J Appl Physiol 115: 153–154, 2013;
doi:10.1152/japplphysiol.00581.2013.

Invited editorial on "Lung membrane conductance and capillary volume derived from the NO and CO transfer in high altitude newcomers"

J. Michael B. Hughes

National Heart and Lung Institute, Imperial College, London, United Kingdom

Submitted 15 May 2013; accepted in final form 15 May 2013

IN ABSTRACTS PUBLISHED IN 1983 (2) and 1984 (3), Borland and Higenbottam from Cambridge, UK, showed that alveolar uptake of inhaled nitric oxide (NO) was, on average, 4.3 times faster than that of simultaneously inhaled carbon monoxide (CO), using the standard single-breath test. This observation, made by chance (1), has been a great stimulus to research in the field of alveolar-capillary gas diffusion (or transfer). A paper (7) that explored further the physiology behind this differential uptake followed in 1989 (see Fig. 1).

Analysis of Diffusing Capacity Measurements

The Roughton-Forster equation [from their classic 1957 paper (16)] is still the model for analysis:

$$1/D_L = 1/Dm + 1/\theta Vc \qquad (1)$$

where $1/D_L$ is the total resistance to gas transfer, 1/Dm is the resistance to passive diffusion from alveolar gas to the red cell membrane, $1/\theta Vc$ is the transfer resistance of red cells, θ is the reaction rate with blood, adjusted to a standard hemoglobin (Hb) concentration, and Vc is the pulmonary capillary volume. Thus Dm and θVc are the membrane and blood conductances for a particular gas [NO (Dm_{NO} and θ_{NO}) or CO (Dm_{CO} and θ_{CO})]. For CO, $1/D_{LCO}$ (D_{LCO} = lung diffusing capacity for CO) can be partitioned into its components by repeating the measurement at a higher alveolar oxygen tension (P_{AO_2}) because there is a linear relationship in vitro between $1/\theta_{CO}$ and P_{AO_2} (16). Thus, from *Eq. 1*, $1/D_{LCO}$ can be plotted against P_{AO_2}, with the *y*-axis intercept representing $1/Dm_{CO}$, and the slope 1/Vc. The strict definition of Dm_{CO} is what remains of $1/D_{LCO}$ at zero P_{AO_2}. Thus plasma is part of Dm_{CO}, as it is non-O_2 combining, like the membranes and interstitium.

The Hypothesis That θ for NO Is Infinite

In the 1970s, the late D. Bargeton and H. Guenard speculated that the Roughton-Forster equation could be solved, with a single maneuver, if a second gas, which reacted with Hb, were added (1); this would counter objections that the measurement in hyperoxia might alter pulmonary vascular pressures and cardiac output and thus Vc itself. In 1987, the "Bordeaux group" [H. Guenard and colleagues (11)] used the "Cambridge" NO-CO single-breath method to measure D_{LNO} (lung diffusing capacity for NO) and D_{LCO} and showed that the Roughton-Forster equation could be rearranged for the two gases:

$$Vc\ (\text{infinite } \theta_{NO}) = (1/\theta_{CO})/(1/D_{LCO} - \alpha/D_{LNO}) \qquad (2)$$

where α is the ratio of physical diffusivities of NO and CO (1.97); at infinite θ_{NO}, D_{LNO} is equivalent to Dm_{NO}, which equals $Dm_{CO} \times 1.97$. θ_{NO} was eliminated on the grounds that the rate of reaction of NO with free Hb was much faster (approximately $\times 250$) than with CO. The objection to this notion is that θ_{NO} relates to whole blood, and not to Hb solutions. The reason for a difference between the reaction kinetics of blood vs. "infinitely thin" solutions of Hb, expressed intuitively, is the "advancing front phenomenon", whereby gas diffusing into a red cell (or Hb solution of finite thickness) reacts "as if" instantaneously with the first Hb molecule it meets, which reduces the local diffusion gradient to zero and slows the uptake process (14). If the reaction of NO with oxyhemoglobin were infinite, θ_{NO} would be entirely diffusion limited, but in fact both θ_{NO} and θ_{CO} are reaction and diffusion limited, with CO weighted by reaction limitation and NO dominated by diffusion limitation. Clearly, θ_{NO} must have a finite value.

In Favor of θ_{NO} Being "Operationally" Infinite

It is only possible to measure θ_{NO} and θ_{NO} in vitro. At P_{AO_2} of 100 Torr, θ_{CO} is 0.58 (10); the ratio θ_{NO}/θ_{CO} varies from 7.7 (9) to 4.8 (8). If diffusion distances in alveolar capillaries in

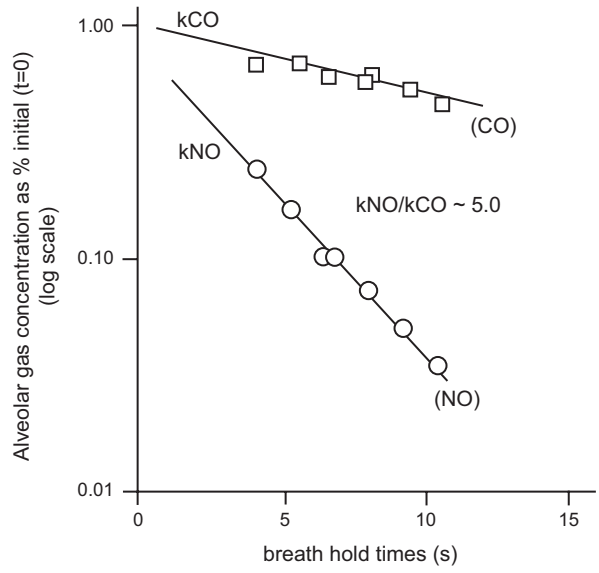

Fig. 1. The first published figure of the uptake of nitric oxide (NO) in relation to the simultaneous uptake of carbon monoxide (CO). Data were collected from a series of breath holds (from 4 to 11 s) in one subject. Log alveolar concentrations were plotted as %initial concentration at breath-hold onset (time $t = 0$) against breath-hold times. From the slopes of gas disappearance, a rate constant (k) proportional to lung diffusing capacity for CO (D_{LCO})/alveolar volume (V_A) and lung diffusing capacity for NO (D_{LNO})/V_A has been calculated. The ratio k_{NO}/k_{CO} is ~5.0. Since D_{LCO} (or D_{LNO}) = $k \times V_A$ (and V_A is common to both), the ratio k_{NO}/k_{CO} is identical to the ratio D_{LNO}/D_{LCO} (12). [Reproduced with permission of the European Respiratory Society, *Eur Respir J*, January 1, 2: 56, 1989 (7)].

Address for reprint requests and other correspondence: J. M. B. Hughes, 4 Cedars Rd., London SW13 0HP, UK (e-mail: mike.hughes@imperial.ac.uk).

vivo were less than for in vitro conditions, this would increase NO reaction rates more than for CO, so the ratio might become quasi-infinite. A stronger argument is that D_{LNO} (4) and θ_{NO} (Ref. 8; R. E. Forster, personal communication) are independent of any P_{O_2} change, unlike D_{LCO} and θ_{CO}.

In Favor of θ_{NO} Being Finite

Borland et al. (5) innovatively modeled NO and CO uptake in a membrane oxygenator, as used in cardiopulmonary bypass surgery. They dissociated Hb from its carrier (the erythrocyte) by lysis with tap water (5); in other experiments (6), in anesthetized dogs, cell-free heme-based oxyglobin was substituted for red cells. In both instances, D_{LNO} increased, but D_{LCO} hardly altered. Thirty-seven percent of the total NO resistance resided in the $1/\theta_{NO}$ Vc component, but this fraction should be treated with caution because of the unusual experimental conditions.

θ_{NO} Finite Compared With θ_{NO} Infinite

In this issue of the *Journal of Applied Physiology*, the "Bordeaux" (13) and "Cambridge, UK" (6) groups appear to have reached an *entente cordiale*! Martinot, Guenard, and colleagues (13) now state that θ_{NO} Vc is finite and should be taken into consideration. Thus *Eq. 2* becomes:

$$\mathrm{Vc}\left(\text{finite }\theta_{NO}\right) = \left[\left(1/\theta_{CO}\right)/\left(1-\alpha/k\right)\right]/\left(1/D_{L_{CO}} - \alpha/D_{L_{NO}}\right) \quad (3)$$

where $k = \theta_{NO}/\theta_{CO}$. They measured simultaneous single-breath D_{LNO} and D_{LCO} in normal subjects, at sea level and after acute exposure to altitude at 4,300 m [only data at rest, after 2/3 days exposure to altitude (high altitude *day 2/3*) will be discussed]. NO is particularly suitable for sea level-altitude comparisons, as it is P_{O_2} independent (4, 8). D_{LCO} at altitude was measured at a $P_{A_{O_2}}$ of 53 Torr. To have matched the sea level normoxic $P_{A_{O_2}}$ would have altered pulmonary vascular pressures and Vc, since there was significant pulmonary arterial hypertension at this stage. A correction of altitude D_{LCO} to the sea level $P_{A_{O_2}}$ equivalent was required, but fortunately this was not large.

At high-altitude *day 2/3*, in relation to sea level, D_{LNO}, D_{LCO}, and alveolar volume (V_A) all increased significantly and D_{LNO}/D_{LCO} decreased by 9%. The Dm_{CO}-to-Vc ratio, which is positively related to D_{LNO}/D_{LCO} change (13), decreased with both the finite and infinite θ_{NO} analysis. With their adaptation of the Roughton-Forster analysis, Vc increased by 30%, more than the 20% increase of Dm_{CO}, irrespective of the θ_{NO} value. The absolute values of Dm and Vc were, of course, different. For the same D_{LNO}, Dm (finite θ_{NO}) was larger than Dm (infinite θ_{NO}), as required by theory, and Vc (finite θ_{NO}) was correspondingly less than Vc (infinite θ_{NO}). So, the physiological message (13)–that the pulmonary hypertension induced by acute altitude exposure increased Vc–did not depend on whether θ_{NO} was assumed to be finite or infinite. Thus, for clinical purposes, θ_{NO} could be regarded as "operationally" infinite.

Direct (D_{LNO}, D_{LCO}) vs. Derived (Dm_{CO}, Vc) Measurements

Martinot et al. (13) used particular values of θ_{NO} (9) and θ_{CO} (10) to derive measurements of the alveolar-capillary Dm and Vc, although different values for θ_{NO} (8) and θ_{CO} (15) exist. No one yet knows how closely the in vitro estimates of θ_{NO} and θ_{CO} mimic the actual in vivo θ values, where the rheological conditions in alveolar septal capillaries and the plasma environment are likely to be different.

The measured D_{LNO} is a new index of alveolar gas transfer. In different clinical situations, the D_{LNO}-to-D_{LCO} ratio (~Dm/Vc) rises and falls in a predictable way (12). D_{LNO}/V_A (k_{NO}) and D_{LCO}/V_A (k_{CO}) respond to VA change differently (Ref. 12, see Fig. 1*B*), the former being driven by Dm/V_A and the latter by Vc/V_A. It is likely that, in the future, these three indexes will provide new clinical insights, while further characterization of θ_{NO} and θ_{CO} will continue to challenge physiologists.

ACKNOWLEDGMENTS

The author thanks R. E. Forster, C. D. Borland, and N. B. Pride for helpful discussions, and D. Simmonds for the artwork.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.M.B.H. drafted manuscript.

REFERENCES

1. **Borland C.** A place for *T*L,NO with *T*L,CO? *Eur Respir J* 31: 918–919, 2008.
2. **Borland C, Chamberlain A, Higenbottam T.** The fate of inhaled nitric oxide (Abstract). *Clin Sci* 65: 37, 1983.
3. **Borland C, Cracknell N, Higenbottam T.** Is the measurement of "DLNO" a true measure of membrane diffusing capacity (Abstract)? *Clin Sci* 67: 41, 1984.
4. **Borland CD, Cox Y.** Effect of varying alveolar oxygen partial pressure on diffusing capacity for nitric oxide and carbon monoxide, membrane diffusing capacity and lung capillary volume. *Clin Sci (Lond)* 81: 759–765, 1991.
5. **Borland CD, Dunningham H, Bottril F, Vuylsteke A.** Can a membrane oxygenator be a model for NO and CO transfer? *J Appl Physiol* 100: 1527–1538, 2006.
6. **Borland CD, Dunningham H, Bottril F, Vuylsteke A, Yilmaz C, Dane DM, Hsia CC.** Significant blood resistance to nitric oxide transfer in the lung. *J Appl Physiol* 108: 1052–1060, 2010.
7. **Borland CD, Higenbottam TW.** A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. *Eur Respir J* 2: 56–63, 1989.
8. **Botros N, Spalthoff S, Zimmerman UJ, Forster RE.** Rate of NO uptake by human erythrocytes at different P_{O_2} (Abstract). *FASEB J* 16: 290, 2002.
9. **Carlsen E, Comroe JH.** The rate of uptake of carbon monoxide and of nitric oxide by normal human erythrocytes and experimentally produced spherocytes. *J Gen Physiol* 42: 83–107, 1958.
10. **Forster RE.** Diffusion of gases across the alveolar membrane. In: *Handbook of Physiology. The Respiratory System. Gas Exchange*. Bethesda, MD: Am. Physiol. Soc., 1987, sect. 3, vol. IV, chapt. 5, p. 71–88.
11. **Guenard H, Varenne N, Vaida P.** Determination of lung capillary blood volume and membrane diffusing capacity by measurement of NO and CO transfer. *Respir Physiol* 70: 113–120, 1987.
12. **Hughes JMB, van der Lee I.** The *T*L,NO/*T*L,CO ratio in pulmonary function test interpretation. *Eur Respir J* 41: 453–461, 2013.
13. **Martinot J, Mule M, de Bisschop C, Overbeck MJ, Le-Dong N, Naeije R, Guénard H.** Lung membrane conductance and capillary volume derived from the NO and CO transfer in high altitude newcomers. *J Appl Physiol*; doi: 10.1152/japplphysiol.01455.2012.
14. **Nicholson P, Roughton FJW.** A theoretical study of the influence of diffusion and chemical reaction velocity on the rate of exchange of carbon monoxide and oxygen between the red corpuscle and the surrounding fluid. *Proc R Soc Lond B Biol Sci* 138: 241–264, 1951.
15. **Reeves RB, Park HK.** CO uptake kinetics of red cells and CO diffusing capacity. *Respir Physiol* 88: 1–21, 1992.
16. **Roughton FJW, Forster RE.** Relative importance of diffusion and chemical reaction in determining rate of exchange of gases in the human lung. *J Appl Physiol* 11: 290–302, 1957.