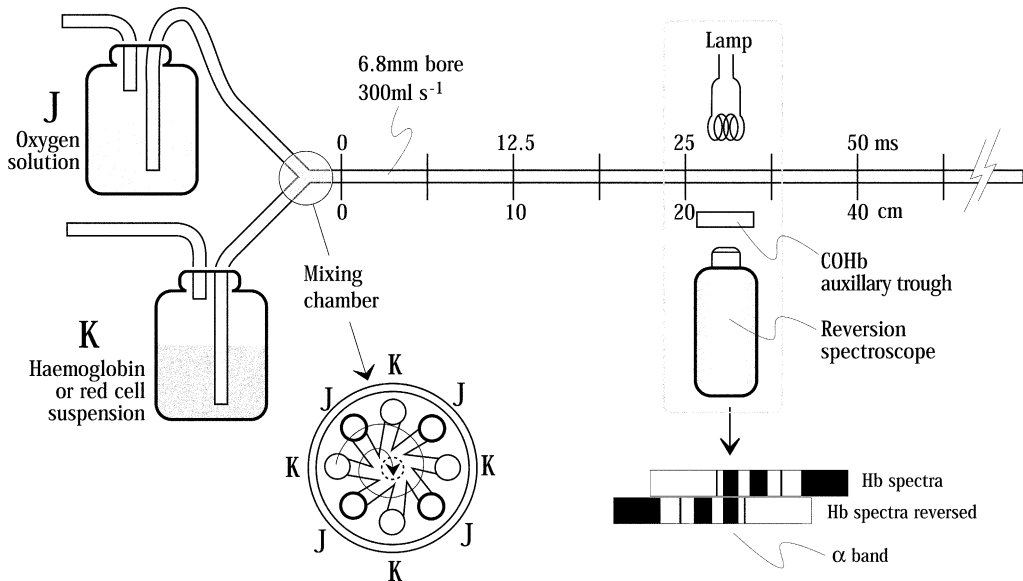

Fig. 2. Outline of apparatus for studying O_2 and CO kinetics in Hb solutions or red cells. Reagents were driven from pressure bottles by compressed N_2 through the mixing chamber at a fixed flow and constant velocity (0.8 cm ms^{-1}) so that 1 cm = 1.25 ms along the observation tube. The reversion spectroscope was set up at intervals, and the $HbO_2 \pm HbCO$ composition determined from the position of the α band on two identical spectra mounted one above the other (with one reversed). Redrawn from Gibson (1959).

only 5 sec. Eventually, the response times of detectors and recorders were able to cope with the fastest reactions, and the stopped-flow rapid reaction technique was introduced by Chance in 1951, and adapted for work with Hb by Gibson in 1954 (see Gibson, 1959). In this method, flow was stopped abruptly in < 1.0 ms after mixing, and a complete kinetic curve was recorded straightaway from the *same* quantum of blood or Hb, which was not possible with the continuous-flow apparatus. The stopped–flow technique is the more convenient option although stagnant plasma/buffer layers (see Section 6.3) form immediately flow ceases. Forster (1987) believes the continuous flow rapid reaction apparatus gives more accurate values for O_2 and CO reaction rates with *blood* (usually termed θ [see Section 3.4] (twice the rate in the case of θ_{CO}), but that stopped flow is useful for measuring changes. Holland (1997), on the other hand, does not accept that there is a systematic difference between the continuous and stopped flow techniques for the O_2–Hb velocity constants.

3.3. Effect of varying P_{O_2} on $HbO_2 + CO \rightarrow HbCO + O_2$ reaction

In 1945, three papers from Roughton and his colleagues appeared consecutively in the same issue of the American Journal of Physiology. The work had been motivated, in part, by the need for better information on the physiological effects of CO inhalation—from the standpoint of the toxic effects of exhaust fumes in confined spaces such as tanks, submarines and the cockpits of fighter planes. In the first, Forbes et al. (1945) studied the rate of CO uptake in normal subjects exposed to varying inspired concentrations (0.5–2%) for different time periods.[4] The development by Roughton and Scholander (1943) of a microgasometric ("bubble") technique had enhanced the accuracy of the measurements of HbCO. 98% oxygen breathing slowed the CO uptake rate at

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[4] Figure 2 in this paper with 27 lines radiating from a central point on the Y-axis like the sun at dawn was always referred to by Roughton as the "Japanese flag"!

rest by 25% compared to air breathing, an effect attributed to slowing of the reaction between CO and Hb in the presence of oxygen. Evidence for this was gained in the second paper. Roughton (1945a), using the Hartridge rapid reaction apparatus and the reversion spectroscope, found that the rate of CO uptake by red cell suspensions was inversely proportional to the buffer P_{O_2} with which it was mixed; in addition, at high P_{O_2}s the velocity constant was about the same for red cells as for Hb solutions, whereas at lower P_{O_2}s the Hb/red cell velocity ratio averaged 1.18. Roughton took this to be good evidence that the kinetics of CO combination with Hb was the same for Hb in solution as when it was incorporated into the cell, and he surmised that at high P_{O_2}, the uptake of CO by red cell suspensions was 'limited by chemical reaction' (see Table 1), in contrast to the situation at low P_{O_2} (diffusion + reaction limitation).

In the final paper (Roughton, 1945b) in this remarkable trio, Roughton calculated "*The average time spent by the blood in the human lung capillary* ..." from the data given in the two previous papers. The argument was tortuous but very ingenious. The basic tenets were: (1) 99% of HbCO formation during steady state CO inhalation occurs during red cell transit of the pulmonary capillary bed; (2) the rate constant of lung uptake (τ_L) of HbCO multiplied by the pulmonary capillary blood volume must, by conservation of matter, equal the rate of HbCO uptake in the total circulation (τ_{tot}) times the total blood volume; (3) since blood flow is common to lung and the systemic circulation, blood volumes will reflect transit times; (4) for τ_{tot}, the ratio (98% O_2/air breathing) had already been established (Forbes et al., 1945); (5) the rate of formation of HbCO in blood in vitro $= m' \cdot [k \cdot (P\bar{c}_{CO}/P\bar{c}_{O_2})] \cdot HbO_2$ where m' is the velocity constant for the combination of CO with HbO_2, and k is a constant converting plasma CO and O_2 contents to partial pressures (Roughton, 1945a). From the data of Forbes et al. (1945) with 98% O_2 and 0.1% CO inhaled, Roughton calculated a pulmonary capillary transit time at rest of 0.73 sec reducing to 0.34 sec on exercise (compare Table 8). With appropriate values for cardiac output, the pulmonary

capillary volume was 60 ml at rest increasing to 95 ml on exercise.[5]

The calculations were a tour de force (and very credible—see Table 8 and Fig. 5), but there was an interesting dénouement in an Appendix (under Roughton's name) to a paper published 12 years later (Roughton et al., 1957) in which Roughton acknowledged the existence of two errors in his 1945 paper. He said "*Probably it was due to the accidental and nearly equal compensation produced by two oppositely signed errors ..... [use of an incorrect calibration curve and neglect of a CO/O_2 kinetic term] ..... that Roughton's 1945 estimates differ so slightly from those obtained by sounder methods available today*". A remarkable example of good fortune!

3.4. Partitioning $D_{L_{CO}}$ into membrane (D_M) and red cell (θVc) conductances

In 1952–3, Forster and his colleagues were repeating Marie Krogh's single breath $D_{L_{CO}}$ experiments. They reported their results in the Journal of Clinical Investigation (Forster et al., 1954a,b).[6] In the first paper (Forster et al., 1954a), which was "*a re-examination of the theory of CO uptake from the lungs*", the term $\theta \cdot Vc$ (see later Eq. (3) for its definition) appeared for the first time—in a 'footnote' on page 2! Forster (being well aware of Roughton's earlier work) had proposed a 'correction' factor (C) to allow for the fact that "*the plasma CO tension was not negligible*". C contained the term $[D_L/\theta \cdot Vc]$ because Forster reasoned that "*the capillary CO tension may be significant, either because the mean capillary COHb concentration is large, or because the diffusing capacity (D_L) is large in relation to the rate of combination of CO and Hb*". $\theta \cdot Vc$ is

equivalent to the diffusing capacity ($\sim$ transfer factor) of pulmonary capillary blood. If $\theta \cdot Vc$ is high, the major part of the inhaled CO diffusing into blood will be 'mopped up' rapidly by haemoglobin so that plasma P_{CO} will be low. Conversely, if D_L (we would now use the term D_M) was high in relation to θVc (as would occur when 100% O_2 was breathed), the scavenging action of Hb would be insufficient and plasma P_{CO} would rise. In fact, Roughton (1945b) had calculated from the data of Forbes et al. (1945) that $P\bar{c}_{CO}$, where $\bar{c}$ refers to the mean capillary pressure, was 0.06 mmHg breathing air and 0.4 mmHg breathing oxygen—an almost sevenfold increase. Since hyperoxia slows the rate at which CO combines with Hb, plasma P_{CO} must rise as the CO arriving is not immediately removed, and this 'back pressure' effect will lower the $D_{L_{CO}}$ when oxygen is breathed, for a given $P_{A_{CO}}$.[7] The outcome, as related by Forster (2000), was a letter to Cambridge asking if Roughton had any data on the effect of P_{O_2} on the rate of combination of CO with Hb in human blood at 37 °C. Roughton's (1945a) data were very preliminary, so he invited himself to the Physiology Department in Philadelphia for 3 months to do the relevant experiments!

Two years later approximately, four papers appeared together in the Journal of Applied Physiology with Forster and Roughton sharing the first authorships between them. Two of these papers (Roughton et al., 1957; Forster et al., 1957a) were concerned with in vitro O_2–CO kinetics of human Hb and red cell suspensions to obtain the relationship between $1/\theta_{CO}$ and P_{O_2} (extending Roughton's (1945a) earlier work), and also with λ, the ratio of the red cell membrane permeability to that of the cell interior. The third paper (Forster et al., 1957b) documented the inverse relationship between $D_{L_{CO}}$ (sb) (and $D_{L_{CO}}$ (ss)) and alveolar P_{O_2}, showing a fall in 'apparent' $D_{L_{CO}}$ of 50–60% at $P_{A_{O_2}} \sim 600$ mmHg versus ~ 100 mmHg. The finale in the quartet was

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[5] Roughton also calculated the cumulative length of all capillaries at rest (970 *miles*) and on exercise (1540 *miles*) from which he estimated D_M (49 ml min^{-1} mmHg^{-1} at rest, 77 on exercise).

[6] Forster submitted these MSS to the JCI from a village post office in Kent (near the cottage of a coauthor, David Bates). The weight was just over some critical threshold for airmail consignment, and the postmistress tried to persuade him to remove a few pages to make the package lighter!

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[7] The CO 'back pressure' effect becomes important in clinical terms when blood HbCO levels are raised, as in heavy cigarette smokers, or when repeated $D_{L_{CO}}$ measurements are made in a single session.

the 'classic' paper by Roughton and Forster (1957) which stated: "*During recent years we have developed a simplified modification of Roughton's mathematical treatment* ...[calculation of pulmonary capillary transit time and blood volume (Roughton, 1945b)] ....*leading finally to the equation*:

$$\frac{1}{D_L} = \frac{1}{D_M} + \frac{1}{\theta \cdot V_c} \qquad (3)$$

where D_M is the true diffusing capacity of the membrane separating the alveolar air from the blood, D_L is the overall diffusing capacity of the lung as measured by the Bohr–Krogh methods, V_c is the total volume in ml of blood in the lung capillaries exposed to the alveolar air, θ is the number of ml of gas taken up by the red cells in 1 ml of blood min^{-1} 1 $mmHg^{-1}$ gradient of partial pressure of dissolved gas between the plasma and the interior of the red cell. A similar equation has been given by Kruhoffer". Note that $\theta \cdot V_c$ is the effective diffusing capacity of blood, and not just the rate of combination of CO with Hb. Kruhoffer (1954), who worked in the same Department in Copenhagen as the Kroghs, had published a similar equation 3 years earlier, but without giving a formal proof, and with erroneous values for θ_{CO}.

This equation was of great significance for several reasons: (a) it emphasized that there was a P_{CO} gradient from pulmonary capillary plasma to the red cell interior that might be as great or greater than the P_{CO} gradient from alveolar gas to plasma; (b) it described a method, based on in vitro kinetics of CO uptake by red cells, for using $D_{L_{CO}}$ measurements at different alveolar P_{O_2}s to obtain the membrane (D_M) and red cell ($\theta \cdot V_c$) conductances (see Fig. 5 for an example); and (c) this methodology could be used to test hypotheses such as the effect of alveolar expansion on D_M (Stam et al., 1983), of anaemia (Rankin et al., 1961; Cotes et al., 1972) on θ, and of exercise (Hsia et al., 1995a) on V_c.

The essence of the Roughton–Forster equation had occurred to Roughton (1945b) 12 years earlier when he wrote " ... *and by plotting the values of the Diffusion Constant [the $D_{L_{CO}}$] at a series of different alveolar O_2 pressures, it might be possible,* *by extrapolation, to arrive at the true Diffusion Constant of the lung with zero back pressure of CO in the blood [i.e. the D_M]. It is hoped it may be possible soon to carry out such experiments.*"

D_M is the diffusion conductance from alveolar gas up to the red cell, and $\theta \cdot V_c$ is the diffusion conductance from the red cell membrane to the haemoglobin molecule. $1/\theta \cdot V_c$ is the oxygen-dependent part of the total diffusion resistance. $D_{L_{CO}} = \dot{V}_{CO}/[P_{A_{CO}} - P_{rc_{CO}}]$ where rc is the red cell; it follows from the Roughton–Forster equation that ($\dot{V}_{CO}$ being common) $1/D_M \sim [P_{A_{CO}} - P_{pl_{CO}}]$ and $1/\theta \cdot V_c \sim [P_{pl_{CO}} - P_{rc_{CO}}]$ where pl is plasma. The ratio $[(1/D_L - 1/D_M)/(1/D_L)]$ is that fraction of the resistance which is within the red cell [R_{rc}/R_{tot}], and it is also proportional to the fraction of the total P_{CO} gradient which is located in the erythrocytes, $[P_{pl_{CO}} - P_{rc_{CO}}]/[P_{A_{CO}} - P_{rc_{CO}}]$. The same considerations apply to $D_{L_{O_2}}$ and P_{O_2}.

Support for the Roughton–Forster equation in vivo came from some rather heroic experiments (Nairn et al., 1965) in which $D_{L_{CO}}$ (sb) was measured in normal volunteers in a hyperbaric chamber where the pressure was raised to 4.8 atm. At this pressure, 6% O_2 in nitrogen was breathed in between measurements of $D_{L_{CO}}$; graduated decompression took 3–4 h! $1/D_{L_{CO}}$ increased linearly with intracapillary P_{O_2} with high correlation coefficients (r = 0.99). $D_{L_{CO}}$ at a $P_{c'_{O_2}}$ of nearly 3000 mmHg was 1/7th of that at sea level breathing air. The red cell resistance at that high P_{O_2} was 90% of the total resistance; this is because of the scarcity of free (unliganded) binding sites on the haemoglobin molecule [as $Hb_4(O_2)_3$] at that P_{O_2}. At that high P_{O_2}, $[P_{pl_{CO}} - P_{rc_{CO}}]$ is 9 times greater than $[P_{A_{CO}} - P_{pl_{CO}}]$.

4. 1950–57: Development of the steady state and modified single-breath $D_{L_{CO}}$

4.1. Why and how the $D_{L_{CO}}$ (sb) was 'rediscovered'

The 1950s was a time when most of the pulmonary function tests in common use today were developed or refined [the FEV_1 had been described by Tiffeneau and Pinelli in 1947 (see

Yernault, 1997)]. A turning point was J.H. Comroe's Beaumont lecture to the Wayne County Medical Society, Detroit, in February 1954, entitled 'The Physiological Diagnosis of Pulmonary Disease', which formed the basis for the book *The Lung* (Comroe et al., 1955), a brilliant explanation for doctors and medical students of the relevance of pulmonary physiology to medicine. Julius Comroe had been asked to be the Editor of the section on Pulmonary Function Tests for the book *Methods in Medical Research* (Comroe, 1950). He intended that the chapter on 'gas–blood diffusion' should deal mainly with the elegant measurements of $D_{L_{O_2}}$ of Lilienthal et al. (1946). He chose Seymour Kety to write it. Kety was not a pulmonary physiologist (he was interested in tracer methods to measure cerebral blood flow) but he undertook a thorough review of all previous work on pulmonary diffusion. He concluded that the Lilienthal method for measuring $D_{L_{O_2}}$ would never achieve clinical usefulness because mixed venous P_{O_2} was needed for an accurate estimation and so cardiac catheterisation would be required (authors' note: this point is arguable). According to Comroe (1975) Kety concluded "*It appears that the determination of $D_{L_{O_2}}$ (from $D_{L_{CO}}$) by Krogh's method would constitute a practicable and clinically useful technique for defining the diffusion characteristics of the alveolar membrane in health and disease*". R.E. Forster, a new recruit to the Department of Physiology at the University of Pennsylvania at the time, records (Forster, 2000) that Comroe (the Head of Department) suggested that he repeat M. Krogh's experiment using a new infra-red CO meter recently made for the Department.

Forster (2000) admitted, in retrospect, that he became bogged down in attempts to improve the sensitivity of the CO meter, and it took the arrival of a colleague, Ward Fowler (obviously sent by Comroe), to get things going. Within a day or two, some measurements of Krogh's $D_{L_{CO}}$ had actually been made! Fowler himself made the timely suggestion that helium should be included in the CO mixture as an inert reference gas (other inert gases have been used, such as methane). This was an important improvement to M. Krogh's method of 1915, as already mentioned, making the proce-

dure simpler (one, not two, expired samples to be made) and the measurement itself less sensitive to inspired gas inhomogeneity; it also meant that breath-holding occurred at full inflation (TLC), a reproducible reference point and one at which patients would be able to hold their breath more easily.

4.2. Development of other techniques for measuring the $D_{L_{CO}}$

Independently, Filley et al. (1954) developed a steady state method for $D_{L_{CO}}$ at the Trudeau Saranac Institute (attached to a TB sanatorium) in New York State. In the UK, stimulated by F.J.W. Roughton, J.C. Gilson and P. Hugh-Jones at the Pneumoconiosis Research Unit, Llandough and D.V. Bates at St Bartholomew's Hospital, London were also developing steady state CO uptake techniques. Roughton was still active and collaborated importantly at this time with Forster and the Philadelphia group in unravelling the physiology of the $D_{L_{CO}}$ in terms of its membrane (D_M) and reactive ($\theta \cdot V_c$) conductances. The fundamental work carried out in several laboratories in the 1950s has been summarized by Forster (1957) in a masterly review. R.W. Hyde (2002) recalls asking Forster in the late 1960s how Dr Roughton spent his time as a retired Professor. He replied "He does cowbird research. He lays innovative scientific eggs in other people's nests and comes back in a year or two to check up on his fledglings".[8]

By the end of 1955, six different techniques (four steady state, a single breath and rebreathing) had been published for measuring $D_{L_{CO}}$ or CO uptake, as outlined in Table 2.

The *fractional CO uptake method* of Bates (1952) (1) was a steady state inhalation technique, not dissimilar to that of Forbes et al. (1945) and others in the 1940s. Basically, CO absorption [$\dot{V}_{CO}$] was measured as a fraction of the inspired CO load ($\dot{V}_I \cdot F_{I_{CO}}$). Since $D_{L_{CO}}$ could be calculated by adding a measurement of $P_{A_{CO}}$, fractional

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[8] For European readers, the cowbird is a species of American blackbird which borrows other bird's nests. Unlike the cuckoo, its young do not eject the rightful owners!

Table 2
Different methods for measuring CO uptake and $D_{L_{CO}}$ in the 1952–57 period

Technique	Calculation	Advantages	Disadvantages
1. Fractional CO uptake (Bates, 1952)	$\dot{V}_{CO}/^{1}\dot{V}_{I_{CO}}$	Simple technically Reduced in emphysema	Not a measure of $D_{L_{CO}}$ Sensitive to hyperventilation
2. $D_{L_{CO}}$ ss (Bates et al., 1955)	$\dot{V}_{CO}/P_{A_{CO}}$ 'alveolar' sampling	Simple technically Suitable for exercise studies	$P_{A_{CO}}$ inaccurate with a sloping alveolar plateau
3. $D_{L,CO}$ ss (Filley et al., 1954)	$\dot{V}_{CO}/P_{A_{CO}}$ $P_{A_{CO}}$ estimated via $P_{a_{CO_2}}$	Side-steps the problem of 'unrepresentative' $P_{A_{CO}}$	Requires arterial sample for $P_{a_{CO_2}}$
4. $D_{L_{CO}}$ ss plus He (Gilson and Hugh-Jones, 1955)	$\dot{V}_{CO}/P_{A_{CO}}$ 'alveolar' sampling	Corrects for $\dot{V}_I/V_A$ inhomogeneity	$P_{A_{CO}}$ inaccurate as in (2)
5. $D_{L_{CO}}$ sb (Ogilvie et al., 1957)	$[k_{CO} \times \dot{V}A]/^{2}Pb*$	Reproducible, reliable. mostly $\dot{V}_I/V_A$ insensitive	Lung volume (TLC) and breath hold unphysiological
6. Rebreathing $D_{L_{CO}}$ (Kruhoffer, 1954)	$[k_{CO} \times ^{3}Vsys]/Pb*$	Volume-weighted DL/VA, a "true" mean value	Unsuitable for emphysema as hyperventilation required

[1] $\dot{V}_{I_{CO}} = \dot{V}_I \cdot F_{I_{CO}}$.
[2] $*Pb = Pb - P_{H_2O}$.
[3] Vsys = volume of rebreathing bag plus initial volume of the lung.

uptake by itself became superfluous. In the *steady state $D_{L_{CO}}$* of Bates et al. (1955) *[$D_{L_{CO}}$ (ss)]* (2) $P_{A_{CO}}$ was estimated in various ways, but there was no easy solution to getting a 'representative' alveolar concentration when there was a sloping alveolar plateau (concentration versus time/volume) during expiration. The *steady state $D_{L_{CO}}$* of Filley et al. (1954) (3) sidestepped the issue of alveolar sampling (as does the classical $\dot{V}A/\dot{Q}$ analysis), substituting an 'effective' alveolar P_{CO}, i.e. the $P_{A_{CO}}$ which would be present throughout the lung if it were homogeneous. $D_{L_{CO}}$ (ss) calculated in this way is a robust measurement but it requires arterial sampling for $P_{a_{CO_2}}$. The *steady state combined CO and helium wash-in* of Gilson and Hugh-Jones (1955) (4) was an ingenious technique designed to see if impairment of CO uptake was due to $\dot{V}_I/V_A$ maldistribution (assessed from the helium wash-in curve), or to impaired alveolar–capillary diffusion per se. They also undertook a two compartment analysis of the helium wash-in curve. In essence, the helium and CO wash-in curves in normal subjects were

compatible with two compartments with different $\dot{V}_I/V_A$, each with the same DL/VA, whereas in emphysema and fibrosis each $\dot{V}_I/V_A$ compartment had a different DL/VA.

In the paper describing the *M. Krogh 'modified' single breath technique* with helium added to the inspired CO mixture (Forster et al., 1954b) (5), there was concern that expired alveolar CO concentrations obtained after different breath holding times (from 10 to 60 sec) did not decline as a single exponential, but 'curved upwards'. Thus, the $[P_{A_{CO_0}} - P_{A_{CO_t}}]$ difference decreased with increasing breath holding time (BHT), so that the calculated $D_{L_{CO}}$ became less. Forster et al. (1954b) concluded that local variations in the slope of alveolar uptake (Krogh's k, the modern K_{CO}, or its equivalent DL/VA [TL/VA]) must be responsible. For example, DL/VA will vary from the apex to the base of the lung. Later investigators, the first being Marshall (1958), have found that the departure of the expired P_{CO} concentration–time curve from the monoexponential in normal subjects is trivial in the first 20 sec.

Nevertheless, there is substantial non-linearity in emphysema where there are large variations in $\dot{V}_I$/V_A and D_L/V_A between lung units, so that the concentrations of helium and CO in an *early* expired sample exceeded that from a *late* 'maximal expiratory' sample by 20%. Forster's view (1983) was that "*the variability in D_L measured by single samples would be so great as to render the data of minimal value for clinical work*". Generously, he added "*My colleagues went ahead despite my views, fortunately*."

Rebreathing $D_{L_{CO}}$ of Kruhoffer (1954)—$D_{L_{CO}}$ *(rb))* (6). Subjects rebreathed from a bag of 6 L capacity with hydrogen (an inert gas) and tracer amounts of ^{14}CO added, taking deep and rapid (25 min^{-1}) breaths. Discrete samples were withdrawn at 12, 22 and 32 sec for gas analysis. The rate of CO uptake ($\sim$[log$_e$CO$_0$/Co$_t$]/BHT), calculated from the sampled CO concentrations and the time interval, was multiplied by the volume of the *lung–bag system* (from H$_2$ dilution) and divided by barometric pressure to give the $D_{L_{CO}}$. Modern practice (Hsia et al., 1995a) is to monitor gas concentrations continuously using a mass spectrometer (C^{18}O for $D_{L_{CO}}$, C$_2$H$_2$ (acetylene) for cardiac output, helium for gas mixing and volumes) from which log gas concentration versus time is plotted and a linear regression calculated. If the respiratory frequency is high and the rebreathing bag is almost emptied with each breath, equilibrium between the alveolar P$_{CO}$ throughout the lung and the rebreathing bag will be maintained throughout the manoeuvre, and the rate of decrease of CO concentration in the system will reflect the overall volume-weighted D_L/V_A of the lung—the 'true' mean D_L/V_A. In contrast, as shown by Lewis et al. (1959), $D_{L_{CO}}$ *(sb)* is 'weighted' by those units with high $\dot{V}_I$/V_A and high D_L/V_A, but is little affected by $\dot{V}_I$/V_A inequality if D_L/V_A is uniform. $D_{L_{CO}}$ *(ss)* underestimates overall $D_{L_{CO}}$ in the presence of uneven $\dot{V}_I$/V_A whether or not D_L/V_A is uniform.

Apart from the rebreathing technique, all the $D_{L_{CO}}$ methods are inherently inaccurate (the steady state methods more so than the single breath technique) in the presence of $\dot{V}_I$/V_A and D_L/V_A inhomogeneities. From a practical point of view, patients with airflow obstruction such as emphysema cannot sustain sufficiently high levels of ventilation to benefit from the 'more representative' rebreathing procedure.

4.3. $D_{L_{CO}}$ measurements in normal subjects and patients with emphysema and lung fibrosis

In normal subjects, the ranges at rest and on exercise for $D_{L_{CO}}$ (ss) by the 'effective' alveolar P$_{CO}$ method (Filley et al., 1954) or the 'weighted' alveolar gas sampling method (Bates et al., 1955) were very similar (Table 3). Marshall (1958), using end-tidal (ET) sampling, obtained higher $D_{L_{CO}}$ (ss) values than when using the technique of Filley et al. (1954), P$_{ET_{CO}}$ being lower than the 'effective' alveolar P$_{CO}$. Marshall (1958) was able to compare $D_{L_{CO}}$ (ss) and $D_{L_{CO}}$ (sb) in the same (normal) subjects, and found that the difference between the two measurements could be wholly explained by the lower lung volume at which the $D_{L_{CO}}$ (ss) measurement was made. In general (Table 3), in resting subjects, $D_{L_{CO}}$ (sb) measurements at TLC are greater than $D_{L_{CO}}$ (ss) measurements, but the difference diminishes on exercise because of a reduction in ventilation and perfusion inhomogeneities.

In emphysema, $D_{L_{CO}}$ by either the steady state or single breath techniques was reduced, but not invariably. 2/6 of Ogilvie et al.'s (1957) and 4/12 of Kjerulf-Jensen and Kruhoffer's (1954) patients with emphysema had a $D_{L_{CO}}$ within the normal range. In generalized lung fibrosis, however, very reduced values of $D_{L_{CO}}$ were the rule (Table 3). In the largest study of emphysema, Bates et al. (1956) also found considerable overlap at rest (but not on exercise) between normal subjects and patients. There are two main reasons for this, (a) methodological, relating to a falsely low P$_{A_{CO}}$ giving an overestimate of $D_{L_{CO}}$, and (b) misclassification of emphysema from clinical data alone; Bates et al. (1956) had two cases which on later review were considered to be predominantly 'bronchospasm'. The interest of their study was that serial measurements showed a decline in $D_{L_{CO}}$ which correlated with a worsening of the clinical condition and prognosis, $D_{L_{CO}}$ being more discriminatory in this regard than other pulmonary function tests, so that the authors could say " ... *this measurement,*

Table 3
Comparisons of $D_{L_{CO}}$ (ss) and $D_{L_{CO}}$ (sb) in the period 1954–61 in normal subjects and patients with emphysema and lung fibrosis (M. Krogh's results also shown)

Author	No. subjects	$D_{L_{CO}}$ (ss)[1]		$D_{L_{CO}}$ (sb)[1]
		Rest	Exercise	Rest
Normal subjects				
Krogh (1915)			30–47[2] (sb)	19–36[2]
Bates (1952)	19	11–29	32–46	
Filley et al. (1954)	11	11–28	23-55	
Ogilvie et al. (1957)	11M			17–38
	9F			16–35
Marshall (1958)	5	19–37		25–49 (TLC)[3]
				22–37 (FRC)[3]
Kruhoffer (1954)	10M			21–31[4]
	5F			19–24[4]
Emphysema				
Bates et al. (1956)	59	3–22		
	6	4–11	4–11	
Ogilvie et al. (1957)	6			4–35
Marshall (1958)	5	8–25[5]		11–22
Kjerulf-Jensen and Kruhoffer (1954)	12			11–25[4]
Holland and Blacket (1961)	19 (rest) 9 (ex)	4–13[5]	9–18[5]	
Fibrosis				
Kjerulf-Jensen and Kruhoffer (1954)	3			6–10[4]
Forster et al. (1954c)	2		5, 17[6]	8, 14[6]
Ogilvie et al. (1957)	4			4–10
Holland and Blacket (1960)	5	4–7[5]	8–11[5]	

[1] ml min^{-1} mmHg^{-1}. For SI units (mmol min^{-1} kPa^{-1}) divide by 3. $P_{A_{O_2}}$ ca 90–110 mmHg.
[2] Numbers joined by a hyphen represent the range.
[3] Comparison of measurements at full inflation (TLC) and at mid-lung volume (FRC).
[4] Rebreathing technique.
[5] Filley method.
[6] Individual results.

or some refinement of it, is an essential part of any complete assessment of respiratory function''.

4.4. Comparison of $D_{L_{O_2}}$ and $D_{L_{CO}}$

$D_{L_{CO}}$ was originally considered a surrogate for $D_{L_{O_2}}$. As we have seen, Marie Krogh (1915) multiplied $D_{L_{O_2}}$ by 1.23 to obtain $D_{L_{O_2}}$, although this is not correct in the light of later theory (Roughton and Forster, 1957) because part of the diffusion resistance to CO resides within the red cell and in the rate of CO combination with haemoglobin. Direct measurement of $D_{L_{O_2}}$ requires an estimate the oxygen diffusion gradient ($P_{A_{O_2}} - P\bar{c}_{O_2}$). If $P_{A_{O_2}}$ and $P\bar{v}_{O_2}$ are known, and if

the lung is sufficiently homogeneous, $P\bar{c}_{O_2}$ can be calculated (as Marie Krogh did) by the technique of Bohr integration (Bohr, 1909). In actual fact, it is the end–capillary P_{O_2} (Pc'_{O_2}), not the $P_{A_{O_2}}$, which must be used in the calculation.

The $P_A - P_a$ difference for O_2 is the sum of two components, an alveolar to *end*-capillary tension difference ($P_A - Pc'$) which is dependent on diffusion (a positive gradient implies diffusion limitation to O_2 exchange) and a $Pc' - P_a$ difference which reflects veno–arterial shunting, either anatomic or physiological. Although, breathing air, $P_{a_{O_2}}$ is significantly less than $P_{A_{O_2}}$ even in normal subjects, in hypoxia the $P_A - Pc'$ difference is negligible except during exercise. Lilienthal et al.

(1946) described an ingenious method of measuring the alveolar to end-capillary tension difference (P_A–Pc') for oxygen. Under conditions of alveolar hypoxia, the Pc'–Pa gradient due to venous admixture is virtually abolished (in normal subjects) because the slope of the HbO_2 dissociation curve is steep at P_{O_2}s 40—55 mmHg, but that due to diffusion limitation (P_A–Pc') is accentuated. The converse applies during normoxia. Measurements of the ideal alveolar–arterial P_{O_2} difference were made at rest and on exercise, breathing air and repeated breathing about 11% inspired O_2; from these data the alveolar to end-capillary oxygen tension difference was derived, and the mean gradient ($[P_A–P\bar{c}]O_2$) was calculated by Bohr integration from the Pc'–$P\bar{v}$ oxygen tension difference. Lilienthal et al. (1946) found that their results for $D_{L_{O_2}}$ at rest and on exercise were in accord with M. Krogh's (1915) calculations of $D_{L_{O_2}}$ from $D_{L_{CO}}$.

In terms of validation for the $D_{L_{CO}}$, the $D_{L_{O_2}}$ was considered to be the 'gold standard'; in the 1950s, differences between $D_{L_{CO}}$ (ss) and $D_{L_{CO}}$ (sb) were thought to be of secondary importance, according to Colin Ogilvie (personal communication). For the comparison, the $D_{L_{O_2}}$ by the Lilienthal et al. (1946) technique, and the $D_{L_{CO}}$ were measured in the steady state, on exercise, breathing 10–11% oxygen, at a $P_{A_{O_2}} < 54$ mmHg. The measurements of $D_{L_{CO}}$ (ss) were repeated shortly afterwards at a normal $P_{A_{O_2}}$. Much later, Meyer et al. (1981) measured $D_{L_{O_2}}$/$D_{L_{CO}}$ simultaneously using a rebreathing technique and the stable oxygen isotope, ^{18}O, also at a low $P_{A_{O_2}}$ (ca. 40 mmHg). The results are given in Table 4.

From Table 4, the mean $D_{L_{O_2}}$/$D_{L_{CO}}$ ratio in hypoxia is 1.36 (omitting the two subjects in Forster et al. (1954c). This translates to a $D_{L_{O_2}}$/$D_{L_{CO}}$ ratio in *normoxia* of 1.71 (1.36×1.26). The reasons for this are the differences in tissue diffusivities (the O_2/CO diffusivity ratio being in the range 1.1–1.4; (Meyer et al., 1981) and in the rates of reaction of O_2 and CO with haemoglobin (θ_{O_2} being about twice θ_{CO}; Forster, 1987). There was no agreement about whether $D_{L_{O_2}}$ or $D_{L_{CO}}$ reached a maximum value on exercise, but more recent work has shown that both increase as a linear function of pulmonary blood flow up to the

maximum exercise level (Johnson et al., 1996, pp. 546). There have been measurements, reviewed by Haab (1981), in which the $D_{L_{O_2}}$/$D_{L_{CO}}$ ratio is < 1.0, but these were carried out at rest or with the single breath technique, situations in which inhomogeneity of D_L, particularly for oxygen, is likely to influence the results.

A low $D_{L_{O_2}}$ (and $D_{L_{CO}}$) is a cause of a fall in arterial oxygen saturation (Sa_{O_2}) on exercise. Baldwin et al. (1949) described a group of patients with restrictive lung disease and lung fibrosis whose Sa_{O_2} had decreased by > 10% in the first minute following exercise. Their finding was confirmed, in a similar group of patients, by Austrian et al. (1951) who, in addition, also measured a low $D_{L_{O_2}}$. They coined the term 'alveolar–capillary block', but the 'block', which refers to *diffusion-limitation*, only plays a significant role on exercise when oxygen demands are increased. When resting $D_{L_{CO}}$ is < 60% predicted in patients with lung fibrosis, worsening of arterial hypoxaemia on exercise is almost invariable.

5. 1958–75: the single breath $D_{L_{CO}}$ ($T_{L_{CO}}$) becomes the method of choice

5.1. Single breath versus steady state $D_{L_{CO}}$

From a scientific point of view in the 1950s, there was no clear-cut answer to the question "Which $D_{L_{CO}}$ method is the best?"—see Table 5. All methods gave reasonably concordant results in normal subjects, and on exercise there was a good correlation between $D_{L_{O_2}}$ and $D_{L_{CO}}$ (ss) (Table 4). But, all $D_{L_{CO}}$ methods were bound to 'fail' to a greater or lesser extent in the face of gross inhomogeneity of ventilation and diffusion per unit alveolar volume, e.g. in emphysema, because no 'correct' estimate of mean alveolar $P_{A_{CO}}$ was feasible, and the Filley measurement of 'effective' $P_{A_{CO}}$ required arterial sampling. The single breath technique, with its rapid inspiration to TLC, might be 'unphysiological', but it did remove some of the maldistribution of ventilation which, in airflow obstruction, was amplified during tidal breathing; the CO back pressure effect was also less.

Table 4
Simultaneous comparison of $D_{L_{O_2}}$ and $D_{L_{CO}}$ (ss)

Authors/subjects	$\dot{V}_{O_2}$ (L min^{-1})	$D_{L_{O_2}}$ (ml min^{-1} mmHg^{-1})	$D_{L_{O_2}}/D_{L_{CO}}$ (hypoxia)	$D_{L_{CO}}$ (hypoxia)/$D_{L_{CO}}$ (normoxia)
Forster et al. (1954c)				
Normal subject	1.1–1.5[1]	65[2]	0.98	1.27
Silicosis patient	1.4	56	1.37	1.28
Shepard et al. (1958)				
Normals ($n = 3$)	1.0–2.5[1]	51[2]	1.4	1.25
Turino et al. (1963)				
Normals ($n = 11$)	1.2	67	1.37	
	1.7	90	1.6	
Meyer et al. (1981)[3]				
Normals ($n = 6$)	1.4	64	1.2	
	2.1	65	1.21	

Two to four observations were made per subject per workload.
[1] Range for up to four measurements.
[2] Mean value for up to four measurements.
[3] Rebreathing technique.

Table 5
Advantages and disadvantages of steady state and single breath $D_{L_{CO}}$

$D_{L_{CO}}$ (sb)	$D_{L_{CO}}$ (ss)
Advantages	
Rapid, reproducible, repeatable	Minimal subject cooperation
Inspiration to TLC reduces ventilatory maldistribution	Measured during normal breathing
HbCO levels low	Easily measured on exercise
Disadvantages	
Breath holding at TLC is unphysiological	Bates method of sampling alveolar P_{CO} is unreliable in airflow obstruction
Sensitive to $\dot{V}I/VA$ maldistribution	Sensitive $++$ to $\dot{V}I/VA$ maldistribution
Sensitive $++$ to DL/VA maldistribution	Sensitive $++$ to DL/VA maldistribution
	Filley method requires arterial sampling

In the end, the decision was a pragmatic one. A busy Pulmonary Function Laboratory wants a test which is rapid, reproducible and repeatable. A turning point was the publication of an article from Philadelphia by Ogilvie et al. (1957) entitled "*A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide*". Colin Ogilvie was a Travelling Fellow of the Postgraduate Federation of the University of London. The article was wide ranging in its examination of the methodological, physiological and clinical aspects of the $D_{L_{CO}}$ (sb), e.g. the optimal breath hold time, dead space wash-out, the effects of intrathoracic pressure, lung volume and body position change, the influence of $P_{A_{O_2}}$ and HbCO levels, exercise, hyperventilation and reproducibility. In normal subjects, the $D_{L_{CO}}$ (sb) was correlated with body surface area. In 28 patients with respiratory disease, $D_{L_{CO}}$ (sb) was reduced in emphysema, and in fibrosis from various causes; importantly, the $D_{L_{CO}}$ was within the normal range in asthma, localised lung disease, pulmonary hypertension and kyphoscoliosis.

Nevertheless, the paper of Ogilvie et al. (1957) aroused little enthusiasm at the time. When Ogilvie gave a seminar in the 'enclave of $D_{L_{O_2}}$' (RL Riley's Laboratory at Johns Hopkins, Baltimore), Moran Campbell, then a research fellow, said "the single breath $D_{L_{CO}}$ is the greatest fraud perpetrated since the South Sea Bubble". He later relented, and it was in use at the Hammersmith Hospital in the early 1960s! With time, Ogilvie et al.'s paper became influential and much quoted. Its tone was pragmatic, such as, "*The absolute error in DL is unknown at present; the greatest usefulness of the measurement lies in comparisons*". In the 1960s,
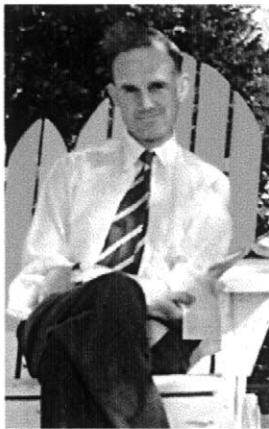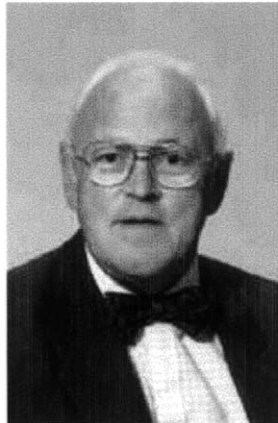
August and Marie Krogh
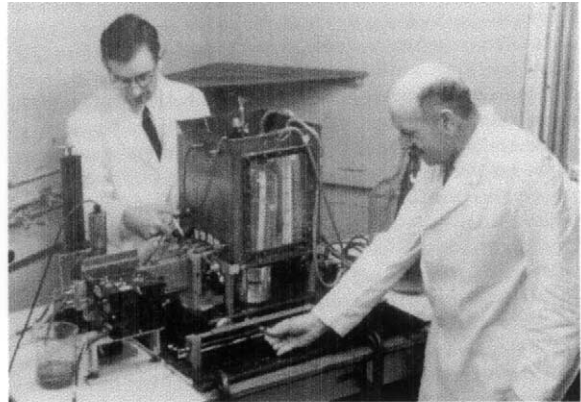
R.E. Forster

F.J.W. Roughton

C.M. Ogilvie

D.V. Bates

H. Hartridge

Fig. 3. August and Marie Krogh were the originators of the DL_{CO} sb technique in 1909–15, the modern version being described by Ogilvie et al. (1957). The DL_{CO} ss was pioneered by Bates (1952), Bates et al. (1955, 1956). The D_M and $\theta \cdot V_c$ analysis was described by Roughton and Forster (1957), and the first measurements of O_2 and CO combination with Hb were made by Hartridge and Roughton (1923a).

DL_{CO} (sb) and DL_{CO} (ss) tests were beginning to establish themselves in Pulmonary Function Laboratories throughout the world, but by the 1970s the DL_{CO} (sb) was starting to take over (Fig. 3).

5.2. DL_{CO} sb [TL_{CO}]: modifications and refinements for clinical use

With Ogilvie et al.'s 1957 paper, the single breath DL_{CO} was launched as a clinical test. Initially, there was concern about "*unnecessarily straining the laboratory facilities of personnel and*

equipment" and that "*there are simpler methods [arterial or capillary P_{O_2} and P_{CO_2} on exercise] giving almost the same information*" (Bjure and Soderholm, 1968)). Nevertheless, in 1965, an automated apparatus for measuring (i) lung volumes, (ii) the alveolar plateau for nitrogen and (iii) the single breath TL_{CO} was described by the staff of the Pneumoconiosis Research Unit (PRU), Llandough (Meade et al., 1965), and came onto the market. Other manufacturers followed suit. Once it was possible for Pulmonary Function Laboratories to buy apparatus off the shelf, any

inhibitions about introducing a complex test like $T_{L_{CO}}$ into routine clinical practice were quickly dispelled, so that by 1975 all serious pulmonary function services were offering the $D_{L_{CO}}$ (sb) as a routine test.

Jones and Meade (1961), also from the PRU, Llandough, published an erudite paper on 'anomalies in the $T_{L_{CO}}$ (sb)', combining theory with observations; their recommendations on the measurement of the breath hold time are now generally accepted.

5.3. Introduction of the term transfer factor [$T_{L_{CO}}$]

JE Cotes in a symposium discussion (Cotes and Meade, 1963) of papers by F.J.W. Roughton and others stated that in relation to the Roughton–Forster equation "*the term diffusing capacity was clearly inappropriate*", and he suggested substituting "*the transfer coefficient, T_L*". Roughton's response was to agree, but he pointed out that *coefficient* often had precise connotations in terms of 'unit area or thickness', and until these were better defined for the lung he felt that the word 'factor' should be used. So, transfer factor ($T_{L_{CO}}$) it became! The $T_{L_{CO}}$ did not 'transfer' (sic) across the Atlantic very easily, and the 'inappropriately named' $D_{L_{CO}}$ still remains in common usage in North America. The term 'transfer coefficient' has been handed on to the D_L/V_A (T_L/V_A) (see below).

5.4. Diffusing capacity (transfer factor) per unit volume D_L/V_A (T_L/V_A)

There has always been confusion about the T_L/V_A (D_L/V_A), the popular misconception being that by dividing $T_{L_{CO}}$ by the alveolar volume (V_A) at which the measurement was made, the $T_{L_{CO}}$ was somehow 'corrected' for volume to give a *specific* $T_{L_{CO}}$, analogous to specific airway conductance or specific lung compliance (neither of which are 'volume independent'). The confusion stems from the fact that T_L/V_A is the equivalent (except for its units) to Krogh's *k*, K_{CO} or $(\log_e[CO_1/CO_2]/BHT$. Thus, $T_{L_{CO}}$ is *derived* from T_L/V_A and V_A, rather than the other way round. In the UK and Europe, the more Krogh-like term K_{CO} is gradually repla-

cing T_L/V_A in recognition of this. The objection to the use of T_L/V_A (K_{CO}) is that it is not independent of lung volume; Forster (1983) was always dubious about it for this reason, and Chinn et al. (1996) consider that, for epidemiological research in particular, the T_L/V_A (K_{CO}) should be abandoned. But, clinicians like the K_{CO} and find it useful, and a defence of the K_{CO} has come from the Hammersmith Hospital (Hughes and Pride, 2001).

6. The Roughton–Forster equation (1958–2003)

6.1. Membrane diffusing capacity (D_M)

Measurements of $D_{M_{CO}}$ in normal subjects (single breath method unless otherwise stated) in the era 1957–1975 are given in Table 6. D_M increases from rest to exercise by 50% (if Forster's data [1957c] are excluded) mostly because of improvements in $\dot{V}_I/V_A$ and D_M/V_C distribution, rather than actual 'unfolding' of the alveolar epithelial surface. At rest, partly because of gravity, blood flow (to which V_C is linked) is uneven in relation to alveolar volume and D_M; on exercise, there is better 'coupling' of D_M and V_C. The obligatory 'coupling' of D_M and V_C is the reason why the Roughton–Forster partitioning of the $D_{L_{CO}}$ into its components (not difficult to do in patients) has not proved useful clinically. The only examples of 'uncoupling' are congestive heart failure (Puri et al., 1995; D_M is reduced when V_C is normal or high), intrapulmonary haemorrhage (Ewan et al., 1976; V_C high, D_M normal or reduced) and adaptation to high altitude (West, 1962; V_C high, D_M normal).

A further example of the importance of 'coupling' of D_M and V_C comes from the finite element model (FEM) of R.L. Johnson and his colleagues, following a suggestion of Federspiel (1989) that D_M was not a fixed quantity for a given alveolar geometry, but was affected by the localisation, i.e. the spacing, of the red cells. Hsia et al. (1995b, 1999) and Frank et al. (1997) computed D_M and $D_{L_{CO}}$ by FEM in a capillary model, using it as the 'gold standard' for Roughton–Forster or morphometric ('Weibel') calculations. Note that in the

Table 6
Diffusing capacity ($D_{L_{CO}}$), membrane diffusing capacity ($D_{M_{CO}}$), pulmonary capillary volume (Vc) and red cell diffusion resistance percentage (Rcap/Rtot% $\sim [1/D_L - 1/D_M]/1/D_L$%) at rest and on exercise in normal subjects, plus estimates of D_M from dithionite-perfused lobes and from morphometric studies

Authors	Status	$D_{L_{CO}}$[1]	$D_{M_{CO}}$[1]	Vc (ml)	Rcap/Rtot%[2]
Forster, 1957[3]	Rest	30	57	80	52
Johnson et al., 1960[4]	Rest	25	48	79	47
Sackner et al., 1975[4,5]	Rest	23	34	98	34
Mean at rest		26	46	86	44
Johnson et al., 1960[6]	Exercise	45	73	142	38
Sackner et al., 1975[6]	Exercise	37	51	149	27
Bates et al., 1960[7]	Exercise	37	55	153	33
Mean on exercise		40	60	148	33
Burns and Shepard, 1979	Isolated dog lobe		187[8]		
Morphometry					
Gehr et al., 1978	Necropsy	70–165	463[8] (272[9])	200	64–83

$1/\theta_{CO}$ values according to Roughton and Forster (1957).

[1] Units are traditional: ml min^{-1} mmHg^{-1}. For SI units (mmol min^{-1} kPa^{-1}) divide by 3.

[2] Referred to $D_{L_{CO}}$ breathing air ($P_{A_{O_2}} \sim 100$–120 mmHg).

[3] Mean (from Table 2 in Forster, 1957) from four studies (various techniques) but excluding Roughton 1945a.

[4] Calculated for $\dot{Q} = 6$ L min^{-1} from regression equations. Single breath technique.

[5] Rebreathing technique.

[6] Calculated for $\dot{Q} = 15$ L min^{-1} from regression equations.

[7] Steady state technique, at mean $\dot{V}_{O_2}$ 1.3 L min^{-1}.

[8] $D_{M_{O_2}}/1.23$. Dog lobes (Burns and Shepard, 1979) scaled to 5.0 L volume.

[9] After correction according to Hsia et al. (1995b), see text.

Roughton–Forster model $1/D_M$ includes the diffusion resistance of the plasma. D_M per red cell, calculated by FEM, decreases as Hct increases because of restricted diffusion pathways between red cells as they bunch together although the 'errors' in the case of the Roughton–Forster analysis are quite small (Hsia et al., 1995b). More importantly, at a fixed 'physiological' capillary Hct (~ 30%, Brudin et al., 1986), bunching and spacing-out of red cells in the alveolar septum could decrease $D_{M_{CO}}$ by up to 30–40%. If cells are bunched together, their adjacent surfaces reduce the combined surface area for diffusion, while there is no CO uptake in the space they have vacated, a concept, in terms of diffusive uptake, not dissimilar to that of alveolar dead space and 'shunt' for $\dot{V}_A/\dot{Q}$. In addition, lowering the haematocrit will affect not just $\theta \cdot Vc$ but D_M as well, because more of the alveolar–capillary membrane is separated ('uncoupled') from the red cell membrane.

There have been two other approaches (see Table 6), both of which would seem to measure an upper–bound value or D_M max. The first is a physiological one; Burns and Shepard (1979) added the powerful reducing agent, sodium dithionite ($Na_2S_2O_4$), to the blood perfusing an isolated dog lobe. $Na_2S_2O_4$ reacts so rapidly with O_2 in plasma that Ppl_{O_2} is effectively zero; thus, $D_{L_{O_2}} = \dot{V}_{O_2}/P_{A_{O_2}}$ and is equivalent to $D_{M_{O_2}}$. In dog lobes, the $D_{M_{CO}}$ (from $D_{M_{O_2}}/1.23$) was 3 times greater than values in humans on exercise (Table 6), subject to reservations about the species difference and the scaling up to human lung volumes.

The other approach is a stereologic and statistical one. The morphometric measurements developed by Weibel (1973) and colleagues (Gehr et al., 1978)) consist of statistical calculations, from microscopic and electron microscopic lung sections, of the area and thickness of the alveolar–capillary membranes, and of the area and length of the pulmonary capillaries. Their calculations of

DL_{O_2} and DM_{O_2} are many times greater than in vivo measurements by any technique (Table 6). DM, adjusted to DM_{CO}, is 463 ml min^{-1} $mmHg^{-1}$, which is $\times 10$ greater than in vivo DM_{CO}. Hsia et al. (1995b) have suggested that the morphometric technique underestimates molecular diffusion distances and overestimates the epithelial surface area, the capillary haematocrit, and the DM_{CO} itself by 1.7 times. Their 'corrected' morphometric DM_{CO} of 272 (Table 6) must be regarded as a 'maximum' which is unattainable in life.

Finally, DM_{CO} is sensitive to the value of $1/\theta_{CO}$ in the Roughton–Forster analysis (see Fig. 5, Table 8 and Section 6.5).

6.2. Diffusing capacity of blood (θ·Vc)

$1/\theta \cdot Vc$ in the Roughton–Forster equation is the transfer resistance per ml blood. To be more accurate, it is the oxygen–dependent part of the total resistance, and as such the plasma is excluded. Partitioning DL_{CO} into DM and Vc is dependent on the reciprocal relationship between $1/\theta$ and intracapillary P_{O_2}:

$$1/\theta = (\alpha + \beta \cdot P_{O_2}) \cdot [Hb\ st/Hb] \qquad (4)$$

where $1/\theta$ is the overall *specific* transfer resistance from the red cell membrane to the haemoglobin molecule, α and β are specific resistances in series, and [Hbst/Hb] is a standardised normal value divided by the haemoglobin concentration of the subject as a fraction of normal. The specific resistance $(1/\theta)$ is the reciprocal of the conductance per ml capillary blood $[\theta \cdot Vc/Vc]$. Traditionally, α is related to λ, the ratio of the permeability of the red cell membrane to the permeability of the red cell interior, but current opinion places most of the diffusion resistance external to the red cell interior in unstirred plasma layers immediately adjacent to the cell membrane (see Section 6.3). β is a temperature- and pH-dependent coefficient linked to the kinetic reactions of CO with Hb; it is a specific resistance linked to the combination of diffusion and reaction within the red cell. Eq. (4), established by Roughton and Forster (1957) using in vitro kinetic data is for red cells, the equivalent

per ml blood of the Roughton–Forster equation for the overall transfer process.

6.3. Unstirred extracellular layers (USL)

Roughton was very intrigued by the differences in reaction rates, particularly for oxygen, between Hb solutions and red cell suspensions (see Table 1), originally described by Hartridge and Roughton (1927), and the question was posed in that paper " *which is of predominating importance, the rate of diffusion of dissolved gas through the corpuscle membrane, or the rate of chemical reaction inside the corpuscle?*". Roughton (1932) made elaborate calculations of the process of simultaneous diffusion and reaction with Hb within simulated red cells, and though the models became more sophisticated in later years (Nicolson and Roughton, 1951), the diffusion resistance of the red cell overall was always greater (for O_2 2:1, for CO somewhat less) than estimates of the combined diffusion and reaction processes within the cell itself. From an early stage (see above), Roughton became convinced that the 'missing' resistance lay within the red cell membrane, introducing the term λ, the ratio of the permeability of the red cell membrane to the cell interior (generally taken to be 1.5–2.5).

The process of combined diffusion and chemical reaction within the red cell is complex. As CO diffuses into the cell, the outer layers of Hb combine with it so rapidly that a 'front' is created (Forster, 1964), i.e. chemical combination reduces the dissolved CO tension and slows diffusion. This process is called the 'advancing front' phenomenon. As the reaction rate increases (e.g. CO to O_2 to NO), the overall red cell specific conductance (θ) reaches a plateau (R.E. Forster, personal communication) because intra-red cell diffusion becomes rate-limiting.

It is easy, in retrospect, to see that a lipid-rich cell membrane cannot be a significant barrier to the transfer of physiological gases, and that the additional resistance must lie outside the membrane. Intuitively, the existence of stagnant pericellular layers in a rapid reaction apparatus with a turbulent flow regime seemed unlikely; Hartridge and Roughton (1927) thought they had shown that

mixing was and remained complete. Nevertheless, the particulate nature of blood is such that stagnant layers form around cells within 2–3 ms after mixing, as well as eddies with mixing times of 3–60 ms. From 1979–85 several groups showed that θ_{O_2} was sensitive to changes in the pericellular environment, being reduced when the diffusivity and solubility of extracellular fluid was lowered by the addition of albumin (Huxley and Kutchai, 1981; Yamaguchi et al., 1985) and enhanced (in the case of the $HbO_2 \rightarrow Hb + O_2$ reaction) when an oxygen scavenger (Na dithionite) was added to the buffer solution (Coin and Olson, 1979; Holland et al., 1985; Yamaguchi et al., 1985). The red cell membrane contributed only 5% of the resistance to red cell entry (Huxley and Kutchai, 1981). Holland et al. (1985) estimated that the effective thickness of the unstirred layer was 0.7–0.9 μm, equal to the half thickness of the red cell, and that it con-

tributed 67% of the total specific resistance ($1/\theta$) in the case of O_2.

Reeves and Park (1992) investigated θ_{CO} in a novel way by enclosing thin films (1.5–6.0 μm) of whole blood (of normal haematocrit) in Gore-Tex, a highly gas permeable open mesh of Teflon fibrils, and then exposing them to step changes of P_{CO} and P_{O_2} (Fig. 4). Values approached those found with Hb solutions (Gibson and Roughton, 1955) indicating that for non-flowing whole blood the diffusion resistance ($1/\theta$) per ml was located predominantly in the reaction with Hb. In Reeves and Park's (1992) equation for $1/\theta$ the α term was negligible (Table 7), implying that there was no unstirred layer. Nevertheless, the properties of blood *flowing* in capillaries mean that the presence of an unstirred layer in vivo cannot be discounted.

The measurements of Roughton and Forster (1957) and of Forster (1987) were made in the continuous-flow rapid reaction apparatus, the former at pH 8.0 and the latter at pH 7.4. Holland (1969) used the stopped flow technique. Reeves and Park's (1992) measurements were on static thin blood films, as just described. The term α in Eq. (4) represents the non-Hb red cell resistance, located in the cell, cell membrane and pericellular unstirred fluid. The contribution of α to the total resistance declines at high P_{O_2} as the Hb-reactive resistance increases (Table 7). In relation to the reactive resistance (Hb reacting with CO), the diffusive resistance (α) in Reeves and Park (1992) is negligible. The higher values for α in the rapid reaction studies may be due to the presence of unstirred plasma/buffer layers (notice α is higher with the stopped flow technique (Holland, 1969) than with the more recent continuous flow measurements (Forster, 1987)).

6.4. Pulmonary capillary volume (Vc) and transit time

From Table 6 Vc at rest is in the range 80–100 ml, increasing to 145 ml on exercise due to recruitment and distension of the capillary bed. Pulmonary capillary haematocrit is 67% of that in the larger vessels (Brudin et al., 1986) due to the Fåhreus–Lindqvist effect, whereby red cells accelerate relative to mean plasma flow in their passage
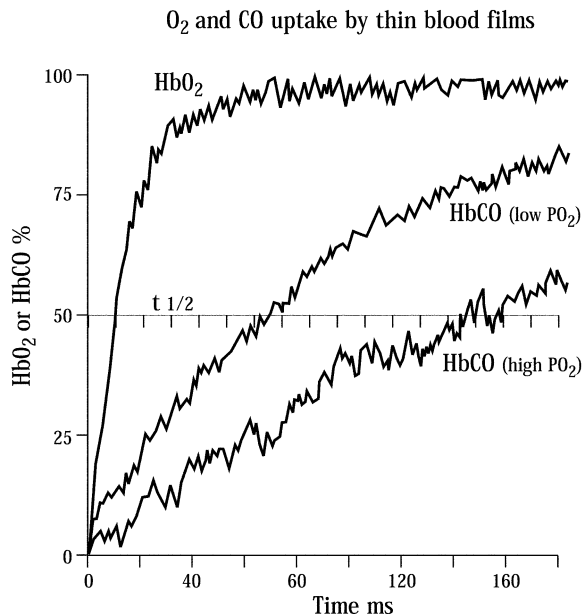

Fig. 4. Time course of percent O_2 and CO binding on a whole blood thin film (sequential measurements) for step change of P_{O_2} 70 mmHg (top curve), P_{O_2} 70 and P_{CO} 70 mmHg (middle curve), P_{O_2} 280 and P_{CO} 70 mmHg (bottom curve). Note $t_{1/2}$ of 8 ms (top), 68 ms (mid) and 150 ms (bottom). Redrawn from Reeves and Park (1992).

Table 7
Published equations for $1/\theta = (\alpha + \beta \cdot P_{O_2})$ with values for $1/\theta$ at P_{O_2} 100 and 500 mmHg (units: ml min^{-1} mmHg^{-1}) at T = 37 °C, and percentage resistance in the non-P_{O_2}, non-Hb dependent term, α, at the corresponding P_{O_2}s (100) (500)

Authors	α	β	$1/\theta$ (100)	$1/\theta$ (500)	$\alpha/[1/\theta]$ (100) (%)	$\alpha/[1/\theta]$ (500) (%)
Roughton and Forster (1957)	0.7	0.0061	1.31	3.75	53	19
Holland (1969)	1.08	0.0065	1.73	4.33	62	25
Forster (1987)	1.3	0.0041	1.71	3.35	76	38
Reeves and Park (1992)	0.0156	0.008	0.816	4.016	1.9	0.39

through the capillary bed. This does not mean that Vc values should be increased by a factor equal to 1.0/0.67, because the 'effective' θ_{CO} will have been reduced by 0.67 (see Eq. (4)), the two factors cancelling out. On exercise, pulmonary capillary temperature may increase by 0.5–1.0 °C (reviewed in Bradley et al., 1976). θ_{CO} increases by 2.5% °C^{-1} (Forster, 1987). This has the effect, which is negligible, of decreasing the calculations of Vc on exercise by 3% °C^{-1} rise (DM increases by 2%).

An advantage of deriving capillary volume (Vc) from the Roughton–Forster equation was that capillary transit time could be calculated as Vc/$\dot{Q}$, where $\dot{Q}$ is cardiac output. Johnson et al. (1960) calculated mean pulmonary capillary transit time, finding a large spread of values at rest (0.7–1.2 sec), but less scatter on exercise with a progressive fall in transit time as a function of cardiac output to 0.5 sec at $\dot{Q} = 18$ L min^{-1}. Note more recent estimates of 0.7–0.8 sec (rest; Table 6) and 0.4 sec (exercise; Fig. 5). These estimates confirm Roughton's (1945b) calculations using a more indirect method.

Mostyn et al. (1963) made the interesting observation that championship swimmers of Olympic or near Olympic standard had a 40% higher DL$_{CO}$ ss at $\dot{V}_{O_2}$ 2.0 L min^{-1}. This was due to a higher pulmonary capillary blood volume (Vc). For the same cardiac output (15 L min^{-1}), the champion swimmers had a Vc of 180 ml versus 120 ml for non-champions, giving them a pulmonary capillary transit time advantage (0.75 vs 0.5 sec). This supernormal DL$_{CO}$ in champion swimmers would seem to be inherited. Further discussion is given by Bates et al. (1966).

6.5. DM and Vc calculated from different equations for $1/\theta$

In Table 8 (rest) and Fig. 5 (exercise), the effect of the equation for $1/\theta$ (Table 7) on the calculation of DM and Vc can be appreciated. The published
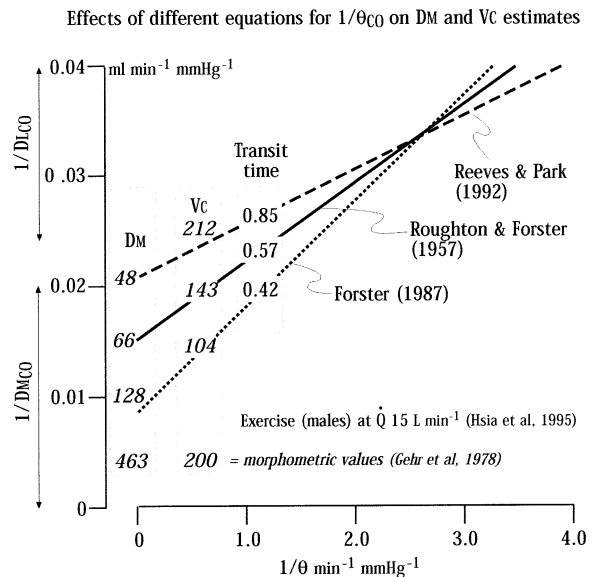

Fig. 5. Reciprocal of DL$_{CO}$ (1/DL$_{CO}$) plotted against $1/\theta$ (according to the Roughton–Forster equation). The y-axis intercept is the membrane diffusion resistance (1/DM) and the slopes are 1/Vc. Data for 1/DL (at P$_{AO2}$ 120 mmHg) and 1/DM, on exercise ($\dot{Q} = 15$ L min^{-1}) taken from regression equations of Hsia et al. (1995a) [their Table 3], and 1/Vc plotted and extrapolated to P$_{AO2}$ 500 mmHg. Solid lines are based on Roughton and Forster (1957) θ_{CO} values, as used by Hsia et al., and interrupted lines represent the same data replotted using the Forster (1987) or Reeves and Park (1992) relationship between $1/\theta$ and P$_{O_2}$. Vertical columns give values for membrane diffusing capacity (DM), pulmonary capillary blood volume (Vc) and capillary transit time with morphometric estimates for comparison.

Table 8
Resting diffusing capacity ($D_{L_{CO}}$), membrane diffusing capacity ($D_{M_{CO}}$), pulmonary capillary volume (Vc) and transit time, and red cell resistance percentage (Rrc/Rtot% ~ [1/D_L(air)−1/D_M]/1/D_L(air)%) , showing effects of different θ_{CO} values

Authors	θ_{CO} values	$D_{L_{CO}}$[1]	$D_{M_{CO}}$[1]	Vc (ml)	Transit time (s)	Rrc/Rtot (%)
Hsia et al., 1995a[2]	1957[3]	25	45	91	1.1	45
Hsia recalculated	1987[4]	25	108	58	0.7	78
Hsia recalculated	1992[5]	25	30	118	1.42	18
Borland and Cox, 1991[6]	1987[4]	28	159[7]	56	0.7	82

T = 37 °C.

[1] Units are traditional: ml min⁻¹ mmHg⁻¹. For SI units (mmol min⁻¹ kPa⁻¹) divide by 3.

[2] Rebreathing technique. Males ($n = 30$). D_L and D_M from regression equations (Hsia et al., 1995a: Table 3] 5.0 L min⁻¹ cardiac output. Vc calculated from Roughton–Forster equation.

[3] θ_{CO} values from Roughton and Forster, 1957.

[4] Forster (1987) θ_{CO} values.

[5] Reeves and Park (1992) θ_{CO} values.

[6] Simultaneous inhalation of CO and NO. Single breath technique. $D_{L_{NO}} = 126$.

[7] $D_{M_{CO}}$ calculated from Eq. (5) using θ_{NO} (Carlsen and Comroe, 1958) and the 1987 θ_{CO} values. 5.0 L min⁻¹ cardiac output (assumed).

data from Hsia et al. (1995a) used the Roughton and Forster (1957) 1/θ values.

There are substantial differences in the calculated values for D_M, Vc and capillary transit time at rest (Table 8) and on exercise (Fig. 5) depending on the 1/θ equation used. The low value for α and higher value for β · P_{O_2} of Reeves and Park (1992) means that D_M is low, Vc and transit time are high and 82% of the transfer resistance (84% on exercise) is in the alveolar-capillary membranes. Conversely, Forster's (1987) equation for 1/θ with the highest α value and the lowest β · P_{O_2} exponent is associated with high D_M values, low Vc and transit times, with most of the transfer resistance associated with the red cell.

6.6. Diffusing capacity for nitric oxide $D_{L_{NO}}$

An important development has been introduction of NO as a marker gas and the simultaneous measurement of $D_{L_{NO}}$ and $D_{L_{CO}}$. Nitric oxide reacts very rapidly with HbO_2 to form metHb and NO_3; in blood, θ_{NO} is nearly 6 times greater than θ_{CO} (4.4 mmHg⁻¹ min⁻¹ vs 0.76 for θ_{CO} at P_{O_2} 100 mmHg; Carlsen and Comroe, 1958; Roughton and Forster, 1957). The method and analysis of $D_{L_{NO}}$ by the single breath or the rebreathing technique is similar to that for the $D_{L_{CO}}$. Borland and Higgenbottam (1989) published results for

$D_{L_{NO}}$, having reported measurements of NO uptake in Abstract form in 1983. Their reason for measuring $D_{L_{NO}}$ initially was that it was an important component of the gas phase of cigarette smoke.

Guenard et al. (1987) reported measurements of D_M and Vc, derived from simultaneous measurements of single breath $D_{L_{NO}}$ and $D_{L_{CO}}$. Their findings of a $D_{L_{NO}}$ in normal subjects of 136 ml min⁻¹ mmHg⁻¹ and a $D_{L_{NO}}/D_{L_{CO}}$ ratio of 4–5 have been confirmed by subsequent work (Tamhane et al., 2001); this ratio does not change on exercise (Tamhane et al., 2001). $D_{L_{NO}}$ (and θ_{NO}), unlike $D_{L_{CO}}$ and θ_{CO}, is not altered by increasing $P_{A_{O_2}}$ (Borland and Cox, 1991). Guenard et al. (1987) argued that since the rate of reaction of NO with haemoglobin itself was 200 times faster than that for CO, the 1/θ·Vc term in the Roughton–Forster equation could be neglected so that [$D_{L_{NO}}/\alpha$] = $D_{M_{CO}}$ where α (1.93) is the physical diffusivity ratio of NO/CO. Therefore, 1/Vc = (1/$D_{L_{CO}}$−α/$D_{L_{NO}}$), and $D_{L_{NO}}$ becomes a surrogate for $D_{M_{CO}}$. This increases the calculated value of $D_{M,CO}$ two-fold, and the $D_{M,CO}/D_{L_{CO}}$ ratio at rest from about 1.7 to 3.5. The weakness of the argument that $\theta_{NO} = \infty$ is that the resistance of the unstirred layers, red cell membrane and the interior of the cell is neglected. Surprisingly, Tamhane et al. (2001) found a $D_{L_{NO}}/D_{M_{CO}}$ ratio at rest and

on exercise of 2.49, greater than the 1.93 ratio predicted if $D_{L_{NO}} \sim D_{M_{NO}}$. Their explanation was that their $1/\theta_{CO}$ values (from Roughton and Forster, 1957) may have underestimated $D_{M_{CO}}$ by 25%. In fact, if θ_{NO} is finite, $D_{M_{NO}}$ must exceed $D_{L_{NO}}$, so the ratio $D_{M_{NO}}/D_{M_{CO}}$ would have been >2.49, and the underestimation of $D_{M_{CO}}$ would have been closer to 100%.

Borland and Cox (1991) and Borland et al. (2001) used Carlsen and Comroe's (1958) value for θ_{NO}, measured in the continuous-flow rapid reaction apparatus (for θ_{CO}, they took Forster's 1987 data). From the Roughton–Forster equations for CO and NO, they derived:

$$D_{M_{NO}} = (\theta_{NO}-2\theta_{CO})/(\theta_{NO}/D_{L_{NO}}-\theta_{CO}/D_{L,CO}) \quad (5)$$

If Eq. (5) is used, $D_{M_{NO}}$ exceeds $D_{L_{NO}}$ about twofold (333 vs 182 ml min^{-1} $mmHg^{-1}$; Borland and Cox, 1991) and $D_{M_{CO}} = 159$ ml min^{-1} $mmHg^{-1}$, some 3.6 times greater than $D_{M_{CO}}$ measured with the conventional Roughton and Forster (1957) θ_{CO} equation (see Table 8). There is, of course, the same uncertainty about the 'true' value for θ_{NO} as there is for θ_{CO}.

7. What is the $D_{L_{CO}}$ ($T_{L_{CO}}$) actually measuring?

Up to 1957, the prevailing view was that the $D_{L_{CO}}$ was a measure of alveolar–capillary membrane diffusion, i.e. D_M. The publication of the Roughton–Forster equation in 1957 changed perceptions by partitioning the total transfer resistance into two approximately equal parts, with half the resistance being in a membrane component ($1/D_M$), and half in pulmonary capillary blood ($1/\theta \cdot V_c$). θ_{CO} was calculated in vitro in the rapid reaction apparatus, but there is no guarantee that this mimics the situation in vivo. $1/\theta_{CO}$ of blood measured in vitro includes a diffusion resistance in series with the reaction resistance, emanating from a stagnant 1.0 μm thick pericellular layer of plasma (Huxley and Kutchai, 1981; Holland et al., 1985). An unstirred plasma layer (greater in the case of measurements with the stopped flow compared with the rapid reaction apparatus) probably also exists in the capillary bed, either pericellular or associated with

the luminal surface of the endothelial cell and with the low haematocrit in the microvasculature (Brudin et al., 1986).

The precise values chosen for θ_{CO} affects the calculations of D_M, V_c, capillary transit time and the transfer resistance partititing (R_{rc}/R_{tot}) quite markedly (Table 8, Fig. 5). The percentage of the transfer resistance located in the red cell itself (R_{rc}/R_{tot}), including the plasma layer, varies from 20 to 80% depending on the values for $1/\theta_{CO}$. On the basis of capillary transit times, re-estimations of $D_{M_{CO}}$ from $D_{L_{NO}}$ and $D_{M_{NO}}$ (Table 8; Borland and Cox, 1991; Borland et al., 2001) and morphometric measurements of D_M (see Section 6.1), R_{rc}/R_{tot} is more likely to be $>50\%$ (possibly $>75\%$) rather than $<50\%$. Other features about $D_{L_{CO}}$ which argue in favour of $1/\theta \cdot V_c$ being an important rate limiting step in alveolar CO uptake are, (i) the effects of anaemia and increases of HbCO on $D_{L_{CO}}$, (ii) the marked reduction in $D_{L_{CO}}$ (with normal vital capacity) in some pulmonary vascular conditions, and (iii) the effect of raising $P_{A_{O_2}}$.

$D_{L_{CO}}$ vs $D_{L_{O_2}}$. At a physiological alveolar P_{O_2} (100 mmHg), the half-time for the $HbO_2+CO \rightarrow HbCO+O_2$ reaction in blood is about 6–7 times longer (slower) than the oxygenation reaction of HbO_2 (from 75 to 97.5%) within the alveoli (Reeves and Park, 1992). This implies that the red cell transfer resistance (R_{rc}/R_{tot}) is less for $D_{L_{O_2}}$ than for $D_{L_{CO}}$, and that $1/D_M$ is a more important rate-limiting step for oxygen transfer than for CO.

For a slowly reacting gas such as CO, the lungs are very well designed; D_M is large, so that red cells are exposed almost nakedly to alveolar gas. On the other hand, for oxygen, the haemoglobin molecule is very well designed with a high θ_{O_2} so that, coupled with a large D_M, diffusion disequilibrium for O_2 between alveolar gas and end-capillary blood in normoxia is negligible in normal lungs, except on severe exercise when $\dot{V}_{O_2} > 2.5$ L min^{-1} (Wagner et al., 1986). Current opinion favours $D_{L_{NO}}$ as a surrogate for $D_{M_{CO}}$; in actual fact, it might be a better surrogate for $D_{L_{O_2}}$. There is a case to be made for simultaneous measurements of $D_{L_{CO}}$ and $D_{L_{NO}}$ as described by Tamhane et al. (2001) with the addition of $D_{L_{O_2}}$

measured with ^{18}O (Meyer et al., 1981). If concordance between $D_{L,NO}$ and $D_{L_{O_2}}$ were to be shown in respiratory disease as well as in health, the value of simultaneous measurements of $D_{L_{CO}}$ and $D_{L,NO}$ would be enhanced.

8. Conclusions

The single breath $D_{L_{CO}}$ ($T_{L_{CO}}$) has proved itself an essential part of the routine pulmonary function screen, equal in value to spirometry. In spite of nearly 100 years research, there is still uncertainty over the relative importance of the alveolar-capillary membranes versus the red cells as rate limiting steps in the overall transfer of carbon monoxide from gas to blood. But this is only a quantitative problem. The essential nature of the $D_{L_{CO}}$ has already been elucidated, F.J.W. Roughton and R.E. Forster having played the major roles.

Marie Krogh's original $D_{L_{CO}}$ sb test was 'rediscovered' in the 1950s and, with W.S. Fowler's modification, it became (with later refinements) a robust and reliable measurement. The steady state technique for measuring $D_{L_{O_2}}$ was an important step forward (Lilienthal et al., 1946), and for a time it looked as if steady state $D_{L_{CO}}$ techniques (Filley et al., 1954; Bates et al., 1955) would prevail. In the end, the practical and quicker single breath technique was adopted, and its clinical application is now widespread.

Acknowledgements

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