OPINION
The centenary (2015) of the transfer factor for carbon monoxide (T_{LCO}): Marie Krogh’s legacy

J Michael B Hughes, Colin D R Borland

THE BIRTH OF THE SINGLE BREATH (SB) T_{LCO}: THE OXYGEN SECRETION CONTROVERSY
Whether or not the lungs actively secreted oxygen, particularly under stressful conditions (severe exertion, alveolar hypoxia), was an argument which continued for more than 50 years (1870–1923), and involved some of the most distinguished respiratory physiologists of that era, such as J S Haldane, Christian Bohr and August Krogh. The denouement, as told by Krogh’s daughter, Bodil Schmidt-Neilsen, began 11 years earlier in 1904 when a Danish medical student, Marie Jørgensen, attended a class taught by August Krogh, an instructor in physiology in Christian Bohr’s department. They were attracted to each other and married in 1905, and Marie (figure 1) joined August (a Nobel Prize winner in physiology and medicine in 1920) in some aspects of his research. The subsequent publication of a paper in 1915, 100 years ago this year, by Marie Krogh in the *Journal of Physiology* was the pivotal moment in the story. The influence of this paper “The diffusion of gases through the lungs of man” continues to this day, long after the oxygen secretion question was settled (in Marie Krogh’s favour). Nowadays, pulmonary function laboratories throughout the world use a modification of her single breath transfer factor for carbon monoxide (T_{LCO, sb}) test, known in North America as the D_{LCO, sb} (carbon monoxide diffusing capacity); it is an essential part of routine lung function screening, and the only non-invasive test (apart from pulse oximetry) of the gas exchanging efficiency of the lung.

The oxygen secretion story has been told many times. A century ago, the idea that Claude Bernard’s *milieu intérieur* might be stabilised by alveolar cells secreting oxygen at times of great demand or shortage would have seemed not unreasonable. There was, after all, the example in nature of O_2 partial pressures (P_{O_2}) in the swim bladders of fish many times higher than the environmental oxygen. Recently, however, Scheid et al have shown that this can occur without active secretion, due to a combination of mechanisms, (A) lactic acid production, (B) reduced haemoglobin affinity and capacity for O_2, (C) a countercurrent *rete mirabile*, and (D) a swim bladder wall made impermeable by guanine crystals. In fact, the only evidence ever produced in favour of alveolar oxygen secretion was the finding that arterial P_{O_2} was greater than alveolar P_{O_2}. This occurred in the years 1890–1912 when methods of measuring P_{O_2} in blood were relatively crude and inaccurate. August Krogh first solved the technical problem of measuring arterial P_{O_2} accurately; the Kroghs found alveolar P_{O_2} was always greater than arterial P_{O_2}. But, the proponents of the secretion theory could always argue that end-capillary P_{O_2} (after subtraction of the contributions from hypoxaemic blood from intrapulmonary and extrapulmonary shunts) might, on severe exercise, have been up to 1 kPa (1–7 mm Hg) above mean alveolar P_{O_2} (which might have been elevated by contamination with dead space P_{O_2}). Thus, an alternative approach was needed.

When CO is inhaled, its great affinity for haemoglobin (Hb) in blood (×230 vs O_2) means that its partial pressure in blood (P_{CO}) stays negligible. The transfer factor for CO (quantity taken up per unit time, per unit P_{CO}), or T_{LCO}, equals the rate of uptake (ΔCO/Δt) from alveolar gas (which is easily measured) times the alveolar volume (VA) at which the measurement is made, divided by the total dry gas pressure (barometric minus water vapour pressure at 37°). The Kroghs measured the T_{LCO} in normal subjects at rest and on exercise; finally, Marie Krogh repeated the measurements with a much improved method. Using an O_2/CO diffusivity ratio of 1.23 (based on their physical properties), she calculated T_{LCO} from the measured T_{LCO} and showed that, with reasonable values for the alveolar-mean capillary P_{O_2} gradient multiplied by the exercise T_{LCO} (= V_{O_2}), the measured oxygen

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consumption (VO₂) of 2.6 L min⁻¹ at the exercise levels studied, could be accounted for solely by passive diffusion. Using the principle of Occam’s razor, there was no need to invoke an additional mechanism.

THE 1915 SINGLE BREATH DₗCO (TₗCO)  
To measure the diffusing capacity (transfer factor) for CO, only alveolar PₐCO and CO uptake (VₗCO) had to be measured. How best to do it? The Kroghs tried first a steady state technique (like the measurement of VO₂) but abandoned it because PₐCO measurements were not reproducible during tidal breathing; a breath hold technique proved more satisfactory. For the same reasons, when the DₗCO was ‘rediscovered’ in the 1950s, the single breath technique became the preferred choice over the steady state. The 1915 DₛₗCO differs from the present day TₛₗCO in some technical details (see Table 1), but, quantitatively, Marie Krogh’s measurements have been confirmed by later investigators (Table 1, note ¶).

NOMENCLATURE AND CALCULATIONS IN 1915  
DₗCO (the term ‘transfer factor’, TₗCO ‹, did not come in until 1963) was diffusion constant, and k, the rate of CO uptake (rate constant per unit PₐCO) now referred to as kₗCO/(Pₐ – PₐH,O) or KₗCO was permeability. Marie Krogh states “The diffusion is determined by two factors, namely the permeability (k) and the mean capacity (∼VA); the relationship TₗCO=KₗCO×VA is what we teach today.” She also says (p. 288) “Two persons may have about the same diffusion constant, … though both mean capacity and the permeability are very different”. See table 3 in a recent review.

1923–1957: UNDERSTANDING THE PHYSIOLOGY OF CO UPTAKE  
In 1915, Marie Krogh said “…… an essentially indifferent gas, like carbon monoxide, must pass through the alveolar epithelium by diffusion alone ….”. This was, at the time, a generally held assumption. The rate of combination of the Hb in the red cell with oxygen, and especially CO, was thought to be instantaneous. In the 1920s, with improved analytical techniques, Hartridge and Roughton in Cambridge (UK) were able to measure the rate of association of CO with solutions of Hb. They showed that the reaction velocity of CO was not instantaneous, but measurable, and that its rate of combination with Hb packed inside red cells was significantly slower than in Hb solutions, implying diffusion as well as reaction resistance to CO uptake within pulmonary capillary blood. Furthermore, the reaction resistance was proportional to red cell PₐCO. Finally, the TₗCO-sb was found to be directly related to alveolar PₐO₂ (note 1/TₗCO is a resistance, TₗCO is a conductance). This era of physiological discovery culminated in the formulation of the famous Roughton–Forster equation, which partitioned the alveolar uptake of CO into membrane (Dₘ) and red cell (θVc) components:

\[
1/DₗCO = 1/Dₘₐₕ⁺ 1/θVc
\]

where 1/Dₘₐₕ is the diffusion resistance from the epithelial surface to the red cell membrane and 1/θVc is the red cell resistance to CO uptake, where θ is rate of CO uptake per mL blood (inversely proportional to PₐCO) and Vc is the pulmonary capillary volume. Subsequently, physiological studies, involving simultaneous nitric oxide (NO) and CO uptake, have shown that 80% of the resistance to CO transfer from alveolar gas to

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Table 1 Comparison of TₗCO-sb methods since Marie Krogh’s original description; shading indicates no change from the earlier date

<table>
<thead>
<tr>
<th>TₗCO (DₗCO)</th>
<th>Marie Krogh 1915</th>
<th>Ogilvie et al 1957</th>
<th>ATS/ERS statement 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume history</td>
<td>1. IVC from RV</td>
<td>1. Rapid IVC from RV</td>
<td>1. Rapid IVC from RV</td>
</tr>
<tr>
<td></td>
<td>2. Expire 0.5 VC (A)</td>
<td>2. Breath hold</td>
<td>2. Breath hold</td>
</tr>
<tr>
<td></td>
<td>4. Expire 0.5 VC (B)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inspired gas composition</td>
<td>0.5–1% CO in air</td>
<td>0.3% CO in air</td>
<td>0.3% CO, 0.01% CH₄ in air or 0.3% CO, 10% He, 18–19% O₂</td>
</tr>
<tr>
<td>Vₐ during the breath hold</td>
<td>c. 60% TLC</td>
<td>TₐC</td>
<td>TLC</td>
</tr>
<tr>
<td>Vₐ calculation for the TₗCO measurement</td>
<td>(IVC–0.5VC)+RV with separate estimate of RV (multibreath H₂)</td>
<td>(IVC+RV) with separate estimate of RV (multibreath He)</td>
<td></td>
</tr>
<tr>
<td>Alveolar CO at t=0</td>
<td>direct sampling from expire (A)</td>
<td>Estimated from FₐCO × Heₐ/Heₑ*</td>
<td>Estimated from FₐCO × Heₐ/Heₑ*</td>
</tr>
<tr>
<td>Breath hold time measurement</td>
<td>(A)–(B) where (A) and (B) are first and second expirations</td>
<td>from start IVC to start alveolar sample collection</td>
<td>from 0.3 duration of IVC to mid-time of alv. collection: Jones and Meade²</td>
</tr>
<tr>
<td>Breath hold time (s)</td>
<td>6.5–7.5 s</td>
<td>10 s</td>
<td>10 s</td>
</tr>
<tr>
<td>Dead space washout (mL)</td>
<td>“Alveolar” sample taken from expirates (A) and (B)</td>
<td>750 mL</td>
<td>750 mL</td>
</tr>
<tr>
<td>Hb correction</td>
<td>None</td>
<td>None</td>
<td>From standard reference equations, for example, Cotes³</td>
</tr>
<tr>
<td>TₗCO at rest: men</td>
<td>10.05 (n=12)</td>
<td>10.6 (n=8)</td>
<td>13.8–9.1</td>
</tr>
<tr>
<td></td>
<td>7.3 (n=3)</td>
<td>8.0 (n=9)</td>
<td>11.2–7.4</td>
</tr>
<tr>
<td>Reference values</td>
<td>None but correlation noted with BSA</td>
<td>Referenced to BSA</td>
<td>Referenced to age, sex and height</td>
</tr>
</tbody>
</table>

*Heₑ and Heₐ: alveolar sample of expired and inspired helium concentrations, respectively.  
†Vₑₚₐₕ: anatomical dead space (usually estimated from body weight, but see ref. 10, p.277).  
‡See reference 11.  
§See reference 12.  
¶SI units: mmol min⁻¹ kPa⁻¹ (×3 for traditional units). For Krogh and Ogilvie et al: age: 18–42 years. ATS/ERS from reference equations (ref 13) for age 30 years, h 1.75 m (men), 1.65 m (women), 90% confidence limits.  
ATS: American Thoracic Society; BSA, body surface area; ERS, European Respiratory Society; FICO, inspired carbon monoxide fraction; Hb, haemoglobin; IVC, inspiratory vital capacity; RV, residual volume; sb, single breath; TLC, total lung capacity; TₗCO, transfer factor for carbon monoxide; Vₐ, alveolar volume; Vc, inspiratory vital capacity.
intracapillary Hb (~1/TlCO·SB) lies in the red cell itself. Thus, it is not unreasonable to describe the TlCO as a ‘window on the pulmonary microcirculation’.

TlCO 1945–1957

There was no clinical follow-up after Marie Krogh’s pioneering 1915 publication, until the TlCO (~DlCO) reappeared in 1957, slightly modified, in a paper by Ogilvie et al. from the University of Pennsylvania, USA. Colin Ogilvie was an English chest physician. The invention of the infrared CO meter in Germany in the early 1940s made CO analysis quicker and more practical. In addition, interest in lung diffusion had been revived in 1945 by a challenging paper from Lilienthal et al. in which the oxygen diffusing capacity (DlO2) was measured. Their method was complex and involved breathing hypoxic gas mixtures (13% O2). At around this time (1949–1951), clinicians had seen patients with lung fibrosis and small lungs with a decrease in arterial O2 saturation (SaO2) >10% on exercise, and pulmonary function laboratories worldwide, playing a key role in this process. However, interest did not return until the TLCO calculated from it are weighted towards the better ventilated regions of the lung.

Slightly modified, this ‘alveolar-capillary block’; we now call it ‘diffusion limitation’.

When resting DlCO is <60% predicted in patients with interstitial lung fibrosis, worsening of arterial hypoxaemia on exercise is extremely common.

Sever Kety, a circulating physiologist, in a review of methods for measuring pulmonary diffusion, realised that Marie Krogh’s use of CO ‘brilliantly sidestepped’ the difficulties involved in measuring DlO2. Julius Comroe in Philadelphia assembled a team, led by Robert Forster, to repeat Krogh’s work with clinical application in mind. Ward Fowler, working in Comroe’s department on alveolar gas distribution, made the novel suggestion of adding an insoluble ‘volume marker’ gas (helium) to the inspired mixture. This, cleverly, eliminated the first expiration (A) because the initial alveolar CO concentration (at t=0) could be calculated from the helium expired at the end of the breath hold as FICO × He2/He1 where e and i or I refer to expired and inspired, respectively (see table 1). Thus, Krogh’s first expiration was avoided, the breath hold was maintained at a reproducible level (~TLC) and only two samples (from the inspired bag and the expired sample) had to be analysed. There had to be an assumption that the inspired CO and helium mixed instantaneously with alveolar gas; while this was unlikely to happen, the preceding fast inspired vital capacity would minimise mixing delays. This was the only modification of substance made to Krogh’s method. Other modifications to Krogh 1915, made in 1957 and subsequently, are listed in table 1. The two most important are (A) standardisation of the calculation of breath hold time, and (B) the substitution of a helium dilution VHe measured during the single breath manoeuvre, for a separately measured residual volume to which the inspiratory vital capacity was added. This saved Pulmonary Function Laboratories from making two separate measurements. But, in the presence of airflow obstruction, it means that the V′He and the TlCO calculated from it are weighted towards the better ventilated lung regions.

TlCO·SB IN 2015

Pulmonary function testing did not have a role in medicine in 1915, and its profile did not rise until the late 1940s and 1950s. Marie Krogh can hardly have foreseen that her single breath method, using CO, designed to answer a specific physiological question, would become, 50 years later, a day in, day out test in Pulmonary Function Laboratories worldwide, playing a key role in functional assessment in respiratory and systemic disease. Clinically, the TlCO·SB (and KCO) are normal in uncomplicated asthma, and abnormal in emphysema, where there is a good correlation with CT scanning indices of lung destruction. In restrictive lung disease, ‘small lungs’, KCO is considerably greater than predicted normal (>120%) in extrapulmonary causes, but <100% predicted with intrapulmonary pathology. Interpretation of a reduced TlCO·SB in disease is helped by consideration of its two separate components (KCO and VHe), to which Marie Krogh first drew attention.

TlNO·SB THE FUTURE

In 1987–1989, two papers were published (although the earlier paper was preceded by the work described in the later publication) in which gaseous NO replaced CO, and the single breath transfer factor for nitric oxide (TlNO) emerged; the theory and methodology were similar to the TlCO. These two groups, from Cambridge, UK, and Bordeaux, France, have since shown that, for NO, 35–40% only of the transfer resistance lies in the red cell (80% for CO). Thus, TlNO·SB is more weighted to the alveolar and capillary surface area, that is, true diffusion, than to the red cell. So, TlNO differs from TlCO·SB, but complements it. Furthermore, by using laboratory derived values for θCO and θNO, DlCO and Vc can be calculated from one single breath manoeuvre. The future may tell us whether the combination of TlNO and TlCO will prove more useful clinically than TlCO·SB alone.

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