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OPINION

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

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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 JS Haldane, Christian Bohr and August Krogh. The denouement, as told by Krogh's daughter, Bodil Schmidt-Neilsen,[1] 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*[2] was the pivotal moment in the story. The influence of this paper "The diffusion of gases through the lungs of man"[2] 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.

Figure 1 Marie Krogh (née Jørgenson), born Christmas day 1874 on the island of Fyn, Denmark, married August Krogh in Copenhagen, March 1905, died 1943 in Copenhagen, aged 69 years.

The oxygen secretion story has been told many times.[1 3 4] 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*[5] 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;[6] the Kroghs found alveolar P_{O_2} was always *greater* than arterial P_{O_2}.[7] 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 ($\Delta CO/\Delta t$) from alveolar gas (which is easily measured) times the alveolar volume (V_A) 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;[8] finally, Marie Krogh repeated the measurements with a much improved method.[2] Using an O_2/CO diffusivity ratio of 1.23 (based on their physical properties), she calculated T_{LO_2} 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_{LO_2} ($= \dot{V}O_2$), the measured oxygen

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consumption ($\dot{V}O_2$) 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 D_{LCO} (T_{LCO})

To measure the diffusing capacity (transfer factor) for CO, only alveolar P_{CO} and CO uptake ($\dot{V}_{CO}$) had to be measured. How best to do it? The Kroghs tried first a steady state technique[8] (like the measurement of $\dot{V}O_2$) but abandoned it because $P_{A_{CO}}$ measurements were not reproducible during tidal breathing; a breath hold technique proved more satisfactory.[2] For the same reasons, when the $D_{L_{CO}}$ was 'rediscovered' in the 1950s, the single breath technique became the preferred choice over the steady state.[9] The 1915 $D_{L_{CO}}$ differs from the present day $T_{L_{CO}}$ 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

D_{LCO} (the term "transfer factor, T_{LCO}", did not come in until 1963[14]) was *diffusion constant*, and *k*, the rate of CO uptake (rate constant) per unit P_{CO} (now referred to as $k_{CO}/(Pb - P_{H_2O})$ or K_{CO}) was *permeability*. Marie Krogh[2] states "The diffusion is determined by two factors, namely the permeability (k) and the mean capacity (~V_A)"; the relationship $T_{LCO}=K_{CO}\times V_A$ is what we teach today.[15] She also says (p. 288)[2] "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.[15]

1923–1957: UNDERSTANDING THE PHYSIOLOGY OF CO UPTAKE

In 1915, Marie Krogh[2] 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.[4] 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 measureable, 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 P_{O_2}. Finally, the T_{LCO}.sb was found to be *directly* related to alveolar P_{O_2} (note $1/T_{LCO}$ is a resistance, T_{LCO} is a conductance).[4]

This era of physiological discovery culminated in the formulation of the famous Roughton–Forster equation,[16] which partitioned the alveolar uptake of CO into membrane (D_M) and red cell (θVc) components:

$$1/D_{LCO} = 1/D_{MCO} + 1/\theta_{CO}Vc \quad (1)$$

where $1/D_{MCO}$ is the diffusion resistance from the epithelial surface to the red cell membrane and $1/\theta Vc$ is the red cell resistance to CO uptake, where θ is rate of CO uptake per mL blood (inversely proportional to P_{O_2}) 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

Table 1 Comparison of T_{LCO}.sb methods since Marie Krogh's original description; shading indicates no change from the earlier date

T_{LCO} (D_{LCO})	Marie Krogh[2] 1915	Ogilvie *et al*[9] 1957	ATS/ERS statement[10] 2005
Volume history	1. IVC from RV 2. Expire 0.5 VC (A) 3. Breath hold 4. Expire 0.5 VC (B)	1. Rapid IVC from RV 2. Breath hold 3. Expire VC	1. Rapid IVC from RV 2. Breath hold 3. Expire VC
Inspired gas composition	0.5–1% CO in air	0.3% CO in air 10% helium	0.3% CO, 0.01% CH_4 in air or 0.3% CO, 10% He, 18–19% O_2
V_A during the breath hold	c. 60% TLC	TLC	TLC
V_A calculation for the T_{LCO} measurement	((IVC– 0.5VC)+RV) with separate estimate of RV (multibreath H_2)	(IVC+RV) with separate estimate of RV (multibreath He)	(IVC×He_i/He_e*)—V_{Danat}† from He dilution during T_{LCO}.sb manoeuvre
Alveolar CO at t=0	direct sampling from expire (A)	Estimated from $F_{ICO}\times He_e/He_i$*	Estimated from $F_{ICO}\times He_e/He_i$*
Breath hold time measurement	(A)–(B) where (A) and (B) are first and second expirations	from start IVC to start alveolar sample collection	from 0.3 duration of IVC to mid-time of alv. collection: Jones and Meade‡
Breath hold time (s)	6.5–7.5 s	10 s	10 s
Dead space washout (mL)	"Alveolar" sample taken from expirates (A) and (B)	750 mL	750 mL
Hb correction	None	None	From standard reference equations, for example, Cotes§
T_{LCO} at rest¶: men women	10.05 (n=12) 7.9 (n=3)	10.6 (n=8) 8.0 (n=9)	13.8–9.1 11.2–7.4
Reference values	None but correlation noted with BSA	Referenced to BSA	Referenced to age, sex and height

*He_e and He_i; alveolar sample of expired and inspired helium concentrations, respectively.
†V_{Danat}; anatomical dead space (usually estimated from body weight, but see ref. 10, p.727).
‡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, ht 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; T_{LCO}, transfer factor for carbon monoxide; V_A, alveolar volume; VC; expiratory vital capacity.

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intracapillary Hb (~1/T_{LCO}.sb) lies in the red cell itself.[17 18] Thus, it is not unreasonable to describe the T_{LCO} as a 'window on the pulmonary microcirculation'.[19]

T_{LCO} 1945–1957

There was no clinical follow-up after Marie Krogh's pioneering 1915 publication, until the T_{LCO} (~D_{LCO}) reappeared in 1957, slightly modified, in a paper by Ogilvie *et al*[9] 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*[20] in which the oxygen diffusing capacity (D_{LO_2}) was measured. Their method was complex and involved breathing hypoxic gas mixtures (13% O_2). At around this time (1949–1951), clinicians had seen patients with lung fibrosis and small lungs with a decrease in arterial O_2 saturation (SaO_2)>10% on exercise, from almost normal values at rest.[21] Austrian *et al*[22] measured a low D_{LO_2} (at rest) in similar patients. They described this as 'alveolar–capillary block'; we now call it 'diffusion limitation'. When resting D_{LCO} is <60% predicted in patients with interstitial lung fibrosis, worsening of arterial hypoxaemia on exercise is extremely common.

Seymour Kety,[23] a circulatory 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 D_{LO_2}. 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 $F_{I_{CO}} \times He_e/He_i$ 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,[11] and (B) the substitution of a helium dilution V_A, 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_A' and the T_{LCO} calculated from it are weighted towards the better ventilated lung regions.

$T_{LCO\cdot SB}$ 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 T_{LCO}.sb (and K_{CO}) are normal in uncomplicated asthma, and abnormal in emphysema, where there is a good correlation with CT scanning indices of lung destruction.[24] In restrictive lung disease, 'small lungs', K_{CO} is considerably greater than predicted normal (>120%) in extrapulmonary causes, but <100% predicted with intrapulmonary pathology.[15] Interpretation of a reduced T_{LCO}.sb in disease is helped by consideration of its two separate components (K_{CO} and V_A),[15] to which Marie Krogh first drew attention.[2]

$T_{LNO\cdot SB}$ THE FUTURE

In 1987–1989, two papers were published[25 26] (although the earlier paper[26] was preceded by the work[27] described in the later publication[25]) in which gaseous NO replaced CO, and the single breath transfer factor for nitric oxide (T_{LNO}) emerged; the theory and methodology were similar to the T_{LCO}. These two groups, from Cambridge, UK[17] and Bordeaux, France,[18] have since shown that, for NO, 35–40% only of the transfer resistance lies in the red cell (80% for CO). Thus, T_{LNO}.sb is more weighted to the alveolar and capillary surface area, that is, true diffusion, than to the red cell. So, T_{LNO} differs from T_{LCO}, but complements it. Furthermore, by using laboratory derived values for θ_{CO} and θ_{NO}, D_{MCO} and Vc can be calculated from one single breath manoeuvre. The future may tell us whether the combination of T_{LNO} and T_{LCO} will prove more useful clinically than T_{LCO} alone.

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