The Roughton–Forster equation for pulmonary diffusion: how it happened

Mike Hughes

National Heart and Lung Institute, Imperial College School of Medicine, Hammersmith Hospital, London, UK.

Corresponding author: Mike Hughes (mike.hughes@imperial.ac.uk)

Shareable abstract (@ERSpublications)
The Roughton–Forster equation of 1957 describes the transfer of oxygen and carbon monoxide from alveolar gas to pulmonary capillaries, as molecular diffusion across membranes and interstitium coupled to the reaction with haemoglobin in red cells https://bit.ly/380cg0D

Cite this article as: Hughes M. The Roughton–Forster equation for pulmonary diffusion: how it happened. *Eur Respir J* 2022; 60: 2200789 [DOI: 10.1183/13993003.00789-2022].

Received: 14 April 2022
Accepted: 13 May 2022

Introduction

The transfer factor of the lung for carbon monoxide (T_{LCO}) and spirometry are our two most valued tests in functional assessment. The understanding of the physiology underpinning T_{LCO} (or D_{LCO}) owes much to Robert Forster's research, including the formulation of the Roughton–Forster equation [1], the topic of this editorial. His other research involved oxygen and CO_2 exchange, the role of carbonic anhydrase, and gas exchange in general. Robert (Bob) had a great sense of humour, and was delightful company (figure 1). He remained interested and engaged in the T_{LCO} until a few months before his death in September 2021.

The Roughton–Forster equation has been one of the jewels of pulmonary gas exchange for 65 years: is it still relevant?

The Roughton–Forster equation for pulmonary diffusion

The T_{LCO}, measured by the single breath and breath holding method, describes carbon monoxide (CO) transfer from alveolar gas to the haemoglobin (Hb) molecule in the pulmonary capillary red cells (CO being a surrogate for oxygen). T_{LCO} is a conductance ($mL \cdot min^{-1} \cdot mmHg^{-1}$ in traditional units, $mmol \cdot min^{-1} \cdot kPa^{-1}$ in SI units) or "ease of transfer"; its reciprocal, $1/T_{LCO}$, is a *resistance*. In the Roughton–Forster equation [1], the resistance to transfer of gas_x (O_2, CO, *etc*.) from alveolus to capillary Hb ($1/T_{Lx}$) is presented as the sum of two resistances in series:

$$1/T_{Lx} = 1/D_{Mx} + 1/\theta_x V_c$$

where $1/D_{Mx}$ is the *diffusion* resistance of the alveolar–capillary membranes and interstitium and plasma, and $1/\theta_x V_c$ is the *reactive* resistance of gas_x with capillary blood; θ_x equals mL of gas_x taken up per mL blood per unit time and partial pressure (P_x): the units are $mL \cdot min^{-1} \cdot mmHg^{-1} \cdot mL^{-1}$ (or just $min^{-1} \cdot mmHg^{-1}$); V_c is the pulmonary capillary blood volume.

The Roughton–Forster equation was a big step forward in the following ways:

1) Until that time (1957), the only resistance to oxygen and CO transfer from gas to blood was thought to be *diffusive*, meaning that $1/T_{LCO} \sim 1/D_{MCO}$.
2) The Roughton–Forster equation stressed the importance of the reactive resistance in pulmonary capillary red cells: approximately 75% of the total resistance to CO alveolar–capillary gas transfer (calculated from [2]). Thus, the low T_{LCO} in anaemia and in idiopathic pulmonary hypertension could be explained.
3) Once the relationship between θ_{CO} and P_{O_2} was established (but *in vitro*), the Roughton–Forster equation could be solved by measuring T_{LCO} at different values of alveolar P_{O_2}, *i.e.* at different values of θ_{CO}. In a plot of $1/T_{LCO}$ (*y*-axis) *versus* $1/\theta_{CO}$ (*x*-axis), the intercept on the *y*-axis would be $1/D_{MCO}$ and the slope $1/V_c$.

FIGURE 1 Robert E. Forster (1919–2021): Professor and Chairman, Department of Physiology, University of Pennsylvania, Philadelphia, USA; President of the American Physiological Society (1966–1967). "Your teachers are your foundation; you only add a little to what they taught you."

1909–1957. How the Roughton–Forster equation came about

1909–1915. Copenhagen: the birth of D_{LCO}

August and Marie Krogh introduced CO as a marker gas for measuring pulmonary diffusion, first with a steady state (ss) method [3] and, subsequently, in a famous paper [4], with a single breath (sb) technique, not so different from the T_{LCO} measurement used in pulmonary function laboratories today. In using CO as a surrogate for O_2, the Kroghs made two assumptions: first, that CO was an "indifferent" gas, traversing the alveolar membranes by diffusion alone, not transported by a "carrier" molecule. The absence of a "transporter" was confirmed some 80 years later [5]. The second assumption was that in low concentrations, CO taken up by capillary blood would combine instantaneously with Hb, meaning that no significant partial pressure for CO (P_{CO}) would occur in the red cell. This meant that in the calculation of D_{LCO}, only alveolar P_{CO} needed to be measured. This assumption was reasonable at the time, since CO had been chosen for its very high affinity for Hb (215× the affinity of O_2).

1922–1945. Cambridge (UK) and Boston (USA): Roughton and colleagues

F.J.W. Roughton (1899–1972) became, in 1921, an assistant to Hamilton Hartridge, a Senior Demonstrator in the Department of Physiology at Cambridge. Hartridge had a genius for invention, particularly of scientific apparatus: notably, the reversion spectroscope for measuring small quantities of CO combined with Hb (HbCO), and the continuous flow rapid reaction apparatus for monitoring the reactions of Hb with CO or O_2 at the millisecond level (previously, the time resolution was minutes) [6]. Less than a year later, Hartridge and Roughton reported, in a letter to *Nature* [7]: "the combination (Hb with oxygen) was a very rapid one, being complete in one hundredth part of a second". This was, for the time, an astonishing result. Roughton wanted to go on and measure the rate of uptake of CO with Hb. His mentor's put-down was "it would be a waste of time: CO is so tightly bound that the reaction would be too fast for us to measure it". Cheekily, Roughton waited until Hartridge went away (to a meeting?), did the experiment, and found that the CO velocity (its association constant) was slower, not faster than oxygen! The reason for the high affinity of CO for Hb is that its *dissociation* constant is 1000 times slower than its *association* constant.

Roughton spent the rest of his life using the continuous flow rapid reaction apparatus to measure O_2 and CO reaction times in dilute solutions of Hb and in intact red cells under hypoxic, normoxic and hyperoxic conditions. The velocity constants were fastest for O_2 combining with deoxygenated Hb in solution and slowest for CO combining with oxyhaemoglobin in red cell suspensions (see table 1 in [6]); they convinced Roughton that the uptake of CO into blood was limited, particularly at high P_{O_2}, by diffusion plus reaction, and not by either process alone.

In World War II, Roughton worked in Boston at the Harvard Fatigue Laboratory. The practical problem was the build-up of CO to toxic levels in the confined spaces of tanks, cockpits and submarines. Roughton and his colleagues measured the rate of accumulation of HbCO in themselves at rest and on exercise, using a "bubble" apparatus designed by P.F. Scholander [8]. Under hyperoxic conditions, the rate of rise of HbCO was slowed by 25% [9]; qualitatively, the same was true for red cells *in vitro* [10]. In the last of

three papers describing their research, Roughton made a most ingenious analysis of HbCO formation during steady state CO breathing (see HUGHES and BATES [6], pp. 122–123), in which he calculated pulmonary capillary blood volume at rest (60 mL) and on exercise (95 mL) [11], values remarkably similar to the estimates when the Roughton–Forster equation came on stream 12 years later.

Roughton's work was of the greatest importance in our understanding pulmonary CO transfer and the D_{LCO}, but he was actually more famous for his exposition of the reactions of CO_2 and bicarbonate in lung and tissue gas exchange, and for his discovery (with Meldrum) of the vital enzyme, carbonic anhydrase [12].

1951–1954. Philadelphia (USA): Forster and colleagues; Copenhagen: Kruhoffer

When Robert Forster (1919–2021) started his research in the Department of Physiology and Pharmacology under Julius Comroe at the University of Pennsylvania, he was assigned a small laboratory in a basement with a new mass spectrometer and CO analyser. Comroe suggested he repeat the D_{LCO} experiments which Marie Krogh had done 37 years earlier, but which had been largely ignored. With help from a more experienced colleague (Ward Fowler), Forster improved the Krogh D_{LCO} (sb), making it practical for everyday use in pulmonary function laboratories [13]. Knowing of Roughton's work on CO in hyperoxia [10, 11], Forster and colleagues showed that D_{LCO} (sb) was reduced as inspired oxygen (F_{IO_2}) was increased [14]; the more definitive paper was published in 1957 [15]. Forster realised that increasing F_{IO_2} must have reduced the access of inspired CO to the O_2–free (unliganded) Hb molecules, and that this quantity must be related to the instantaneous pulmonary capillary blood volume (V_c) [16]; in addition, the rate of reaction must have slowed, as Roughton had showed [10]. Forster and co-workers introduced a correction factor (C) to account for a significant red cell P_{CO}, with the term θV_c (the term θ appears only in a footnote!) where θ was, in essence, the diffusing capacity of 1 mL of blood, and V_c was the blood content of pulmonary capillaries accessible to the inhaled CO [16]. "C" included the ratio $D/\theta V_c$, where D was D_M, since Forster reasoned (as had Roughton) that red cell P_{CO} (considered insignificant previously) would become important 1) if HbCO was high due to previous CO exposure (*e.g.* smoking), or 2) if membrane diffusion (D_M) greatly exceeded the rate at which CO combined with the available Hb ($\sim \theta V_c$). This rise of P_{CO} would be "instantaneous" (*i.e.* confined to the pulmonary capillary transit) since the reaction Hb+CO would continue in arterial blood with a fall in P_{CO}. These concepts were the essence of the Roughton–Forster equation.

To complicate matters, Kruhoffer (from the same department the Kroghs had worked in) in the same year published measurements of D_{LCO} at low and high alveolar P_{O_2} using the radioisotope ^{14}C [17]. Although Kruhoffer's equation for D_{MCO} and V_c was almost the same as Roughton and Forster's, he had no reliable values for $1/\theta_{CO}$ as a function of P_{O_2} and was unable to calculate D_{MCO} or V_c.

1954–1957. Forster contacts Roughton: the synthesis

Realising the importance of the $D_{MCO}/\theta V_c$ ratio and knowing he needed information on the $1/\theta$–P_{O_2} relationship in human blood, Forster wrote to Roughton in Cambridge. Roughton replied that his own measurements were not good enough, and could he come to Philadelphia with his own continuous flow rapid reaction apparatus and make the measurements there? It was arranged! Two years later, four papers appeared consecutively in the *Journal of Applied Physiology*, the first two documenting the kinetics of O_2 and CO combination with Hb solutions and with intact red cells [18, 19], the third [15] investigating more rigorously the relationship between D_{LCO} (sb) or D_{LCO} (ss) and alveolar P_{O_2}, and the fourth [1] giving the formal proof of the Roughton–Forster equation and calculating D_{MCO} and V_c from measurements of D_{LCO} (sb) at different levels of alveolar P_{O_2} and $1/\theta$ (calculated from the *in vitro* data).

1957–2022. Clinical applications of the D_{MCO} and V_c calculations

The physiological insight from the D_{MCO} and V_c analysis has been considerable, but the diagnostic gain in various respiratory conditions has, on the whole, been disappointing. This is mainly because there is interdependence between D_M and V_c, between the diffusion pathway and the distribution and shape of red cells in the capillary bed [20, 21]. Clearly, D_{MCO} can only be recorded if Hb is in the vicinity to combine with the incoming CO; D_M must be matched to V_c for CO exchange to take place. The only mismatch is in mild to moderate congestive heart failure in which a selective loss of D_{MCO} occurs [22, 23].

The introduction of the diffusing capacity for nitric oxide (D_{LNO}) in 1987–1989 [24, 25] has changed things [26, 27]. The ratio D_{LNO}/T_{LCO} is about 5.0. This is because 1) NO is twice as diffusible as CO (D_{MNO}/D_{MCO} is ~2.0) and 2) the reactive resistance of NO is lower ($1/\theta_{NO} << 1/\theta_{CO}$). The D_{LNO}/T_{LCO} ratio is related to D_{MCO}/V_c [28], and may act as a surrogate for the Roughton–Forster analysis. More measurements of D_{LNO} and T_{LCO} in a clinical context are awaited with interest.

Conclusion

This article is a tribute to the life and work of Robert E. Forster, the distinguished respiratory physiologist, who died in September 2021 at the age of 101. His work at the University of Pennsylvania led to the introduction in 1957 [13] of the single breath carbon monoxide diffusing capacity (D_{LCO} (sb); now called the CO transfer factor, T_{LCO}), which is used in pulmonary function laboratories throughout the world. The 1957 Roughton–Forster equation focused attention on the role of the pulmonary microcirculation in blood–gas transfer. The recent addition of the D_{LNO} to the well-established T_{LCO} has revived interest in the clinical application of Roughton and Forster's membrane diffusing capacity (D_M) and capillary volume (V_c) analysis.

Conflict of interest: None declared.

References

1 Roughton FJW, Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J Appl Physiol* 1957; 11: 290–302.
2 Hsia CCW, McBrayer DG, Ramanathan M. Reference values of pulmonary diffusing capacity during exercise by a rebreathing technique. *Am J Respir Crit Care Med* 1995; 152: 658–665.
3 Krogh A, Krogh M. On the rate of diffusion of carbonic oxide into the lungs of man. *Scand Arch Physiol* 1909; 23: 236–247.
4 Krogh M. The diffusion of gases through the lungs of man. *J Physiol* 1915; 49: 271–300.
5 Jones HA, Clark JC, Davies EE, *et al.* Rate of uptake of carbon monoxide at different inspired concentrations in humans. *J Appl Physiol Respir Environ Exerc Physiol* 1982; 52: 109–113.
6 Hughes JMB, Bates DV. Historical review: the carbon monoxide diffusing capacity (D_{LCO}) and its membrane (D_m) and red cell ($\theta.V_c$) components. *Respir Physiol Neurobiol* 2003; 138: 115–142.
7 Hartridge H, Roughton FJW. Measurement of the rates of oxidation and reduction of haemoglobin. *Nature* 1923; 111: 325–326.
8 Roughton FJW, Scholander PF. Micro gasometric estimation of the blood gases. I Oxygen. II Carbon monoxide. *J Biol Chem* 1943; 148: 541–563.
9 Forbes WH, Sargent F, Roughton FJW. Rate of CO uptake by normal men. *Am J Physiol* 1945; 143: 594–608.
10 Roughton FJW. The kinetics of the reaction $CO+O_2Hb \rightarrow O_2+COHb$ in human blood at body temperature. *Am J Physiol* 1945; 143: 609–620.
11 Roughton FJW. The average time spent by the blood in the human lung capillary and its relation to the rates of CO uptake and elimination in man. *Am J Physiol* 1945; 143: 621–633.
12 Meldrum NU, Roughton FJW. Carbonic anhydrase. Its preparation and properties. *J Physiol (Lond)* 1933; 80: 113–142.
13 Ogilvie CM, Forster RE, Blakemore WS, *et al.* A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J Clin Invest* 1957; 36: 1–17.
14 Forster RE, Fowler WS, Bates DV. The absorption of carbon monoxide by the lungs during breathholding. *J Clin Invest* 1954; 33: 1135–1145.
15 Forster RE, Roughton FJW, Cander L, *et al.* Apparent pulmonary diffusing capacity for CO at varying alveolar O_2 tensions. *J Appl Physiol* 1957; 11: 277–289.
16 Forster RE, Fowler WS, Bates DV. Considerations on the uptake of carbon monoxide by the lungs. *J Clin Invest* 1954; 33: 1128–1134.
17 Kruhoffer P. Lung diffusion coefficient for CO in normal human subjects by means of $C^{14}O$. *Acta Physiol Scand* 1954; 32: 106–123.
18 Forster RE, Roughton FJW, Kreuzer F, *et al.* Photocalorimetric determination of the rate of uptake of CO and O_2 by reduced human red cell suspensions at 37°C. *J Appl Physiol* 1957; 11: 260–268.
19 Roughton FJW, Forster RE, Cander L. Rate at which carbon monoxide replaces oxygen from combination with human hemoglobin in solution and in the red cell. *J Appl Physiol* 1957; 11: 269–276.
20 Frank AO, Chuong CJ, Johnson RL. A finite-element model of oxygen diffusion in the pulmonary capillaries. *J Appl Physiol (1985)* 1997; 82: 2036–2044.
21 Hsia CCW, Chuong CJC, Johnson RL. Red cell distortion and conceptual basis of diffusing capacity estimates: finite element analysis. *J Appl Physiol (1985)* 1997; 83: 1397–1404.
22 Puri S, Baker LB, Dutka DP, *et al.* Reduced alveolar-capillary membrane diffusing capacity in chronic heart failure. Its pathophysiological relevance and relationship to exercise performance. *Circulation* 1995; 91: 2769–2774.
23 Guazzi M, Pontone G, Brambilla R, *et al.* Alveolar-capillary membrane conductance. A novel prognostic indicator in chronic heart failure. *Eur Heart J* 2002; 23: 467–476.

24 Guènard H, Varene N, Vaida P. Determination of lung capillary blood volume and membrane diffusing capacity in man by the measurements of NO and CO transfer. *Respir Physiol* 1987; 70: 113–120.

25 Borland CD, Higenbottam TW. A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. *Eur Respir J* 1989; 2: 56–63.

26 Borland C, Guènard H. The history of the pulmonary diffusing capacity for nitric oxide DL,NO. *Respir Physiol Neurobiol* 2017; 241: 3–6.

27 Zavorsky GS, Hsia CCW, Hughes JMB, *et al.* Standardisation and application of the single-breath determination of nitric oxide uptake in the lung. *Eur Respir J* 2017; 49: 1600962.

28 Hughes JMB, Dinh-Xuan AT. The DLNO/DLCO ratio: physiological significance and clinical implications. *Respir Physiol Neurobiol* 2017; 241: 17–22.