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A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide

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ABSTRACT: Pulmonary diffusing capacity (D_L) for carbon monoxide (CO) and nitric oxide (NO) were simultaneously measured in man using the single breath method, by adding 40 ppm of NO to the inspired gas and analysing the expirate for NO by a chemiluminescent method. The mean ratio of D_{LNO} to D_{LCO} in thirteen subjects was 4.3 (SD 0.3), mean D_{LNO}= 49 mmol·min^{-1}·kPa^{-1} (SD 10) and mean D_{LCO}=11 mmol·min^{-1}·kPa^{-1} (SD 2). An increase in alveolar oxygen concentration from a mean of 18 to 68% in five subjects was associated with a 54% fall in D_{LCO} but no change in D_{LNO}. A reduction of lung volume from total lung capacity (TLC) (mean of 7 *l*) to a mean volume of 3.9 *l* in five subjects caused a fall in both D_{LNO} (by 34%) and D_{LCO} (by 8%). With 175 watts cycle exercise in three subjects the D_{LCO} rose by 45% and D_{LNO} by 25%. Since NO reacts much faster with haemoglobin than CO, D_{LNO} should be influenced much less by reaction with haemoglobin, and perhaps represents a better index for the diffusing capacity of the alveolar-capillary membrane (Dm) than D_{LCO}.
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Pulmonary diffusing capacity measured by carbon monoxide D_{LCO} [1] reflects both diffusion, and the rate of combination of CO with haemoglobin (Hb) [2, 3], Nitric oxide (NO) combines with dissolved reduced haemoglobin *in vitro* 400 times faster than CO [4]. The rate of reaction of NO with oxyhaemoglobin is of a similar magnitude [5]. The dissociation of NO from HbNO is slower than for CO from HbCO [4, 6]. ROUGHTON and FORSTER [3] considered the pulmonary diffusing capacity for carbon monoxide as composed of two components. An "extra-erythrocyte" step, Dm, consisting of transfer of gas from the alveolus to the red cell and an "intra-erythrocyte" step, the process of entry into the red cell and combination with oxyhaemoglobin, θVc. Theoretically a gas like NO with a rapid rate of combination with haemoglobin could offer a means of measuring the "extra-erythrocyte" gas transfer, Dm_{NO}.

In concentrations of 1000 ppm NO is toxic, and reduces the oxygen carrying capacity of blood by the formation of methaemoglobin [7]. NO is oxidized in air to nitrogen dioxide (NO_2) which is more soluble in water and is a reactive "oxidant" which can injure lung tissue [7]. However, the rate of oxidation of NO is proportional to the square of its concentration and proportional to the oxygen concentration [8]. A concentration of NO of 40 ppm avoids direct toxicity [7, 9] and significant oxidation to NO_2 within the duration of the test [8]. As additional evidence of low acute toxicity, a single "puff" of cigarette smoke contains a ten-fold higher concentration of NO than 40 ppm [10]. Furthermore, evidence is accumulating that NO may be a naturally occuring substance and it has been suggested that endothelium-derived relaxing factor is NO [11].

We report a study using a conventional single breath method to simultaneously measure D_{LCO} and D_{LNO} using an inspired gas concentration of NO of 40 ppm. We have also studied the relative effects on D_{LCO} and D_{LNO} of increased alveolar oxygen concentration, changes in lung volume and exercise.

Methods

The NO analyser

A chemiluminescent analyser [12, 13] was used (Chemlab Ltd, Hornchurch, Essex, U.K.). This technique is the standard physical reference method for measurement of low concentrations of nitrogen oxides both in polluted atmospheres [14] and in cigarette smoke [15]. Within the analyser, ozone is used to

oxidize NO to NO_2 in an "excited" form, yielding photons of light which are detected and quantified via a photomultiplier. The instrument can detect concentrations of NO between 1–1000 ppm. We tested our instrument for linearity, signal-to-noise ratio, reproducibility and response time. We also tested for interference from NO_2, N_2O, NO and CO_2 [16]. The instrument was calibrated daily using a certified concentration of NO in nitrogen of 53 ppm.

Measurement of diffusing capacity

Standard single breath gas transfer equipment (Transfertest, P.K. Morgan, Chatham, Kent, U.K.), which has analysers for helium (He), CO and oxygen (O_2), was used. Each analyser was calibrated daily using a test gas mixture supplied by the manufacturer (CO 0.3%; He 14%; O_2 18%; N_2 65%). The response of each analyser was linear. The He analyser was adjusted for oxygen concentration using an in-built calibration device. In addition to sampling the inhaled and exhaled gases for helium, CO and oxygen concentrations, NO was also sampled.

Measurements of DLCO and DLNO were made simultaneously. A gas mixture containing approximately 40 ppm NO in 14% He, 0.3% CO, 18% O_2 in nitrogen was made up in the inspirate bag immediately before each test by adding 0.3 *l* of 1000 ppm NO in N_2 (Hilger Analytical Ltd, Margate, Kent, U.K.) to 7*l* of the standard gas transfer test mixture (P.K. Morgan, Chatham, Kent, U.K.). The gas volume was measured with the dry spirometer of the "Transfer Test" equipment. The inspirate gas bag was checked for NO concentration before measurements to avoid the risk of inhaling toxic concentrations.

By rapid inspiration of the gas mixture from residual volume (RV) to total lung capacity (TLC) a single breath DL measurement was performed. During expiration a washout volume of 0.75*l* was used and an alveolar sample of 1 *l* was collected for analysis [17]. At least 5 min elapsed between each manoeuvre. The duration of breath-hold was taken to include 0.7 of the inspiration time and 0.5 of the sample collection time [17]. The total duration of the breath-hold was reduced from the recommended 9–11 s to 7.5 s to give an alveolar NO concentration of 3 ppm. Empirically we had found that a breath-hold of greater than 9–11 s resulted in an alveolar concentration of 1 ppm NO or less, unless an unacceptably high inspiratory concentration was used.

Analyses of the alveolar samples were performed immediately after the manoeuvre. The inspiratory mixture was then analysed, approximately 5 min elapsing between the two analyses. At a temperature of 20°C and an oxygen concentration of 18% the concentration of NO of 40 ppm would have fallen to 38.2 ppm after 5 min as a result of oxidation of NO to NO_2. The exact NO concentration at the time of the single breath could be calculated knowing the time that had elapsed from filling the inspirate bag (t):

$$\frac{1}{NO_t} = \frac{1}{NO_0} + \frac{1}{2K(o_2)_t} \quad (1)$$

where K is the rate constant for oxidation [8, 18] of NO. We checked these published kinetics by measuring the rate of decline in concentration of NO in known oxygen concentrations. The value that we obtained for K was 7.1×10^{-10} $ppm^{-2} \cdot min^{-1}$ which compared favourably with the published figure of 7.6×10^{-10} $ppm^{-2} \cdot min^{-1}$ [8] corrected to our laboratory temperature and pressure.

Alveolar air samples were passed through soda lime and calcium chloride before analysis for CO and He. On the other hand, for NO, samples were passed directly into the analyser as a small loss of NO was observed if the sample was passed *via* these agents.

In the three smokers who were studied, the DLCO was calculated after correcting the alveolar CO for back pressure of CO measured by rebreathing [19].

Calculation of DLCO and DLNO

DLCO was calculated from inspired and alveolar concentrations of He and CO and breath-hold by the standard method. The single breath He dilution value for alveolar concentration was used in the calculation [17]. An adjustment was made for the instrument deadspace. DLNO was calculated in the same manner, substituting NO for CO values. However, the measured alveolar concentrations of NO were multiplied by a factor of 1.1 and were substituted in the DL calculations to allow for the reduction in volume which occurs when CO_2 and water vapour are removed from the samples for He and CO analysis [19]. We assumed no alveolar back pressure for NO and an exponential rate of decline in alveolar NO in the calculation. These assumptions were justified by experimental observations reported below.

Simultaneous measurement of DLCO and DLNO in volunteers

Thirteen normal volunteers, free of respiratory disease, were recruited from our staff. All provided informed consent and the study was approved by the hospital's Ethical Committee. In all subjects, the following variables of lung function were within ±2 SD of the predicted values; forced expiratory volume in one second (FEV_1) (Vitalograph, Maidstone, Kent, U.K.) TLC (whole body plethysmograph) and DLCO by the single breath method (Transfer Test, P.K. Morgan, Chatham, Kent, U.K.). A simultaneous measurement of DLNO and DLCO was made at rest in each subject. Three replicates were performed by every person. To test for mutual interference of DLNO and DLCO five subjects undertook separate measurements of DLNO and DLCO alone, and the values obtained were compared with measurements made simultaneously with the gas mixture containing both gases.

Measurement of alveolar NO at varying times of breath-hold

Combined DLCO and DLNO measurements were carried out on two subjects but at different breath-hold times ranging from 4–10 s. The alveolar NO at each time interval was calculated as a fraction of the initial concentration.

$$\frac{F_{ANO_t}}{F_{ANO_o}} = \frac{F_{ANO_t}}{F_{INO}} \times \frac{F_{IHe}}{F_{AHe}} \quad (2)$$

where FA is the fractional concentration in the alveolar gas, FI the fractional concentration in the inspired air and He refers to helium. The subscripts o and t refer to initial and final values.

Measurement of back tension of NO and CO in smokers

In eight tobacco smokers and two nonsmokers alveolar concentrations of CO and NO were sampled following a 20 s breath-hold at TLC [20]. Alveolar concentrations were also measured in one smoker and one nonsmoker after rebreathing oxygen for 4 min. The smokers' daily tobacco consumptions were recorded as was the last time they had smoked.

Measurement of DL at two alveolar oxygen concentrations

Simultaneous single breath measurements of DLCO and DLNO were performed by five normal subjects using the method described. On separate days a gas mixture of CO/NO/He was made up in air as described and was also made up with an increased oxygen concentration [19]. Alveolar CO and O_2 (and NO in two subjects) were recorded after rebreathing oxygen for 4 min. To ensure alveolar hyperoxia at the time of the test, 100% oxygen was breathed by each subject for 5 min via a mask (Adult oxygen mask, Intersurgical Ltd, Rugby, England).

Diffusing capacity of the alveolar capillary membrane for CO (Dmco) was calculated in the usual way [19]. This requires calculation of O for CO *in vivo* from knowledge of pulmonary capillary oxygen tension (Pco_2), HbCO concentration and Hb concentration together with ROUGHTON and FORSTER's value of OCO *in vitro* [3, 19]. The Hb and HbCO were measured from venous blood samples using a spectrophotometer (Il 282, Instrument Laboratories, Andover, Mass, USA) [21]. Pco_2 was in turn calculated from alveolar oxygen tension and resting oxygen uptake (Vo_2). This latter value was assumed to be 12 mmol·min^{-1} [19] but the exact value chosen makes little difference to the final value for Dmco [19].

Measurement of DL at different lung volumes

Five normal subjects were studied at rest in a sitting position. The solenoid operated valve on the "transfer test" equipment (P.K. Morgan, Chatham, Kent, U.K.) was set to either a full inspiration, TLC, or to a volume of approximately TLC/2. Two replicate measurements were made on the subjects at each lung volume.

The measurement of DL at varying levels of exercise

Three normal subjects were studied on a bicycle ergometer which was mechanically braked (Tunturi, Turku, Finland). DL was measured in each at rest, and during exercise of 50, 75, 125 and 175 watts work at 60 rev per min. Each subject cycled or rested at the required level for 4 min, oxygen uptake was measured for the last 2 min and then, whilst still maintaining the exercise, a single breath DL measurement was made. In addition to the DL measurements the rate of oxygen consumption was recorded at each level of work. The oxygen concentration of mixed expired gas in a 5 *l* "baffle" box with a low resistance 2-way valve (Otis McKerrow type) was measured using a paramagnetic oxygen analyser (P.K. Morgan, Chatham, Kent, U.K.). The expired volume was recorded with a heated Fleisch pneumotachograph, differential manometer and integrator (P.K. Morgan, Chatham, Kent, U.K.). Oxygen consumption rate was calculated from exhaled volume and oxygen concentration [22]. Two replicate measurements were made from each subject at each exercise level.

Experimental protocol and analysis of results

No more than four single measurements of DL were made per person each day. For each subject the studies were performed at least two hours after a meal and at the same time of day on each occasion. Within each study the order of the different tests was randomized. One subject was a smoker who maintained his usual smoking pattern, and each estimate of DLCO was adjusted for CO "back pressure" [19]. Two-way analysis of variance was used, factors being subjects and various levels of each test *i.e.*, oxygen concentrations, lung volume or level of exercise. To compare the percentage fall in DLCO and DLNO with reducing lung volume a two sample t test was used. In each study the test of significance was set for the whole experiment to test each hypothesis *e.g.*, exercise has no effect on DL. The standard error terms include within and between individual variation.

Results

The response of the NO analyser was linear from 1–5 ppm but for concentrations 5–30 ppm a minor departure from linearity was noted,with a slight underestimate

Table 1. – Comparison of DLNO and DLCO measured together and separately

Subject	DLCO alone*	DLCO with NO	DLNO alone	DLNO with CO
1	13.3 (0.8)	14.3 (0.4)	57.6 (1.0)	59.6 (1.2)
2	11.9 (0.3)	11.9 (0.3)	57.9 (5.6)	55.2 (1.6)
3	13.7 (0.3)	13.5 (0.3)	53.5 (3.6)	61.0 (0.7)
4	12.0 (1.1)	12.0 (1.1)	49.4 (4.7)	50.2 (4.1)
5	8.9 (0.3)	8.7 (0.3)	47.5 (1.6)	42.6 (2.8)

*Breath-hold time of 10 s. The remaining values are measured with a breath-hold time of 7.5 s. Mean values (SD), three replicates ($mmol \cdot min^{-1} \cdot kPa^{-1}$).

of NO concentration. This loss of linearity reduced DLNO measurements by 0.05 $mmol \cdot min^{-1} \cdot kPa^{-1}$ or less, so that no correction was required. The "noise" level of the instrument was 0.5 ppm [14]. The "signal-to-noise" ratio was 2 at 1 ppm, increasing to 100 at 50 ppm. Repeated analyses of the same concentration of NO agreed to within 10% at 1 ppm and to within 1% at 50 ppm. The time to 95% response after a stepwise increase in concentration was 24 s.

No interference was observed with NO_2 or N_2O. A concentration of 50% CO_2 caused a 1 ppm reduction in the signal from the NO analyser with a 40 ppm NO mixture.

Figure 1 shows that the decline in alveolar NO and that of the CO concentration is logarithmic when plotted as a function of time. There was no alveolar back tension for NO detected in either the two nonsmokers or the eight smokers after a 20 s breath-hold. This was despite the finding of alveolar CO concentrations ranging from 16–34 ppm in the smokers. No alveolar back pressure for NO could be obtained in the one smoker and one nonsmoker after 4 min of rebreathing O_2. The smoker's CO back pressure was 70 ppm at an alveolar oxygen concentration of 75%.

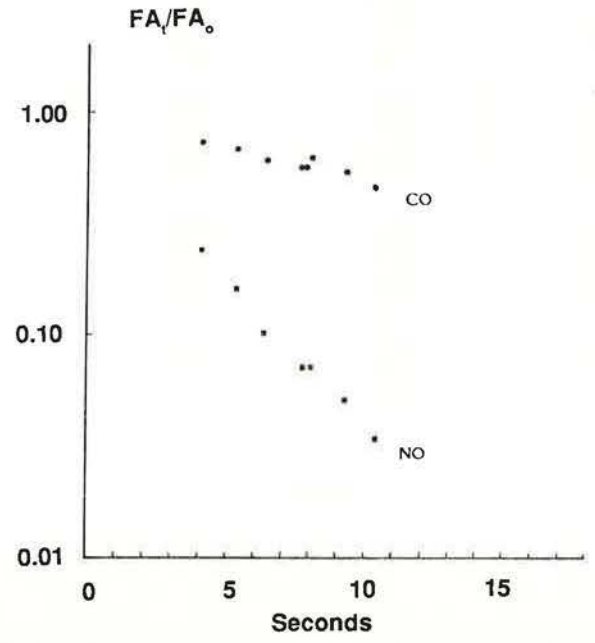

Fig. 1. – Decline in alveolar NO and CO with varying breath-hold times (one subject). Alveolar concentrations at each time (FA_t) are expressed as a fraction of the initial alveolar concentration (FA_o).

DLNO exceeded DLCO by a mean ratio of 4.3 (SD 0.3), average DLCO=11.6 (SD 2.4) {39 (SD 7) $ml \cdot min^{-1} \cdot mm\ Hg^{-1}$} and average DLNO=49.1 (SD 10.2) {147 (SD 31) $ml \cdot min^{-1} \cdot mmHg^{-1}$} n=13. Individual values for DLCO and DLNO were significantly correlated ($r=0.96$, $n=13$, $p<0.001$). Table 1 shows that the presence of CO or NO in the simultaneous test single breath gas mixture did not interfere with the uptake of either CO or NO. The mean values (standard deviation) for the variables from which DLCO and DLNO were calculated are presented in table 2. The coefficient of variation, including within and between day variation, was 6.6% for DLCO and 6.4% for DLNO in our subjects. This compares favourably with published values for variation in DLCO [17, 19].

An increase in alveolar oxygen concentration from an average of 18.6 to 68.5% caused a mean fall in DLCO from11.3 (SD 1.2) to 6.5 (SD 1.2) $mmol \cdot min^{-1} \cdot kPa^{-1}$

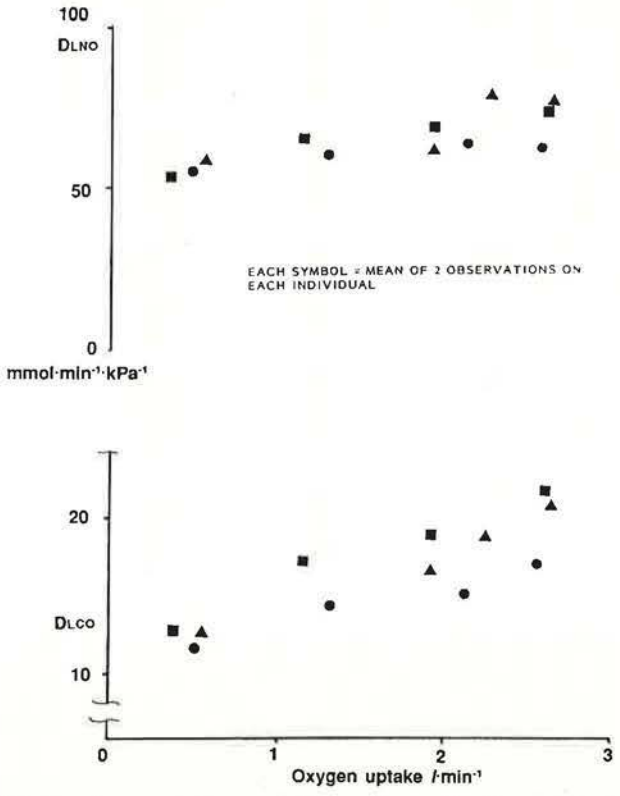

Fig. 2. – Increase in DLNO and DLCO with exercise (three subjects). Rest is represented by the lowest values of oxygen uptake; exercise is represented by the remaining higher values.

Table 2. – Average data from which DLCO and DLNO were calculated

	He%	CO% ppm	NO	O_2%	Breath-hold time s
Inhaled	12.8(1)	0.27(0.008)	38.9(13.8)	17.7(1.6)	7.3(0.4)
Exhaled	10.0(1)	0.11(0.02)	2.2 (1)		
	VA *l*		DLCO mmol·min^{-1}·kPa^{-1}		DLNO mmol·min^{-1}·kPa^{-1}
	6.1 (1.5)		11.6 (2.4)		49.1 (102)

Mean values from 13 subjects, 3 replicates were taken (SD).

($p<0.001$) (33.7 (SD 3.6) to 19.4 (SD 3.6) ml·min^{-1}·mm H^{-1}) but DLNO was unchanged; 51.2 (SD 6.2) compared to 51.4 (SD 5.8) mmol·min^{-1}·kPa^{-1} (153 (SD 18.5) to 153 (SD 17.3) ml·min^{-1}·mmHg^{-1}) ($p>0.05$ NS).

The reduction in alveolar volume, on average from 7 *l* to 3.9 *l* produced a mean fall in DLCO from 12.9 (SD 1.6) to 11.9 (SD 1.9) mmol·min^{-1}·kPa^{-1} ($p<0.01$), (38.5 (4.8) to 35.5 (5.7) ml·min^{-1}·mmHg^{-1}·*l*$^{-1}$ a fall of 8% although in one subject there was an increase in DLCO (table 3). Carbon monoxide transfer coefficient (KCO) rose from 1.9 (SD 0.2) to 3.1 (SD 0.2) mmol·min^{-1}·kPa^{-1}·*l*$^{-1}$ (48%), (9.3 (0.6) to 5.7 (0.6) ml·min^{-1}·mmHg^{-1}·*l*$^{-1}$). For DLNO the fall was rather greater: 54.5(SD 7.0) to 38.6 (SD 6.6) mmol·min^{-1}·kPa^{-1} ($p<0.001$) (163 (21) to 115 (19.7) ml·min^{-1}· mmHg^{-1}) a fall of 34%. Nitric oxide transfer coefficient (KNO) rose from 7.9 (SD 0.8) mmol·min^{-1}·kPa^{-1}·*l* $^{-1}$ to 10.1 (1.1) (24%) (23.6 (2.4) to 30.1 (3.3) ml·min^{-1}·mmHg^{-1}·*l*$^{-1}$). The difference between the falls in DLCO and DLNO with lung volume were highly significant ($p<0.01$).

With exercise, from rest to 175 watts with an increase in the rate of oxygen consumption from 0.4 (SD 0.01) *l*·min^{-1} (17.9 (SD 0.5) mmol·min^{-1}) to 2.6 (SD 0.1)*l*·min^{-1} (116 (SD 4.5) mmol·min^{-1}) the DLCO increased from 12.3 (SD 0.7) to 19.5 (SD 1.7) mmol·min^{-1}·kPa·$^{-1}$(36.7 (2.1) to 58.2 (5.1) ml·min^{-1}·mmHg^{-1}) an increase of 45% ($p<0.001$). The DLNO increased from 53.7 (SD 2.2) to 70 (SD 9.5) mmol·min^{-1}·kPa^{-1} (160 (6.6) to 209 (28) ml·min^{-1}·mmHg^{-1}) an increase of 26% ($p<0.001$) (fig. 2).

Table 3. – Effect of changing lung volume on DLCO and DLNO

Subject	VA *l*	DLCO mmol·min^{-1}·kPa^{-1}	KCO mmol·min^{-1}·kPa^{-1}·*l*$^{-1}$	DLNO mmol·min^{-1}·kPa^{-1}	KNO mmol·min^{-1}·kPa^{-1}·*l*$^{-1}$
CB	4.7	13.5	2.9	45.8	9.8
	4.5	13.5	3.0	33.9	7.5
	7.8	13.8	1.8	60.1	7.7
	8.0	13.6	1.7	56.7	7.1
NC	3.6	11.9	3.3	40.5	11.3
	4.0	11.8	3.0	39.1	9.8
	7.9	14.4	1.8	59.1	7.5
	8.0	12.4	1.6	53.8	6.7
MF	2.8	9.8	3.5	30.6	10.9
	2.7	8.6	3.2	29.2	10.8
	5.0	11.5	2.3	45.2	9.0
	4.7	9.4	2.0	40.9	8.7
IH	3.4	11.2	3.3	36.6	10.8
	3.6	10.4	2.9	36.6	10.2
	7.3	14.4	2.0	63.4	8.7
	7.3	14.1	1.9	59.9	8.2
PW	4.7	14.3	3.0	46.4	9.9
	4.9	13.8	2.8	47.6	9.7
	6.9	12.6	1.8	50.6	7.3
	7.1	13.0	1.8	55.3	7.8

Discussion

We have demonstrated that DLNO is four times as great as DLCO at rest and at normal O_2 levels. Unlike DLCO, DLNO appears to be independent of hyperoxia, but DLNO appears to be more dependent on lung volume. As with DLCO the DLNO increases on exercise. We originally described the simultaneous measurement of KNO

and Kco using the single breath measurement in 1983 [23]. Since then other authors have described similar measurements at rest in man [24] and animals [25]. Both groups obtained similar differences between DLNO and DLCO as we reported previously [23] and show in this study. The difference between DLNO and DLCO is not a result of a reaction of NO with lung tissue. Both *in vitro* and *in vivo* studies using radiolabelled NO demonstrate that NO passes rapidly into the blood, and does not react with lung tissue [26, 27]. These observations will be considered by referring to two widely used models of gas transfer.

The ROUGHTON and FORSTER model [3] considers transfer of CO as two resistances in series, $1/DL=1/Dm+1/\theta Vc$ where 1/Dm represents the "extra-erythrocyte" phase of diffusion across epithelium, interstitium, endothelium and plasma and $1/\theta$ Vc represents the "intra-erythrocyte" entry of CO into the red cell and displacement of oxygen from oxyhaemoglobin. θ is the rate of uptake of CO by blood at 37°C per unit volume, per unit partial pressure. Vc is capillary blood volume. In the original model both steps were thought to offer equal resistance to gas transfer. Like CO, NO is relatively insoluble in water, at 37°C solubility of CO is 0.0184 $ml \cdot ml^{-1}$ [28] whilst the solubility of NO is 0.035 $ml \cdot ml^{-1}$ [28]. As NO reacts virtually irreversibly with haemoglobin we have applied the ROUGHTON and FORSTER model to DLNO. NO combines with Hbo_2 [5] and Hb [4] in solution about four hundred times more rapidly than CO [4]. Although by analogy with CO [3] enclosing the haemoglobin within an erythrocyte may slow the reaction somewhat we should still conclude that θ NO will greatly exceed θ CO and that $1/\theta$ NOVc will tend towards zero. Thus, DLNO should approximate DmNO.

If DLNO does approximate to DmNO it should be close to a predicted value of DmNO calculated knowing Dmco and the diffusivity of NO. Dmco was calculated from knowledge of DLCO at two oxygen concentrations using the standard formulae and values of θ at known Hb, Hbco and calculated Pco_2 [19]. Assuming the "extra-erythocytic" resistance to be an aqueous layer, NO diffusivity (water solubility/the square root of molecular weight) should exceed CO diffusivity by 1.8. From our five subjects we would therefore predict DmNO to be approximately 34 $mmol \cdot min^{-1} \cdot kPa^{-1}$. In fact the average measured value of DLNO was 51 $mmol \cdot min^{-1} \cdot kPa^{-1}$. Considering the inaccuracies of the assumptions involved in calculating Dmco these figures are in reasonable agreement, particularly as it is now believed that Dmco is greater than ROUGHTON and FORSTER originally suggested. Indeed morphological estimates of Dmco and Dmo_2 [2, 29] considerably exceed the physiological measurements. If DLNO approximated DmNO then it would be expected that, as we have observed, DLNO will be independent of hyperoxia but will by comparison to DLCO be more dependant on the lung volume at which it is measured. Indeed, the smaller rise in KNO compared with Kco at the reduced lung volume might suggest that NO uptake is more dependent on alveolar volume than on the pulmonary capillary blood volume compared with CO uptake.

An alternative model for alveolar gas uptake has been proposed by PIIPER and SCHEID [30]. This model uses the application of Fick's law of diffusion to mass gas transfer from a single idealized alveolar unit across a tissue membrane to a moving capillary bloodstream. Transfer across the membrane includes gas diffusion and reaction with the blood. Total conductance (G) is defined by mass transfer divided by total effective partial pressure difference (alveolar air minus end capillary blood partial pressure):

$$G = QB \ (1 - e \ D/QB) \qquad (3)$$

where B is the capacitance coefficient [30].

Transfer of a gas may be limited by diffusion (D) or perfusion (Q) depending on its ratio of water to blood solubility which in turn determines D/QB. The blood solubility of a gas is given by its dissociation curve which for NO has never been calculated. However the greater affinity of NO for Hb in solution suggests that BNO greatly exceeds Bco. It can therefore be predicted that NO uptake is diffusion limited to a greater extent than CO. For a diffusion limited gas G=D and D=M/(PA - PV). PV for CO is assumed to be zero. We have similarly assumed PvNO to be zero because of the slow dissociation of HbNO (6) and because NO cannot be regenerated from methaemoglobin and nitrate [31] together with our failure to demonstrate an alveolar back tension for NO even in tobacco smokers who are continually exposed to NO. In this model D is assumed to be a composite conductance of alveolar gas phase mixing, diffusion through alveolar epithelium, interstitium, endothelium and plasma to the erythrocyte, together with chemical combination with oxyhaemoglobin. For CO the model includes regional inhomogeneities of D/VA and V/VA. Despite the composite nature of D, uptake of CO and as we have shown also for uptake of NO, D obeys first order kinetics. The diffusivity of NO was calculated in the previous section as almost double that of CO. Accordingly, as DLNO is fourfold greater than DLCO the difference cannot be explained by diffusion alone but also by the more rapid rate of reaction of NO with Hbo_2. This idea is consistent with the failure of DLNO to fall with hyperoxia compared with DLCO as follows but does not help to explain the effect of change in lung volume.

The kinetics of the reaction of CO with oxyhaemoglobin are believed to approximate:

$$\frac{d(Hbco)}{dt} = \frac{m(Hbo_2) \times (CO)}{(O_2)} \qquad (4)$$

where m is the rate coefficient.

It is easily appreciated that increasing the oxygen partial pressure above that necessary to achieve 100% Hbo_2 saturation will slow the rate of formation of Hbco. Conversely the kinetics of the reaction of NO

with Hbo_2 are believed to be:

$$\frac{-d(NO)}{dt} = K(NO) \times (Hbo_2) \quad (5)$$

If the pulmonary capillary oxyhaemoglobin saturation is approximately 100%, any further increase in pulmonary capillary oxygen partial pressure will have little effect on the rate of combination of NO with oxyhaemoglobin.

If this explanation is correct then it implies that the published rate constants for NO combining with Hb and Hbo_2 in solution considerably over-estimate the rate of combination with oxyhaemoglobin in the intact red cell in the pulmonary capillary. To clarify this uncertainty there is a need to measure the rate of combination of NO with oxyhaemoglobin in the red cell. The effect of haematocrit should also be investigated: if DLNO only reflects extra-erythocytic transfer it should be independent of haematocrit, on the other hand if it mainly reflects the rate of gas combining with the erythrocyte haemoglobin then haematocrit will affect DLNO.

In conclusion measurement of the diffusing capacity using nitric oxide DLNO in concentrations of 40 ppm can be performed without apparent hazard. Measurement of DLNO and DLCO can be achieved using an NO analyser and commercially available gas transfer measurement apparatus. It may offer a direct measure of Dm for NO (and by extrapolation CO) more rapidly and without the need for making measurements at two oxygen concentrations. On the other hand, it remains possible that the fourfold greater value of DLNO compared to DLCO is a direct result of the differing kinetics of the reaction of NO and CO with the red cell. Further research is needed to clarify these issues.

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Mesure simultanée en respiration unique de la capacité de diffusion pulmonaire par le NO et le CO. C. Borland, T. Higenbottam.

RÉSUMÉ: Les capacités de diffusion pulmonaire (DL) pour le CO et pour le NO ont été mesurées simultanément chez l'homme par la méthode de respiration unique, par l'addition de 40 ppm de NO aux gaz inspirés et par l'analyse des gaz expirés par une méthode chimio-luminescente pour le NO. Le rapport moyen de DLNO sur DLCO chez 13 sujets est de 4.3 (SD 0.3), la DLNO moyenne étant de 49 $mmol \cdot min^{-1} \cdot kPa^{-1}$ (SD 10), et la DLCO moyenne étant de l's $mmol \cdot min^{-1} \cdot kPa^{-1}$ (SD 2). Une augmentation de la concentration de l'oxygène alvéolaire à partir d'une moyenne de 18 jusqu'à 68 % chez cinq sujets fut associée à une chute de la DLCO de 54 %, mais n'entraîne aucune modification de la DLNO. Une réduction du volume pulmonaire depuis la capacité pulmonaire totale (CPT) (moyenne de 7 litres) à un volume moyen de 3.9 litres chez cinq sujets, a provoqué une chute à la fois de la DLNO (34 %) et de la DLCO (8 %). Après un effort de 175 watts sur bicyclette chez trois sujets, la DLCO augmente de 45 %, et la DLNO de 25 %. Puisque NO réagit beaucoup plus rapidement avec l'hémoglobine que CO, la DLNO devrait être influencée d'autant moins par la réaction avec l'hémoglobine, et représente peut-être un meilleur index de la capacité de diffusion de la membrane alvéolo-capillaire (Dm) que la DLCO.
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