CARBON MONOXIDE TRANSFER IN PIG LUNGS DURING MECHANICAL VENTILATION

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CARBON MONOXIDE TRANSFER IN PIG LUNGS DURING MECHANICAL VENTILATION

Koolmonoxide-overdracht
in longen van varkens tijdens beademing

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CONTENTS

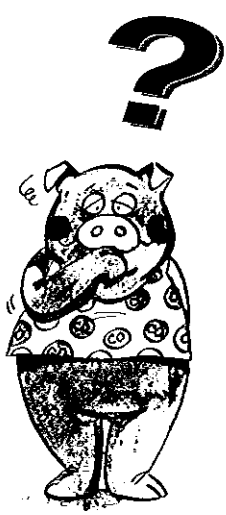
"Men that look no further than their outsides, think health an appurtenance unto life, and quarrel with their constitutions for being sick; but I that have examined the parts of man, and know upon what tender filaments that Fabric hangs, do wonder that we are not always so; and considering the thousand doors that lead to death, do thank my God that we can die but once."

- Mensen die niet verder dan de oppervlakte kijken, denken dat gezondheid een attribuut van het leven is, en richten verwijten tot hun gestel als ze ziek zijn. Maar ik, die weet, op hoe uiterst fijne draden dat weefsel hangt, verwonder mij, dat wij ooit gezond zijn; en als ik de duizenden poorten zie, die tot de dood leiden, dank ik God dat wij maar ééns kunnen sterven. -

SIR THOMAS BROWNE, Religio Medici (1656)

CHAPTER 1

INTRODUCTION



BACKGROUND AND OBJECTIVES OF THIS THESIS

The volume of blood in the lungs comprises about 10-20% of the total blood volume. This relatively small volume will be extremely susceptible to small changes in the output of the two ventricles, as it is located between them. Versprille *et al.* [25] reported transient changes in pulmonary blood volume due to differences between input into (i.e. right ventricular output) and output from (i.e. left ventricular output) the pulmonary circulation during each ventilatory cycle in mechanical ventilation. A decrease in total pulmonary blood volume was found during inflation which recovered during expiration. We wondered whether the capillary part of the pulmonary blood volume, which is a main determinant of gas transfer, would be similarly affected by forced inflation. The capillary blood volume cannot be measured directly in intact subjects. However, its volume can be derived by estimation of the carbon monoxide transfer in the lungs and using the Roughton-Forster relationship [20,27]. Although the carbon monoxide transfer has been studied in animals, in which forced inflation is inherent, the effect of mechanically ventilatory conditions on carbon monoxide transfer is scarcely documented in the literature. Also, knowledge of the filling state of the pulmonary vascular bed under conditions of mechanical ventilation is limited, especially with regard to the capillary part.

This thesis comprises studies of gas transfer in the lungs during mechanical ventilation, which have been obtained in healthy pigs. The objectives of this thesis were: 1) to adapt the breath-holding technique, as used during spontaneous breathing for estimation of gas transfer, to conditions of mechanical ventilation; and 2) to evaluate the effect of changes in lung volume on pulmonary gas transfer and capillary blood volume.

In this first chapter some general aspects will be considered against the background of this thesis. Firstly, an outline is given of gas transfer in general, followed by methods for estimation of carbon monoxide transfer. Hereafter, the derivation of capillary blood volume is explained. Subsequently, the circulatory responses to changes in intrapulmonary pressure during mechanical ventilation are considered and compared with those during spontaneous breathing. Finally, an outline of this thesis is given.

GAS EXCHANGE IN THE LUNGS: GENERAL ASPECTS

An essential function of the lungs is exchange of gases, uptake of oxygen (O_2) and removal of carbon dioxide (CO_2), across the blood-gas barrier which separates the blood in the capillaries from the alveolar gas.

In general, diffusion of a substance through a space is entirely due to the random motion of its molecules which causes it to move from a point of high concentration to a point of low concentration. The rate of diffusion is proportional to the concentration difference. Different substances diffuse at different rates even if the concentration difference is the same; this variation in diffusivity is characterized by the coefficient of diffusion (k). Since the diffusion process is dependent on the random motion of molecules, gases with smaller molecules diffuse more rapidly than those with larger ones. In fact, k varies in inverse proportion to the square root of the molecular weight.

With respect to the diffusion of respiratory gases, we are mainly concerned with differences in partial pressure instead of differences in concentration. In a gas phase, the partial pressure of a gas is strictly related to its concentration, whereas in liquids, the concentration of a gas is equal to its partial pressure multiplied by its solubility (α). For example, the concentration gradient of CO_2, which is 20 times as soluble in plasma as is O_2, is 20 times steeper than that of O_2 for the same partial pressure gradient.

The respiratory gases have to pass the blood-gas barrier in the lungs, which is a compromise between the anatomical necessity to separate blood and air and the physiological necessity to bring them together. Three anatomical zones may be considered: alveolar gas; alveolar-capillary membrane, consisting of alveolar epithelium, interstitial layer and capillary endothelium; and capillary blood. The diffusing capacity of the lungs (DL) for a particular gas is defined as the volume of gas at standardized conditions (i.e. STPD[1]) passing from the alveoli into the capillary blood stream per unit of time when the partial pressure difference between alveolar gas and the blood phase equals one:

$$DL_{CO} = \frac{volume\ gas\ in\ STPD\ diffusing}{(alveolar\ tension - capillary\ tension) \times time} \qquad (1)$$

[1] Standard Temperature (0°C) Pressure (760 mmHg) Dry (zero water vapour pressure)

Traditional units are ml STPD.min^{-1}.$mmHg^{-1}$; in this thesis DL is expressed in SI units, i.e. $\mu mol.sec^{-1}.kPa^{-1}$. DL in SI units equals 5.582 × DL in traditional units. Two points should be noted. Firstly, DL is expressed in terms of the partial pressure difference and not of the concentration difference. Therefore, for a particular gas, DL is related to both its coefficient of diffusion (k) and its solubility (α). Secondly, the definition of DL ignores any specific feature of the membrane, e.g. area, thickness, geometry, through which the gas diffuses. This is inevitable, since the membrane is not susceptible to measurement in vivo. The result is that DL is, to some extent, proportional to the size of the lungs. DL is therefore often expressed per litre lung volume.

The measurement of DL for a particular gas depends on knowledge of its total rate of exchange across the alveolar membrane together with its partial pressure in the alveoli and in the capillary blood. It is relatively easy to measure the quantity of O_2 or CO_2 entering or leaving the body, but the difference between the partial pressures in alveoli and capillary blood is considerably more difficult to estimate. In the case of CO_2, this difference is so minute that it is regarded as non-existent (for practical purposes). Hence DL for CO_2 cannot be estimated. Regarding O_2, the partial pressure in the alveoli could be derived by means of the alveolar gas equation. However, the estimation of its partial pressure in the pulmonary capillaries is much more complex. During passage of blood through the pulmonary capillary bed, the capillary O_2 tension is continually increasing by uptake of O_2 from alveolar gas. To derive a mean capillary O_2 tension, mixed venous and end-capillary O_2 tension, as well as the mathematical description of the change in O_2 tension with capillary passage time have to be known. Mixed-venous O_2 tension can be measured, but catheterization of the pulmonary artery to sample blood is a precondition for it. Sampling of end-capillary blood is not possible, and thus the end-capillary O_2 tension cannot be measured, also due to some venous admixture before the blood reaches the arterial system. Because of these difficulties, DL of carbon monoxide (CO) has been considered as a substitute for O_2. The affinity of haemoglobin for carbon monoxide is so high that when breathing a low concentration of this gas, the partial pressure of CO in the capillaries usually can be ignored, with the exception of studies in e.g. smokers. In general, however, the estimation of DL of carbon monoxide requires the determination of the uptake of carbon monoxide per minute and its partial pressure in the alveoli.

METHODS TO DETERMINE CARBON MONOXIDE TRANSFER

Single-Breath or Breath-Holding Technique

The single-breath technique has usually been applied for measurement of the diffusing capacity of the lungs during spontaneous breathing [8,12,14,16-18,23,26]. This technique was introduced by Krogh [17] in 1915. In her method a maximal inspiration of a gas mixture containing air and CO was made from residual volume and immediately followed by an expiration of at least one litre. Then, breath was held at the remaining lung volume for 6 to 10 seconds, followed by a maximal expiration. An equation was developed in which the uptake of CO from the lungs during breath-holding was described using the CO concentrations of both expiratory gas volumes, assuming an exponential decrease in the decay of CO concentration:

$$FA_{CO(t)} = FA_{CO(0)} \times exp\,(-DL_{CO}\, P_b\, t\, /V_A) \qquad (2)$$

where $FA_{CO(t)}$ and $FA_{CO(0)}$ are alveolar CO concentrations at time t (i.e. Krogh's final sample) and at time 0 (i.e. Krogh's initial sample) respectively; P_b is barometric pressure minus 47 mmHg (= saturated water vapour pressure at 37°C); t is time in seconds between delivery of the two gas samples; and V_A is total alveolar gas volume in ml STPD. The actual alveolar volume was estimated by adding the inspired volume, derived from a spirogram, to an independent measure of the residual volume which was derived with use of a multiple-breath dilution technique. Krogh assumed that: 1) the CO concentrations in the first and second sample were representative of all alveolar gas, and 2) CO tension in the pulmonary capillary plasma was negligible. The use of two samples taken at different degrees of expiration could lead to errors, since differently ventilated alveoli may contribute in varying proportions to the expired air throughout expiration.

The method was modified by Forster *et al.* [12] and Ogilvie *et al.* [18]. By adding helium (He) to the test gas as a means of estimating the initial CO concentration ($FA_{CO(0)}$) in the lungs:

$$\frac{FA_{He}}{FI_{He}} = \frac{FA_{CO(0)}}{FA_{CO(t)}} \qquad (3)$$

where FI_{He} and FI_{CO} are inspiratory He and CO concentration respectively, the initial sample of Krogh became superfluous. In the calculations, they assumed that He was (practically) insoluble in blood and tissue; He and CO diluted in a comparable way; and that CO was not absorbed by blood or tissue to any significant extent until the inspired and residual gases were mixed.

In both methods, the inhalation of test gas, expiration of alveolar gas and the collection of the alveolar sample were assumed to be instantaneous. Jones and Meade [16] showed that this assumption led to errors in the estimation of DL_{CO} due to the uptake of CO during inhalation and expiration as the diffusion equation was only valid for breath-holding. They introduced some alterations in the calculation of DL_{CO} that would minimize these errors: 1) CO uptake was considered to begin after three-tenths of the inhalation time to correct for the finite time of inhalation, and 2) a small alveolar gas sample was collected immediately following wash out of the dead space to minimize errors during expiration. Since then, variations of the Jones and Meade technique have been described, with alterations in the size and timing of alveolar samples [3,6,9].

Wagner *et al.* [26] continuously measured CO during expiration and by doing so, they were able to show that delaying the time of sample collection caused DL_{CO} to increase. However, when mean alveolar volume was taken into account, the calculated DL_{CO} became approximately constant. This was confirmed by results of Cotton *et al.* [8]. They showed that when the times of breath-holding were calculated according to the postulate that gas entering the lung first exits last, and when a separate time-weighted mean alveolar volume (instead of a total alveolar volume) was calculated, DL_{CO} no longer increased when measured from gas sampled later in expiration.

In all these (conventional) methods, DL_{CO} is calculated from a single equation (see equation 2) which is essentially only accurate for the breath-holding period but is applied to a manoeuvre that includes inhalation and expiration in addition to breath-holding. These methods rely on strict standardization procedures to minimize errors, i.e. short inspiratory and expiratory times relative to breath-hold time. It is therefore not surprising that since the introduction of the single-breath technique for measurement of DL_{CO}, several recommendations for standardization have been published [3,7,9]. Instead of being dependent on strict standardization of the manoeuvre and the need of corrections, Graham *et al.* [13,14] developed a new method of calculating DL_{CO}. This method was based on three differential equations to

describe the diffusion in the lung, one for each phase of the single-breath manoeuvre, i.e. inhalation, breath-holding and expiration. Values for $D_{L_{CO}}$ were obtained which were minimally affected by variations in the pattern of the single-breath manoeuvre and in size and timing of the alveolar gas sample [14].

Multiple-breath Techniques

REBREATHING TECHNIQUE - The subject is rebreathing for about 15-30 seconds from a bag containing a gas mixture of air, He and a low concentration of CO. Usually, rebreathing volumes are large, about vital capacity (i.e. maximal volume which can be inspired after maximal expiration) and respiratory rate is high, about 30 per minute. The rebreathing promotes thorough mixing of gas from the bag with alveolar gas, thereby probably diminishing alveolar gas inhomogeneities. Lung volume is derived from the He dilution. $D_{L_{CO}}$ can be calculated in a comparable way as in the single-breath technique using either the slope of the logarithmic decay in CO concentration of the bag versus time or the initial and final bag CO concentrations.

STEADY-STATE TECHNIQUE - The subject is breathing from a gas tank containing a gas mixture of air and a low concentration of CO. During the test period, mixed expired gas is monitored until a steady-state is reached. $D_{L_{CO}}$ is then calculated as CO uptake derived from inspired and expired amount of CO, divided by alveolar CO tension. The latter varies throughout the respiratory cycle and cannot be determined directly. Two methods, introduced by respectively Filley *et al.* [10] and Bates *et al.* [4], have been used to estimate mean alveolar CO tension. Filley used a modification of the alveolar gas equation to derive mean alveolar CO tension, whereas Bates assumed mean alveolar CO tension to be equal to end-tidal CO tension.

In both multiple-breath techniques, $D_{L_{CO}}$ is estimated at a changing lung volume between end-expiratory lung volume and the sum of end-expiratory lung volume and tidal volume. Usually, the end-expiratory lung volume plus half of the tidal volume is used in the calculations. Although the CO load of both techniques is (relatively) larger than for the single-breath technique, the CO load of the steady-state technique will be considerable. During this procedure, the subject is inspiring a constant CO concentration for several minutes. The CO tension in blood will gradually increase, thereby violating the assumption that the CO tension in the pulmonary capillary bed is zero.

PULMONARY CAPILLARY BLOOD VOLUME AND MEMBRANE DIFFUSING CAPACITY

DL_{CO} is the rate of passage of CO (i.e. flow) divided by the partial pressure difference. The resistance to this passage of gas would be the pressure difference divided by the rate of flow. Thus, the resistance to the diffusion of CO is $1/DL_{CO}$. Roughton and Forster have shown that the resistance of the lungs to diffusion of carbon monoxide equals the sum of two resistances connected in series: 1) the resistance of the alveolar-capillary membrane to diffusion of CO, i.e. from alveolar gas to the surface of the red cells ($1/D_M$), and 2) the resistance to passage of CO across the red cell membrane into its interior and chemical binding to haemoglobin ($1/\theta_{CO} \cdot 1/Q_c$) [20].

$$\frac{1}{DL_{CO}} = \frac{1}{D_M} + \frac{1}{\theta_{CO} \times Q_c} \qquad (4)$$

The rate of uptake of CO by haemoglobin in the red cells is symbolized by θ_{CO} and may be calculated from the relationship of $1/\theta_{CO}$ versus mean capillary O_2 tension [20].

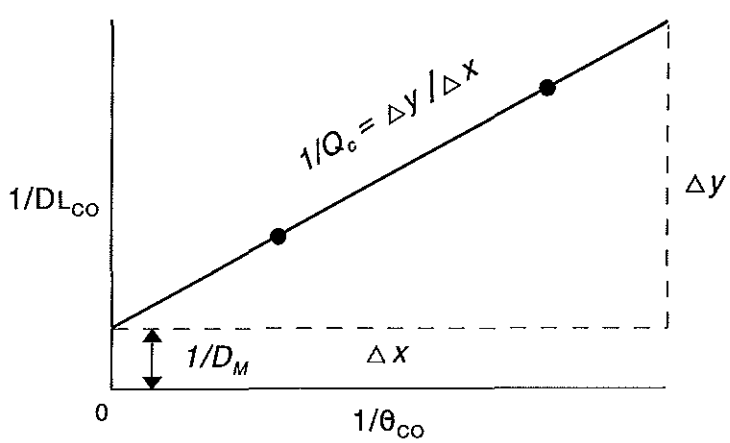


FIGURE 1. Diagram to show the relationship between the reciprocal of carbon monoxide transfer of the lungs ($1/DL_{CO}$) and the reciprocal of the rate of uptake of carbon monoxide by haemoglobin in the red cells ($1/\theta_{CO}$). From this relationship, the diffusing capacity of the pulmonary membrane (D_M) and capillary blood volume (Q_c) can be derived.

The total pulmonary diffusing capacity for CO decreases with respect to air breathing when high concentrations of O_2 are inhaled. This is because CO molecules have to compete with O_2 molecules for binding sites of haemoglobin. Although the affinity of haemoglobin for CO is much greater than for O_2 (225 times greater in human [1]), the rate of combination of CO with reduced (i.e. all binding sites free) haemoglobin is slower than for O_2 [11]. The reaction is slower still when O_2 is displaced from oxyhaemoglobin by CO. Thus the reaction rate of CO with haemoglobin (θ_{CO}) is reduced when the oxygen saturation of haemoglobin is high. When repeating measurements of DL_{CO} at different values of θ_{CO} (obtained by inhaling different concentrations of O_2 and so varying O_2 saturation of haemoglobin), D_M and Q_c can be solved mathematically or graphically by plotting $1/DL_{CO}$ versus $1/\theta_{CO}$ (FIGURE 1). The intercept of the line represents $1/D_M$ and the slope $1/Q_c$. Since D_M is derived by extrapolation of a line, it will be more subject to inaccuracy than Q_c. θ_{CO} normally is expressed for whole blood with a O_2 capacity of 0.2 ml per ml blood, i.e. haemoglobin concentration of 14.4 $g.dl^{-1}$ (=8.9 $mmol.L^{-1}$). Therefore, when measurements of DL_{CO} are obtained at different haemoglobin concentrations, θ_{CO} has to be adjusted to the actual concentration by multiplying it with $Hb_{actual}/14.4$.

MECHANICAL VENTILATION AND SPONTANEOUS BREATHING

During ventilation, the volume changes of the lungs are entirely passive and controlled by forces external to the lungs. During spontaneous breathing, the external forces are delivered by the respiratory muscles, whereas during mechanical ventilation a pressure difference is created between alveolar and ambient air by means of a pump (ventilator). The lungs always tend to recoil to its deflated volume. In contrast, the thoracic cage tends to expand to a volume somewhat larger than Functional Residual Capacity. FRC is defined as the lung volume at end-expiration during normal breathing in rest conditions while respiratory muscle forces are zero. Then the inward elastic recoil forces of the lungs, expressed in terms of recoil pressure (P_L), are balanced by the outward recoil pressure of the thoracic cage (P_C).

The effect of lung inflation by spontaneous breathing on intrathoracic (or 'intrapleural') pressure is different to that by mechanical ventilation. FIGURE 2A represents the situation at FRC during spontaneous breathing, and also at end-

expiratory volume during mechanical ventilation with muscle paralysis and zero end-expiratory pressure. The recoil pressure of the lungs (P_L) and the thorax (P_C) are balanced. The intrathoracic pressure (P_{it}) is equal to $-P_L$ as well as to $-P_C$ when alveolar pressure is equal to ambient air pressure and the absolute value of ambient air pressure is reset to zero.

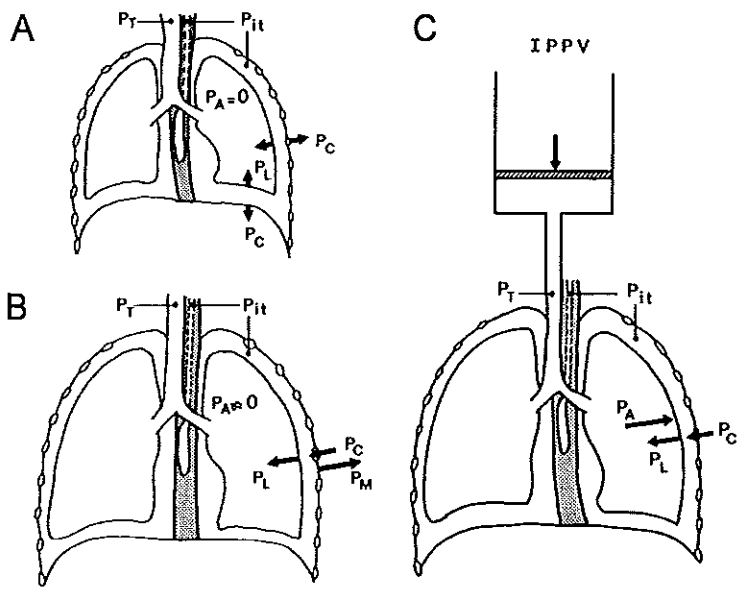


FIGURE 2. Determinants of intrathoracic pressure during spontaneous breathing (A and B) and mechanical ventilation (C). P_A alveolar pressure; P_T tracheal pressure; P_{it} intrathoracic pressure; P_L pressure of the recoil forces of the lungs; P_C pressure of the recoil forces of the thoracic cage; P_M pressure delivered by respiratory muscles, IPPV intermittent-positive-pressure ventilation.

A. At end-expiratory (FRC) level, P_A is equal to ambient air pressure and the recoil pressures of lungs and thorax are balanced: $P_{it} = -P_L = -P_C$ when P_A is reset to zero.

B. After a deep inspiration when P_A is equal to ambient air pressure, P_M balances recoil pressures of lungs and thorax which are now both directed towards expiration: $P_{it} = -P_L = +P_C - P_M$

C. After a deep inflation during mechanical ventilation, when $P_M=0$, an intrapulmonary pressure (P_A) is needed to balance both recoil pressures of lungs and thorax: $P_{it} = -P_L + P_A = +P_c$

(This figure was obtained from Versprille [24])

A deep (spontaneous) inspiration (shown in FIGURE 2B) will increase lung recoil pressure. During inspiration, thoracic recoil pressure will first decrease and when inspiration continues it will increase in the opposite direction. Thus at the end of inspiration, P_L will exert a negative and P_C a positive pressure on the intrathoracic structures, thereby enhancing expiration. To prevent the lungs from expiration, a counteracting pressure is needed which is delivered by the inspiratory muscles (P_M). Then, the intrathoracic pressure will become more negative with regard to end-expiration and equals $-P_L$ as well as $-P_M+P_C$. Thus during spontaneous breathing, the intrathoracic pressure is negative and depends on the recoil pressure of the lungs.

In FIGURE 2C, a similar situation is created as in FIGURE 2B, but now lung inflation is established by the ventilator (intermittent-positive-pressure ventilation). For balance of the recoil pressures of both the lungs and the thorax, another force than from the inspiratory muscles which are paralysed is needed. This force is the alveolar (or intrapulmonary) pressure, P_A, which is measured as tracheal pressure, P_T. Then the intrathoracic pressure equals $+P_A-P_L$ as well as $+P_C$. During mechanical ventilation and muscle paralysis, intrathoracic pressure becomes positive and depends on the thoracic recoil pressure, which in turn depend on lung (i.e. thoracic) volume. Thus intrathoracic pressure does not depend on alveolar pressure and the recoil pressure of the lungs, because if P_L increases, P_A increases as much. When the recoil pressure of the lungs increase (e.g. in the respiratory distress syndrome by stiffening of the lungs) and thoracic volume is kept constant, the intrapulmonary pressure will increase to the same amount, whereas intrathoracic pressure will be unchanged. However, stiffening of the lungs could cause intrathoracic pressure to be changed indirectly, namely via a change in lung volume which will be lower.

The rise in intrathoracic pressure during forced inflation (mechanical ventilation) causes central venous pressure to increase. This increase in central venous pressure is smaller than the increase in intrathoracic pressure, implying a fall in filling (transmural) pressure in the central veins and right atrium [25]. The rise in central venous pressure causes a fall in venous return and right ventricular output. The left ventricular output follows the fall in output at the right side with a delay of several beats. Thus during inflation, the input into the pulmonary circulation (right ventricular output) is smaller than the output from it (left ventricular output), resulting in a decrease in total pulmonary blood volume which recovers during expiration. Furthermore, increasing inflation volume has been associated with a larger decrease in

pulmonary blood volume [25]. As stated above, during spontaneous breathing changes in intrathoracic pressure are different. Lung inflation will result in a more negative intrathoracic pressure thereby enhancing venous return and cardiac output [15].

Mechanical ventilation has been applied for improvement of gas exchange. During mechanical ventilation, two types of intrapulmonary pressure are usually imposed on the lungs and the intrathoracic structures, causing different effects on the systemic and pulmonary circulation. Besides the inevitable dynamic change in pressure within each ventilatory cycle, which has been discussed above, often a static type of positive end-expiratory pressure (PEEP) is applied.

Positive end-expiratory pressure increases end-expiratory volume by preventing the collapse of terminal airspaces during expiration and by alveolar recruitment [19], which may improve arterial oxygenation in patients with the respiratory distress syndrome [2,19]. However, when PEEP increases, cardiac output decreases [5,21,22]. This negative effect of PEEP on cardiac output counteracts its beneficial effect on arterial oxygenation.

OUTLINE OF THESIS

For determination of carbon monoxide transfer in the lungs, a single-breath technique was used. The main reason for choosing this technique is that we were interested in changes in capillary blood volume during inflation and that using this technique different alveolar volumes could be applied easily. This is much more complicated to do so with use of the multiple-breath techniques as large changes in lung volume occur during the procedure. Since measurements were obtained in healthy pigs and thus ventilation-perfusion inhomogeneities were assumed to be minimal, the rebreathing technique was also not regarded superior to the single-breath technique. Furthermore, the calculation of CO transfer is more complex for the rebreathing than for the single-breath technique. Because of its much larger CO load, the steady-state method was not considered for use.

The single-breath technique, as used during spontaneous breathing, was adapted for use in mechanically ventilated subjects and called the inspiratory pause procedure. This is decribed in *CHAPTER 2*. Data were obtained in anaesthetized and paralysed healthy pigs. Firstly, the effect of inspiratory pause time was studied; this was done to evaluate

the decay of alveolar carbon monoxide fraction with time which was assumed to be exponential. Secondly, DL_{CO} was studied at different alveolar volumes by changing either PEEP (2 and 10 cmH_2O) or inflation volume (15, 20, 25 and 30 $ml.kg^{-1}$).

For derivation of the capillary blood volume, we rely on estimates of the diffusing capacity and theta (the rate of uptake of carbon monoxide by haemoglobin in red cells). Theta depends on oxygen tension. The relationship between the two variables, theta and O_2 tension, was not known for pig blood. As there may be differences between species, this relationship was studied in pig blood of which the results are presented in *CHAPTER 3*. Theta was obtained with use of a continuous-flow rapid-mixing apparatus on diluted pig blood at different oxygen pressures.

In *CHAPTER 4* capillary blood volume and membrane conductance were derived by obtaining estimates for the carbon monoxide transfer at three different values of theta which was done by ventilating with 30, 60 and 94% oxygen. Theta was derived using the equation given in chapter 3. The effect of changing alveolar volume, by inflating 15, 20, 25 and 30 ml per kg body weight, on capillary blood volume was studied.

In an additional study, presented in *CHAPTER 5*, the relationship between changes in capillary blood volume and total pulmonary blood volume was studied, as lung inflation may affect both blood volumes differently. In addition to capillary blood volume estimates, which were derived as described in chapter 4 using the Roughton-Forster equation, independent estimates of total pulmonary blood volume were obtained within the same pig, both at the same inflation volumes (15, 20 and 25 ml per kg body weight). Total pulmonary blood volume was estimated with use of a double-indicator-dilution technique using heat and conductivity as indicators.

Finally in *CHAPTER 6*, problems encountered in the determination and interpretation of carbon monoxide transfer are considered. Furthermore, the pig as a model in CO transfer studies is discussed and some suggestions for future research are presented.

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"Was jedermann für ausgemacht hält, verdient am meisten untersucht zu werden."

- Wat iedereen voor uitgemaakte zaak houdt, verdient het allermeest onderzocht te worden. -

LICHTENBERG

CHAPTER 2

TRANSFER OF CARBON MONOXIDE DURING AN INSPIRATORY PAUSE PROCEDURE IN MECHANICALLY VENTILATED PIGS

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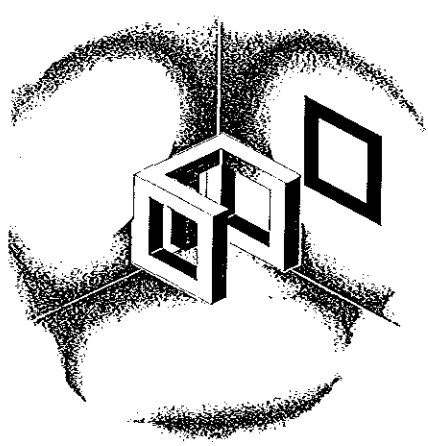

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INTRODUCTION

The pulmonary diffusing capacity (DL_{CO}) has been extensively studied in spontaneously breathing humans with use of the single-breath (SB) technique [4,8,15,18]. In normal humans, an increase in DL_{CO} has been associated with expansion of the lungs [18].

During mechanical ventilation, lung inflation causes an increase in intrathoracic pressure, which impedes venous return and consequently cardiac output. Because of phase differences between decreases in inflow and outflow of the pulmonary circulation, a decrease in pulmonary blood volume will occur [20]. These changes are the opposite of those during spontaneous inhalation and may affect gas transfer differently. Therefore, we questioned whether the relationship between DL_{CO} and alveolar lung volume (V_A) as has been found during spontaneous breathing will also hold during mechanical ventilation.

The effect of increasing alveolar volume on DL_{CO} has hardly systematically been studied in anaesthetized, paralysed and mechanically ventilated humans or animals. Some authors, however, mentioned the possible effect of forced inflation itself on DL_{CO} by the increase in intrathoracic pressure [14,17]. In just a few studies, the main objective was to assess DL_{CO} under conditions of mechanical ventilation [2,6,13]. Comparison of the results from different studies is not simple, not only because of differences in measuring technique and calculation procedure, but also because of variations in the applied manoeuvre.

The objectives of this study were to 1) evaluate a standardized, computer-controlled single-breath technique for measurement of DL_{CO} and 2) analyse the effect of increasing alveolar volume on DL_{CO} during mechanical ventilation in anaesthetized and paralysed healthy pigs.

METHODS

Surgical Procedures

All experiments were performed according to the "Guide for Care and Use of Laboratory Animals" published by the US National Institutes of Health [NIH publication 85-23, revised 1985] and approved by the Animal Care Committee of the Erasmus University Rotterdam, the Netherlands.

Fourteen pigs (mean *(SD)* body weight 11.7 *(1.0)* kg) were anaesthetized with an intraperitoneal injection of pentobarbital sodium (30 $mg.kg^{-1}$), followed by a continuous intravenous infusion of 8.5 $mg.kg^{-1}.h^{-1}$. They were placed in supine position on a thermo-controlled operation table to maintain a body temperature of about 38°C. After tracheostomy, they were connected to a computer-controlled ventilator.

A catheter was inserted via the right common carotid artery into the aortic arch for measurement of arterial blood pressure and blood sampling. A Swan-Ganz catheter was placed into the left pulmonary artery via the right external jugular vein to measure pulmonary arterial blood pressure and core temperature and sample blood. Sampled blood was replaced by saline. A four lumen catheter, which was also inserted through the right external jugular vein, was used to measure central venous pressure and infuse pentobarbital and, after surgery, tubocurarine (0.2 $mg.kg^{-1}.h^{-1}$). The pressure catheters were continuously flushed at a rate of 3 $ml.h^{-1}$ with saline containing a low dose of heparin (10 I.U. per ml) to avoid clotting.

Experimental Procedures

The pigs received volume-controlled ventilation after tracheostomy. The ventilatory frequency was set at 10 breaths per minute (inspiratory vs. expiratory ratio 40:60), and a positive end-expiratory pressure (PEEP) of 2 cmH_2O was applied. Tidal volume was adjusted to an arterial CO_2 tension of 5.1-5.6 kPa and was kept constant throughout the experiment. The animals were ventilated with room air or a gas mixture containing 30% O_2 in N_2. Normal mechanical ventilation and the procedures for DL_{CO} determination were performed with a microcomputer-controlled ventilator [9] (FIGURE 1). DL_{CO} was determined with use of an inspiratory pause (IP) procedure to simulate the standard single-breath technique. This IP procedure consisted of an inflation, an inspiratory pause and an expiration (FIGURE 2). The flow rates of both inflation and expiration were constant. Two series of experiments were performed.

STUDY 1 - To evaluate if the decay of carbon monoxide with time was exponential after forced inflation, which is a precondition for application of the conventional formula to calculate DL_{CO} [1], CO transfer was measured after different IP times in a group of five pigs at two levels of end-expiratory V_A by imposing a PEEP of 2 or 10 cmH_2O (hereafter referred to as $PEEP_2$ and $PEEP_{10}$ respectively). In both conditions a volume of 25 $ml.kg^{-1}$ of the test gas was administered in 2.4 seconds. The IP times were 1, 2, 4, 6 and 8 seconds at $PEEP_2$ and 1, 2, 3, and 6 seconds at $PEEP_{10}$ and were

randomly imposed. Expiration lasted 3.6 seconds. The test gas mixture contained approximately 0.3% labelled carbon monoxide ($C^{18}O$), 10% helium (He) and balance air. The IP procedures were performed at intervals of at least 5 minutes for washing out of test gases and regaining haemodynamic stability.

STUDY 2 - The goal was to study the effect of V_A on DL_{CO} and DL_{CO}/V_A. This was done in another group of nine pigs by inflating the lungs with 15, 20, 25 or 30 $ml.kg^{-1}$ of the test gas at a constant PEEP of 2 cmH_2O. Inflation and expiration times were both 2.4 seconds and IP time was set at 7.2 seconds. A mixture of approximately 0.3% $C^{18}O$, 5% He, 30% O_2 and balance N_2 was used for the IP procedures. The inflation volumes were randomly applied at intervals of at least 5 minutes.

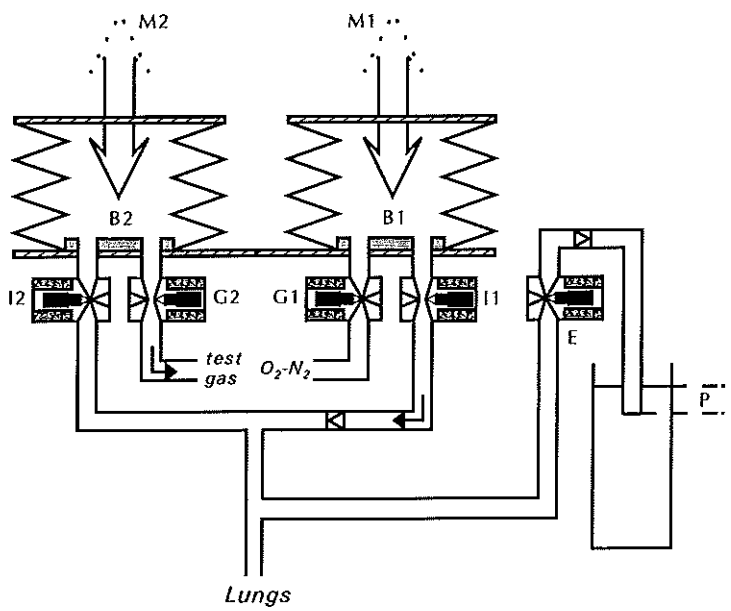

FIGURE 1. Diagram of the pump assembly used for continuous ventilation and inspiratory pause (IP) procedures

B1 and B2, concertina bellows used for continuous ventilation and IP procedures respectively; M1 and M2, motors for moving bellows forward and backward; I1 and I2, inspiratory solenoid valves; E, expiratory solenoid valve; G1 and G2, inlet solenoid valves for supply of gas mixtures, i.e. O_2-N_2 mixture and $C^{18}O$ test gas mixture respectively; P, positive end-expiratory pressure valve; ⋈ uni-directional valves. *Continuous ventilation*: during inflation I1 and G2 are open (shown in diagram); during expiration E, G1 and G2 are open. IP procedures: during inflation, inspiratory pause and controlled expiration I2 and G1 are open.

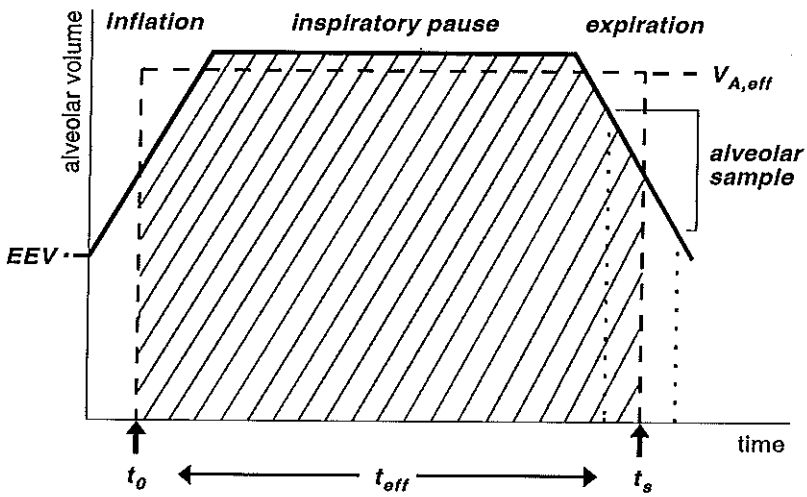

FIGURE 2. IP procedure to determine DL_{CO}
Alveolar volume is plotted on the Y-axis against time on the X-axis, both in arbitrary units, for a representative IP procedure. t_s is the time at the midpoint of the alveolar sample and t_0 is the time at the corresponding volume level during inflation. For each determination we assumed that $C^{18}O$ was taken up by the blood from time t_0 to t_s (= effective time, t_{eff}) and from an alveolar volume equal to the shaded area under the volume-time plot divided by t_{eff} (= effective or time-weighted mean alveolar volume, $V_{A,eff}$).

Measured and Calculated Data

Arterial and mixed-venous blood gas tensions for O_2 and CO_2 and haemoglobin concentration were determined with an automatic blood gas system (ABL510, Radiometer, Copenhagen, Denmark). Oxyhaemoglobin (HbO_2) and carboxy-haemoglobin (HbCO) were determined with a haemoximeter (OSM3, Radiometer, Copenhagen, Denmark). Blood pressures and tracheal pressure were monitored to control steady-state conditions. ECG as well as blood pressures were sampled by a computer at 250 Hz prior to and after a series of IP procedures for calculation of baseline values of heart rate and blood pressures, averaged over one ventilatory cycle, and to check stability throughout the series. For the same reason, cardiac output was estimated with use of the direct Fick method for oxygen.

Gas flow rates were measured with a Godart-Statham pneumotachograph (Fleisch no.0, Switzerland) attached to a differential pressure transducer. Volume changes during inflation and expiration were obtained by integration of the flow signal. Gas fractions were continuously measured during the IP procedures with a mass spectrometer (Perkin-Elmer, MGA 1100, Pomona, California USA) which sampled gas at 1 $ml.s^{-1}$. A correction was made for the delay time of 280 ms of the mass spectrometer relative to the flow signal. For each IP procedure the end-expiratory alveolar volume was calculated from the He-mass balance. In the calculation, both the Fowler respiratory dead space for CO_2 [7] and the apparatus dead space were taken into account.

During the IP procedures signals of flow and gas fractions were sampled by a computer at a rate of 250 Hz. The recorded $C^{18}O$ and He fractions were displayed against expiratory volume. Dead space wash-out volume was determined by indicating the start of the alveolar plateau by eye. The $C^{18}O$ and He fractions of the expired gas volume excluding the dead space wash out volume and the last part of the expired gas were averaged. We will refer to this expired gas volume as alveolar sample. An effective diffusion time (t_{eff}) and an effective alveolar volume ($V_{A,eff}$) from which $C^{18}O$ was taken up were estimated in a similar way as has been described by Cotton *et al.* [4] for single-breath measurements (FIGURE 2).

Analysis

DL_{CO} was calculated using the following formula:

$$DL_{CO} = ln\left(\frac{FA_{C^{18}O(t_0)}}{FA_{C^{18}O(t_s)}}\right) \times \frac{1}{t_{eff}} \times \frac{V_{A,eff}}{22.4 \times 10^{-3} \times P_A}$$

where DL_{CO} is the transfer factor of $C^{18}O$ in $\mu mol.s^{-1}.kPa^{-1}$; $FA_{C^{18}O}(t_0)$ is the alveolar $C^{18}O$ fraction at the start of $C^{18}O$ uptake estimated from He dilution; $FA_{C^{18}O}(t_s)$ is the mean $C^{18}O$ fraction of the alveolar sample; t_{eff} is in seconds; $V_{A,eff}$ is in L STPD; $22.4x10^{-6}$ is the volume in ml STPD of 1 µmol of gas; P_A is the total dry pressure in kPa in the alveolar gas estimated as $P_B+P_T-P_{H2O,s}$ where P_B is the barometric pressure, P_T tracheal pressure and $P_{H2O,s}$ saturated water vapour pressure at the prevailing body temperature. DL_{CO}/V_A is expressed in $\mu mol.s^{-1}.kPa^{-1}.L^{-1}$, where V_A is in litres BTPS.

Carbon Monoxide Back Pressure

Back pressure was estimated prior and after a series of IP procedures using the Haldane relationship [5]. For this purpose, HbCO, HbO_2 and oxygen tension were determined in an arterial blood sample. The Haldane equation's constant M reported for pig blood is 130 [12]. A linear interpolation between the back pressure before and after a series of IP procedures was performed. Back pressure was converted to fraction and subtracted from both alveolar CO fractions at time t_0 and t_s.

Statistical Analysis

The Student's *t*-test for paired samples was used to compare haemodynamic and oxygenation values prior to and after each series of IP procedures, and to compare CO transfer values obtained at $PEEP_2$ with those at $PEEP_{10}$. Multiple regression analysis was used to look at the variation, *within* pigs, in CO transfer indices with increasing t_{eff} (study 1), and with increasing V_A (study 2). Pig was treated as a categorical variable using dummy variables. Partial regression coefficients with 95% confidence intervals (95%CI) are given. The statistical analysis was performed with the Statistical Package for Social Sciences 4.0; differences were considered statistically significant when $p<0.05$.

RESULTS

Baseline Haemodynamic and Oxygenation Variables

Baseline haemodynamic and oxygenation variables did not show significant differences between values obtained prior to and after each series of IP procedures (TABLE 1), except for a moderate decrease in cardiac output (0.16 *(0.13)* $ml.s^{-1}.kg^{-1}$; mean *(SD)*) in study 2.

In study 1, aortic pressure, heart rate and cardiac output were lower and central venous and pulmonary artery pressure were higher at $PEEP_{10}$ compared to $PEEP_2$ ($p<0.05$). At $PEEP_{10}$, also slight increases in arterial oxygen pressure and saturation were found.

TABLE 1. Haemodynamic and oxygenation values prior to and after a series of IP procedures for both studies

	STUDY 1 (n=5)				STUDY 2 (n=9)	
	$PEEP_2$ series		$PEEP_{10}$ series			
	prior	after	prior	after	prior	after
P_{ao} (mmHg)	98 (12)	102 (11)	76 (10)	81 (8)	91 (6)	93 (12)
P_{pa} (mmHg)	17.5 (2.2)	16.8 (3.0)	19.3 (2.6)	20.5 (2.3)	13.7 (2.2)	13.7 (2.2)
P_{cv} (mmHg)	2.7 (0.8)	2.7 (0.8)	5.5 (0.8)	5.6 (0.9)	2.0 (0.6)	2.1 (0.6)
HR (min^{-1})	188 (24)	186 (25)	169 (45)	144 (20)	146 (25)	151 (31)
Q' ($ml.s^{-1}.kg^{-1}$)	2.19 (0.57)	2.42† (0.69)	1.37 (0.43)	1.48† (0.21)	2.29 (0.43)	2.13‡ (0.35)
P_{aO2} (kPa)	11.8 (1.2)	12.0 (1.1)	12.9 (2.1)	12.7 (1.1)	19.6 (1.3)	19.7 (0.7)
S_{aO2} (%)	95.1 (1.8)	95.9 (1.4)	96.3 (2.3)	96.4 (1.1)	99.2 (0.5)	99.3 (0.4)

P_{ao} aortic pressure; **P_{pa}** pulmonary artery pressure; **P_{cv}** central venous pressure; **HR** heart rate; **Q'** cardiac output; **P_{aO2}** arterial oxygen pressure; **S_{aO2}** arterial oxygen saturation. Values are means (*SD*); † n=3; ‡ $p<0.05$ prior vs after, paired *t*-test

Study 1

EFFECT OF INCREASING IP TIME - Using multiple regression analysis, the model assuming an exponential decay with increasing t_{eff} fitted well (proportion of variance explained, i.e. R square, was 0.98 and 0.93 for $PEEP_2$ and $PEEP_{10}$ respectively). The constant, i.e. intercept, was not significantly different from zero, i.e. ln(1) corresponded with $t_{eff}=0$ ($p=0.21$ and 0.17 for $PEEP_2$ and $PEEP_{10}$ respectively). Therefore individual regression equations of the form $\ln[FA_{c^{18}O}(t_s)/FA_{c^{18}O}(t_0)] = -a \times t_{eff}$ were derived which are presented in FIGURE 3. After removal of the variance due to pigs, the mean exponential decrease in alveolar CO fraction per second of t_{eff} was respectively 0.110 (95%CI: 0.102-0.118) at $PEEP_2$ and 0.077 (95%CI: 0.062-0.092) at $PEEP_{10}$.

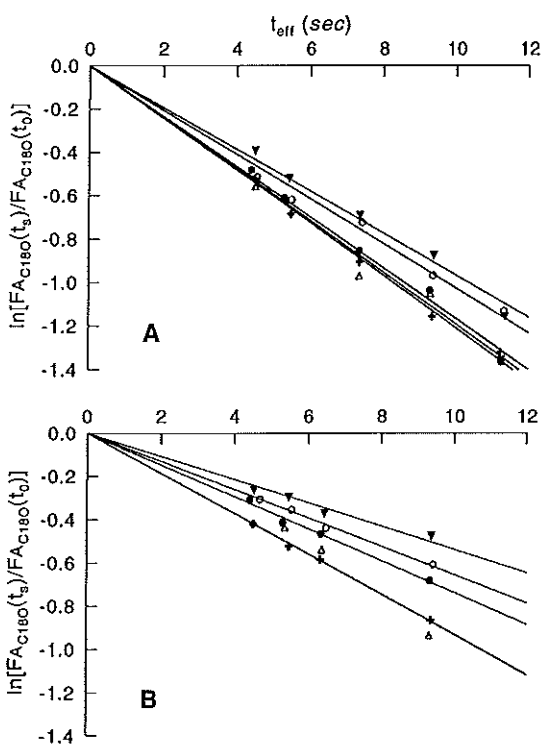

FIGURE 3. Decay of alveolar carbon monoxide fraction with effective time
The natural logarithmic decrease of the alveolar CO fraction is plotted on the Y-axis against the effective time of CO uptake on the X-axis for $PEEP_2$ (**A**) and $PEEP_{10}$ (**B**). Each symbol represents measurements in one pig. Individual linear regression lines through the origin are shown.

DL_{CO}/V_A did not change with increasing t_{eff} at both $PEEP_2$ and $PEEP_{10}$ ($p=0.80$ and 0.76 respectively). A slight increase was found for DL_{CO} with increasing t_{eff}, which was, after removal of the variation due to pigs, on average 0.54 (95%CI: 0.34-0.74) $\mu mol.s^{-1}.kPa^{-1}$ at $PEEP_2$ and 0.66 (95%CI: 0.34-0.97) $\mu mol.s^{-1}.kPa^{-1}$ at $PEEP_{10}$ for each second increase in t_{eff}. Further analysis with use of the Bonferroni *t*-test showed that DL_{CO} was significantly lower for procedures with an IP $\leq$ 2 seconds than for procedures with an IP > 2 seconds, the latter yielding constant DL_{CO} values.

EFFECT OF V_A - To test the effect of an increase in V_A by increasing PEEP on CO transfer, only values obtained after an IP > 2 seconds were used and averaged. DL_{CO}/V_A was larger at $PEEP_2$ than at $PEEP_{10}$ ($p<0.001$), whereas DL_{CO} did not change ($p=0.55$) (FIGURE 4).

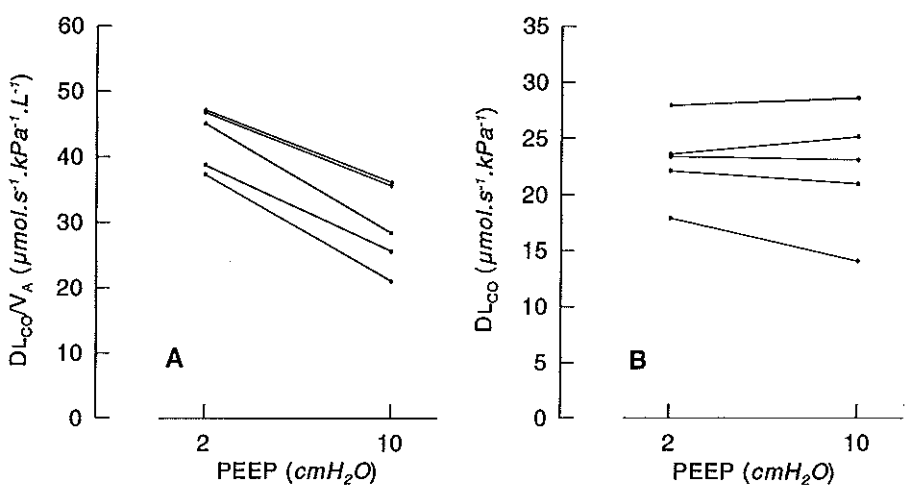

FIGURE 4. DL_{CO}/V_A and DL_{CO} at $PEEP_2$ compared with values at $PEEP_{10}$
DL_{CO} per L BTPS alveolar volume (**A**) and DL_{CO} (**B**) are plotted vertically for $PEEP_2$ and $PEEP_{10}$. Each point represents the individual average of values obtained after IP times > 2 seconds. Each individual's responses are connected by straight lines (n=5). * $p<0.001$, compared to $PEEP_2$

Study 2

EFFECT OF V_A - With increasing inflation volumes, i.e. increasing V_A, DL_{CO}/V_A linearly decreased and DL_{CO} did not change significantly (FIGURE 5).

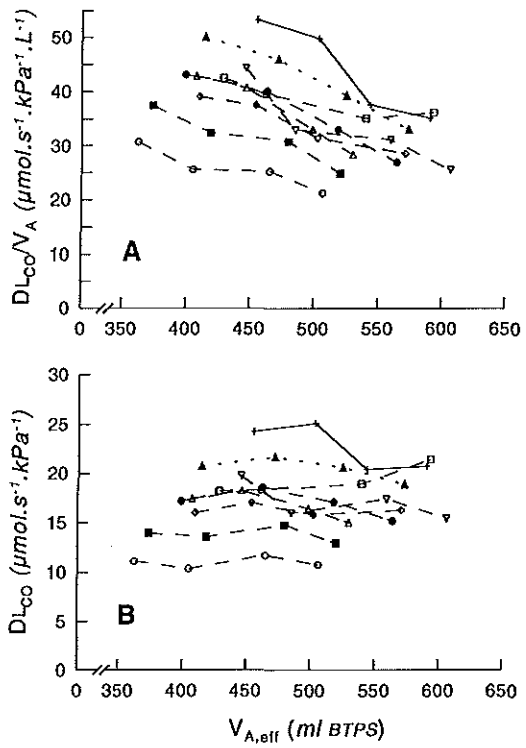

FIGURE 5. *DL_{CO}/V_A and DL_{CO} versus $V_{A,eff}$*
DL_{CO} per L BTPS alveolar volume (**A**) and DL_{CO} (**B**) are plotted on the Y-axis against $V_{A,eff}$ in ml BTPS on the X-axis. Each individual's responses are connected by straight lines (n=9). Adjusted for differences between pigs, DL_{CO}/V_A decreased on average 88 (95%CI: 72-105) µmol.s^{-1}.kPa^{-1}.L^{-1} for each L BTPS increase in V_{Aeff}. DL_{CO} decreased on average 7.7 (95%CI: -0.4-15.7) µmol.s^{-1}.kPa^{-1} for each L BTPS increase in $V_{A,eff}$, which was just not significant.

DISCUSSION

Haemodynamic and Oxygenation Variables

Baseline values throughout all series of observations were stable. Only in study 2, a slight decrease in cardiac output (7%) was found. We suppose that its effect on the DL_{CO} estimates was insignificant, since the order of IP procedures was randomized.

The differences in haemodynamic baseline values between $PEEP_2$ and $PEEP_{10}$ were as expected: an increase in central venous pressure and a decrease in cardiac output and aortic pressure.

IP Procedure

The IP procedure during mechanical ventilation can be regarded as the equivalent of the single-breath procedure during spontaneous breathing. For both procedures, DL_{CO} is calculated from a single equation which only holds for the breath-holding period. To minimize errors in DL_{CO} calculation, short inspiratory and expiratory times relative to breath-hold time are required. Graham *et al.* [8] described another approach of calculating DL_{CO}. They used separate equations for all three phases of the SB procedure (3-EQ method) rather than forcing the procedure to fit the breath-hold equation. With use of the 3-EQ method, they found that DL_{CO} estimated at total lung capacity did not change with variations in the pattern of the SB procedure. However, as long as the SB procedure was performed properly, the results obtained with the conventional methods were not different from those obtained with the 3-EQ method. The conventional method described by Jones and Meade [10] also yielded the same results if variations in breath-hold time, inhaled and exhaled flow rate occurred. However, if the collection of the sample during the expiration was delayed, an increase in the calculated DL_{CO} was found [8]. Cotton *et al.* [4] have shown that this marked increase in DL_{CO} by a delay in the sampling could be eliminated if appropriate corrections for time and volume of carbon monoxide uptake were made.

We used the averaged He and CO fractions of the largest possible alveolar sample to obtain an overall DL_{CO} for the whole lung. A small alveolar gas sample early or late in expiration would not have been representative of all lung compartments, because of a slightly declining alveolar plateau of helium during expiration. Kelly *et al.* [11] also found such sloping alveolar plateau in mechanically ventilated pigs, which appeared to be steeper than in dogs under similar conditions. A reason for this difference might be the absence of a collateral ventilation in pigs [21].

The advantage of the IP procedure is that the parameters can be completely controlled and standardized, and thus variations in the pattern of the procedure, as occurring during spontaneous breathing, are precluded and comparison between and within subjects is facilitated. Compared to the rebreathing procedure [2,13,14], the IP procedure is simpler and faster. Furthermore, with use of the IP procedure changes in

alveolar volume can be easily applied for study of its effect (imposed by mechanical ventilation) on DL_{CO}.

DECAY OF CO - It appeared to be appropriate to use the chosen effective time in the calculation of DL_{CO}/V_A, because the extrapolated uptake of carbon monoxide at time zero was zero, which it should be theoretically. Furthermore, the results presented in FIGURE 3 indicated that for IP procedures in healthy mechanically ventilated pigs the decay in alveolar carbon monoxide concentration was exponential, as has generally been accepted for SB procedures in spontaneously breathing humans [1].

Sikand and Piiper [17] found on the average a non-exponential fall of the alveolar carbon monoxide concentration as the time of apnoea was varied from 3 to 30 seconds in anaesthetized dogs. Unfortunately, they did not provide data on the decay of alveolar carbon monoxide concentration in individual animals. Nevertheless, the first part of the logarithmic carbon monoxide disappearance curve, from 0 to 12 seconds, seemed rather linear. As they stated in the discussion, the alveolar samples may have been contaminated by gas from the dead space resulting in an underestimation of carbon monoxide decay for the longer times of apnoea.

DL_{CO} - DL_{CO} remained constant using IP times > 2 seconds but was significantly lower for shorter IP times. Since helium was less diluted after IP times of 1 and 2 seconds as compared to longer IP times, a smaller maximal V_A was derived and this could explain the lower DL_{CO} values. Although for short IP times, the inflated gas was less well mixed with the alveolar gas, it appeared that the net CO uptake per litre alveolar volume (DL_{CO}/V_A) was constant and independent of IP time. Cotton *et al.* [4] also found reduced values for DL_{CO} in normal subjects after a 2-seconds breath hold which they attributed to stratification of inhaled and alveolar gas. Theoretically, a minimal time of 2.4-3.1 seconds is required for optimum mixing of inflated gas with alveolar gas [3]. In study 2, an IP time of 7.2 seconds was chosen resulting in an effective time of CO uptake of about 10 seconds, which is in accordance with the ATS recommendations for the single-breath technique [1].

Fisher and Hyde [6] found higher DL_{CO} values at shorter breath-hold times in anaesthetized, paralysed and mechanically ventilated dogs, which they ascribed to a non-exponential carbon monoxide decay. Another plausible explanation could be their use of the maximal alveolar volume during breath-hold in the calculation of DL_{CO} in stead of a time-weighted mean alveolar volume, i.e. the effective volume of which carbon monoxide is taken up. The maximal alveolar volume will be larger than the

time-weighted mean alveolar volume and their difference will increase with decreasing breath-hold time [4,8]. Therefore, DL_{CO} will be overestimated, and the more so at shorter breath-hold times.

Dependency of Carbon Monoxide Transfer on V_A

With increasing V_A, imposed by increasing either inflation volume or PEEP, DL_{CO}/V_A significantly decreased, whereas DL_{CO} did not change. A similar result was found by Meyer *et al.* [4], who measured DL_{CO} in anaesthetized, paralysed and mechanically ventilated dogs using a rebreathing technique. However, the effect of lung volume, changed by an increase in PEEP, on CO transfer was studied in only one dog. In the study of Macnaughton *et al.* [13], DL_{CO} was measured with a rebreathing technique during intermittent positive pressure ventilation in healthy volunteers who were neither anaesthetized nor paralysed. DL_{CO} was significantly lower at $PEEP_{10}$ compared to values at ZEEP ($=PEEP_0$). In spontaneously breathing subjects, the single-breath technique revealed an increase in DL_{CO} at increasing alveolar volume [18].

In contrast with a voluntary increase in lung volume (i.e. spontaneous inhalation), a forced increase in lung volume (i.e. forced inflation or application of PEEP) reduces cardiac output by an increase in intrathoracic pressure which impedes venous return. The increase in intrathoracic pressure during mechanical ventilation with muscle paralysis depends on the thoracic recoil pressures which depend on lung (i.e. thoracic) volume and is independent of intrapulmonary pressure [19]. Thus, if thoracic volume is kept constant, intrathoracic pressure will not change, even if intrapulmonary pressure increases by an increase in lung recoil forces (e.g. by stiffening of the lung).

Moreover, phase differences between the changes in inflow and outflow of the pulmonary circulation during forced inflation cause pulmonary blood volume to decrease [20]. We assume that the pulmonary capillary blood volume (Q_c) is involved in this decrease, which will affect gas transfer adversely as was already emphasized by Fisher *et al.* [6]. According to the Roughton-Forster relationship [16], the resistance of the lung to gas transfer ($1/DL_{CO}$) equals the sum of the resistances of the pulmonary membrane, i.e. $1/D_M$, and blood, i.e. $1/(\theta_{CO} \cdot Q_c)$, where θ_{CO} is the rate of uptake of carbon monoxide by red cells per unit blood volume. Theoretically, expansion of the lungs will result in an increase in the surface area which will be relatively smaller than the concomitant increase in lung volume. This is in line with the increase in the

diffusing capacity of the pulmonary membrane (D_M) with an increase in V_A and a decrease in D_M per litre V_A as was found by Stam *et al.* [18] in spontaneously breathing volunteers. If similar relationships hold during forced inflation, then the constant DL_{CO} with increasing V_A could be explained by an increasing D_M and a decreasing Q_c.

We conclude that 1) the inspiratory pause procedure is suitable for determination of carbon monoxide transfer in mechanically ventilated healthy subjects, because it is a well standardized method and the alveolar carbon monoxide decay is exponential. However, for optimal mixing of inflated gas with alveolar gas, an inspiratory pause time of at least 3 seconds is recommended; 2) since an increase in alveolar membrane diffusing capacity with increasing alveolar volume is expected, the constant DL_{CO} could be attributed to a concomitant decrease in pulmonary capillary blood volume.

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"Om bondig te schrijven,
moet men over veel woorden beschikken."

OTTO WEISS

CHAPTER 3

THE RATE OF UPTAKE OF CARBON MONOXIDE BY HAEMOGLOBIN IN PIG ERYTHROCYTES AS A FUNCTION OF OXYGEN TENSION

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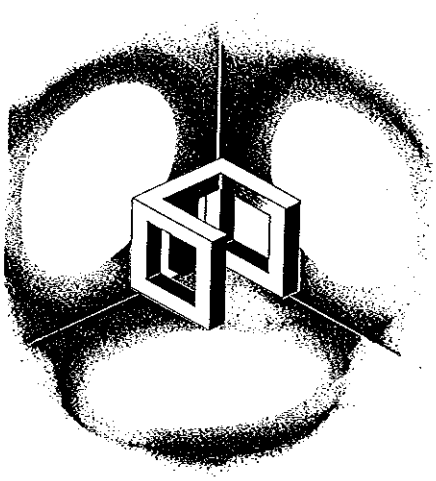

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INTRODUCTION

Mechanical ventilation has been applied in critically ill patients to improve gas transfer in the lungs. We have used pigs to study the effects of mechanical ventilation on the pulmonary circulation [24]. In healthy pigs, a decrease in total pulmonary blood volume was observed during inflation which recovers during expiration [24]. This decrease is caused by a shift of blood from the pulmonary circulation into the systemic circulation. We wonder to what extent this decrease in total pulmonary blood volume comes from the capillary part, which is one of the determinants of gas transfer. Furthermore, we are interested in changes in pulmonary capillary blood volume by mechanical ventilation in pigs with respiratory distress for which we developed two models of respiratory distress.

To estimate the pulmonary capillary blood volume, the Roughton-Forster relationship [21] has been extensively used:

$$\frac{1}{DL_{CO}} = \frac{1}{D_M} + \frac{1}{\theta_{CO} \times Q_c} \qquad (1)$$

where DL_{CO} and D_M represent the carbon monoxide (CO) diffusion capacity of the total lung and the pulmonary membrane respectively (ml CO per min per mmHg); θ_{CO}, the rate of CO uptake in ml CO per ml of whole blood (with a capacity of 0.2 ml CO per ml) per min per mmHg; Q_c, the pulmonary capillary blood volume in ml.[1] Q_c can be calculated by measuring the diffusing capacity at different oxygen tensions (P_{O2}), all greater than 150 mmHg, if D_M and Q_c are assumed to be constant and the relationship of θ_{CO} versus P_{O2} is known.

Although θ_{CO} has been studied in several species, it has never been determined in pig blood. Furthermore, there is a discrepancy in the literature as to whether θ_{CO} is species dependent or not. With use of a stop-flow apparatus, both Holland [15] and Lawson [19] found higher θ_{CO} values for dog than for human. The report of Lawson revealed higher absolute values for both dog and human compared to those of Holland. Measurements of θ_{CO} with use of the continuous-flow technique did, however, not reveal any differences between dog and human [6]. With the stop-flow technique, after cessation of flow, a stagnant layer is rapidly formed around the erythrocytes which acts

[1] *In this chapter, DL and θ are expressed in Traditional Units (i.e. ml CO, min and mmHg)*

as an additional diffusion barrier [5], resulting in an underestimate of θ_{CO}. The continuous-flow apparatus, on the other hand, maintains turbulent mixing up until the point of observation and while there is theoretically a stagnant layer around the red cells, it certainly is much less than in the stop-flow apparatus.

This study was initiated to measure θ_{CO} for CO in pig blood as a function of P_{O2} at 37°C and pH 7.4 with use of the continuous-flow technique. Results were compared with those obtained for human [9,18] and dog [6] blood under the same conditions.

METHODS

For a precise description of the continuous-flow rapid-mixing technique, we refer to Forster *et al.* [11] and Crapo *et al.* [6]. The method is based on mixing of an erythrocyte suspension containing no carboxyhaemoglobin (HbCO) and a buffer solution containing CO, both equilibrated at the same pre-set P_{O2}. This mixture is driven through a straight 1.6 mm bore observation tube into an observation chamber with a linear flow velocity rate of about 240 cm per second. These conditions will be sufficient to maintain turbulent flow, creating an essentially square front so that the averaged elapsed reaction time from mixing to observation chamber at any point will be reasonably precise. The reaction time is varied by using observation tubes of different lengths. The extent of the reaction of haemoglobin with CO is determined from the difference in transmission of light passing through the flowing mixture and subsequently through two narrow band interference filters with peaks at 567 and 583 nm. A study of Gottliebsen *et al.* (unpublished) revealed no significant difference in θ_{CO} using either the difference in light transmission or the difference in optical density.

Solutions and Suspensions

The measurements were performed on blood of five different breeding sows (2.5-4 years, 160-210 kg, mixed breed) who were in good health. Approximately 450 ml venous blood were drawn at the farm from each pig into a standard clinical transfusion bag containing 63 ml Citrate-Phosphate-Dextrose-Adenine-solution (CPDA) and delivered to us at the Medical School (Philadelphia, USA). Except for the first pig, all of the measurements on each blood sample were completed within 8 days. It required a

day to obtain data at each P_{O2}. In the case of the first pig our procedures were not as practised and the studies extended over 2 weeks.

For each experiment, 37.5 ml of blood were diluted to 1,500 ml with isotonic phosphate buffer (composition 3.0 mM KH_2PO_4, 14.1 mM Na_2HPO_4 and 130 mM NaCl, pH 7.4). After deaeration, half of this cell suspension was saturated with CO, i.e. *CO cell standard*, and the other half was equilibrated with a gas mixture without CO containing a pre-set P_{O2} and balance nitrogen, i.e. *CO-free cell standard*. The isotonic phosphate buffer was used to prepare two solutions. The first solution (*CO buffer*) was equilibrated, after deaeration, with a gas mixture of the pre-set P_{O2}, a CO tension (P_{CO}) of about 160 mmHg and balance N_2. The second solution (*CO-free buffer*) was equilibrated without CO using the same pre-set P_{O2} and balance N_2. For a given experiment the P_{O2} in the buffer solutions and the cell suspensions were about the same.

As a check on the equilibration procedure, samples were anaerobically drawn from the cell suspensions and the buffer solutions to measure the final P_{O2} (Radiometer Copenhagen, PHM 73 pH and BMS Mk2). P_{CO} was measured only in the CO buffer solution with use of the apparatus developed by Coburn *et al.* for measurement of CO in blood [4]. For this purpose, we used 250 ml N_2 to washout CO in a 2 ml sample. At least 15 minutes were taken to fill the tonometer with the washout gas. Then the gas was passed through an infra-red CO analyzer (Beckman Instruments, Inc., Fullerton, California). The CO concentration was obtained from a calibration curve, then P_{CO} was derived with use of Henry's law.

Experimental Procedure and Calculations

The temperature of the reactant fluids was maintained at 37°C by either water jackets or immersion in water baths. The cell suspensions were stirred during the course of the experiments to prevent the cells from settling.

The flow of each reactant fluid into the observation tube, which was approximately the same, was measured for derivation of the reaction time. At each reaction time, differences in light transmission were measured for the following mixing procedures: *1)* CO-free cell standard with CO-free buffer to obtain the zero level (0% HbCO), reading Z_1; *2)* CO-free cell standard with CO buffer to obtain the extent of the reaction, reading R_1; *3)* CO cell with CO buffer to obtain the fully saturated level (100% HbCO), reading S; and *4) and 5)* mixtures 2) and 1) again, yielding readings R_2

and Z_2 respectively to correct for drift during the course of the experiment. The saturation of Hb with CO in the mixture of CO-free cell standard and CO buffer was calculated according to:

$$\frac{\%HbCO}{100} = \frac{(R_1 - Z_1) + (R_2 - Z_2)}{(S - Z_1) + (S - Z_2)} \qquad (2)$$

The average %HbCO was used when more than one run was performed for a given reaction time. Each %HbCO was plotted against reaction time and a curve was drawn by eye through these points. The initial slope (s_i), expressed as the fraction HbCO per second, was obtained as the tangent to this curve at time is zero. Then θ_{CO} was determined with the following formula:

$$\theta_{CO} = s_i \times \frac{60 \times 0.2}{P_{CO}} \qquad (3)$$

where 60 is the seconds in one minute; 0.2 is the assumed standard blood CO capacity in ml CO per ml blood, which was used to compare our results with data on human [9,18] and dog [6] blood; and P_{CO} is the CO pressure in mmHg at the start of the reaction, i.e. half the pressure in the CO buffer. For each pig, θ_{CO} was plotted against P_{O2}.

Lysis Control

At the end of each experiment, we collected CO-free cell suspension mixed with CO-free buffer and CO cell suspension mixed with CO buffer solution at the outlet of the observation tube. These mixtures were spun down at 3000 rpm at room temperature for 5 to 10 minutes. Spectrophotometric analyses were done on the supernatant to check for free Hb and methaemoglobin. Both were expressed as a percentage of total haemoglobin content of the CPDA blood used for preparation of the suspensions; dilution factors were taken into account. Haemoglobin contents were derived using the method as described by Shinowara [23]. Methaemoglobin was estimated from the difference in height of the recorded absorption peak at 630 nm in the absence and presence of sodium cyanide [8] only in samples of the last four pigs.

Statistical Analysis

For each pig, a simple linear regression equation of $1/\theta_{CO}$ on P_{O2} was calculated. Analysis of covariance (ANCOVA) [25] was used for testing hypotheses about equality of the five regression coefficients, i.e. slopes, and elevations. If it was concluded that the lines coincide, then a common (i.e. weighted) regression equation was calculated [25] and compared with regression equations for human [9,18] and dog [6] blood obtained under the same conditions with the continuous-flow technique. If the hypothesis of equal slopes was rejected, then a multiple comparison test, the Tukey test [25], was performed to determine between which slopes differences occur. Differences were considered statistically significant when $p < 0.05$.

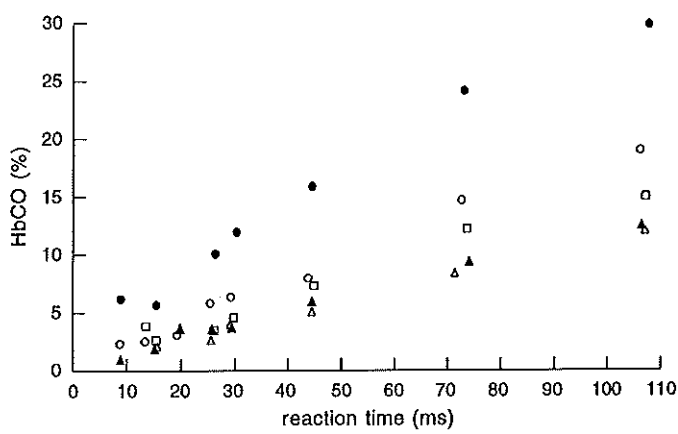

FIGURE 1. HbCO (%) is plotted on the Y-axis versus reaction time (ms) on the X-axis for five different P_{O2} levels: ● 134 mmHg; ○ 258 mmHg; □ 357 mmHg; ▲ 434 mmHg; Δ 599 mmHg. Data points are from measurements on blood of pig 3.

RESULTS

Lysis

The percentage of lysis in the reactant mixtures sampled at the end of each experiment on the blood of the last four pigs ranged from 0.05 to 1.5% which we consider not significant. In reactant mixtures of blood of the first pig, lysis rose up to 8.5%. In the samples of the last four pigs, no methaemoglobin could be detected (detection limit about 0.5%).

TABLE 1. Linear regression statistics of $1/\theta_{CO} = a + b \times P_{O2}$

subject	a	SE(a)	b	SE(b)	r
pig 1	0.29	0.20	0.0084	0.00062	0.9920
pig 2	0.90	0.27	0.0080	0.00105	0.9747
pig 3	0.73	0.39	0.0085	0.00099	0.9801
pig 4	0.32	0.57	0.0088	0.00148	0.9601
pig 5	0.96	0.27	0.0082	0.00066	0.9935
pig *	0.63	0.14	0.0084	0.00041	0.9793
human †	1.30	0.09	0.0041	0.00026	0.9940
dog ‡	1.45	0.34	0.0042	0.00111	0.9106

a is the Y-intercept of each regression line in (ml blood.min.mmHg)/ml CO; *b* is the slope of each regression line in (ml blood.min)/ml CO; SE(*a*) and SE(*b*) are the standard error of the Y-intercept and slope respectively; *r* is Pearson correlation coefficient; * common regression line of pig 1-5; † based on data obtained in 1983 [9,18]; ‡ based on data obtained in 1989 [6]

Theta

The change in saturation of Hb with CO with time was measured at 37°C and pH of 7.4 at five different P_{O2} levels in four pigs and at four different P_{O2}'s in one pig. P_{CO} values ranged between 150 and 156 mmHg. An example of the percentage HbCO versus reaction time at different P_{O2} levels is shown in FIGURE 1.

Regression statistics of $1/\theta_{CO}$ on P_{O2} of each pig are presented in TABLE 1. Regression coefficients, i.e. slopes, were not significantly different ($p=0.98$; ANCOVA). Elevations were just not significantly different ($p=0.06$; ANCOVA). Since the regression lines were not concluded to be different, a common regression coefficient as well as a common Y intercept were calculated (TABLE 1). The 95 percent confidence interval of the common slope was [0.0075;0.0092]. Our present data points and the resulting common regression line of pig erythrocytes and the data points and regression lines of $1/\theta_{CO}$ on P_{O2} of human erythrocytes in 1983 [9,18] and of dog

erythrocytes in 1989 [6] at 37°C and pH 7.4 obtained with a continuous-flow rapid-mixing apparatus are plotted in FIGURE 2. The regression statistics are given in TABLE 1. The slopes of the three species are not all equal ($p<0.001$; ANCOVA). After applying the Tukey multiple comparison test, the following was concluded: $slope_{human} = slope_{dog} < slope_{pig}$.

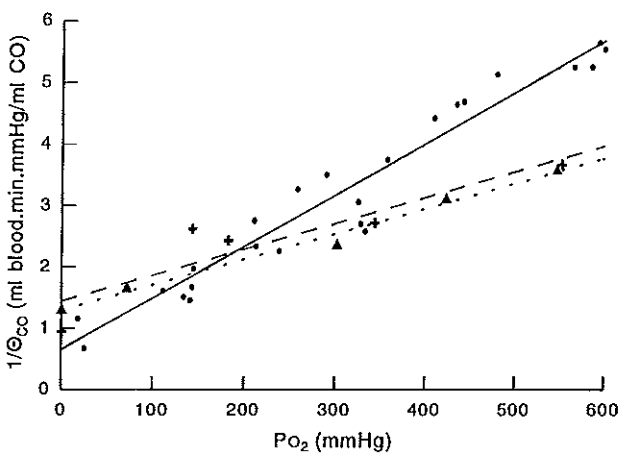

FIGURE 2. Graph of $1/\theta_{CO}$ in ml.min.mmHg/ml versus P_{O2} in mmHg
Solid line is the common linear regression line based on the data points (•) of this study (5 pigs). Dashed and broken line are the linear regression lines obtained at 37°C and pH 7.4 using a continuous-flow rapid-mixing apparatus on respectively human erythrocytes in 1983 [9,18] (▲; data points human) and dog erythrocytes in 1989 [6] (+; data points dog).

DISCUSSION

Critical Comments Concerning Experimental Design

MEASURING TECHNIQUE - Rapid-mixing apparatus, either stop-flow or continuous-flow, has been used to obtain almost all measurements of gas uptake by red cells or haemoglobin solution. However, using the stop-flow rapid-mixing technique, unstirred layers, which act as an additional diffusion barrier, are rapidly formed around the red cells even after high turbulence mixing [5], leading to an underestimate of θ_{CO}. Reeves and Park [20] state that such an additional diffusion barrier is also likely to exist in the

continuous-flow rapid-mixing apparatus. To this, they ascribe the difference found between solution and red cell kinetics with use of the continuous-flow technique. We cannot exclude the existence of stagnant layers, however, they should be much thinner than with use of the stop-flow technique.

Recently, Reeves and Park [20] used an ingenious technique developed by Heidelberger and Reeves [12,13] in which a thin (2 to 4 μm) layer of blood surrounded by gas is suddenly exposed to CO and the time course of HbCO formation is monitored by two colour spectrophotometry. By studying this a thin layer, i.e. 1 to 2 cells in thickness, they hoped to exclude significant diffusion resistances outside the red cells, although obviously the ligand gas must diffuse through some plasma to reach the cell surface. Their values for θ_{CO} are similar to those obtained with a continuous-flow rapid-mixing apparatus at $P_{O2} > 300$ mmHg, but higher at lower P_{O2}. According to these authors, this difference could be explained by a reduction in θ_{CO} resulting from stagnant layers of fluid around the red cells which would occur in all rapid-mixing apparatuses but not in their thin film. However, the experimental evidence they cite in support of this argument all pertains to stop-flow apparatus; there is no experimental evidence concerning a stagnant layer in continuous-flow apparatus. Forster *et al.* found that θ_{CO} in human red blood cells is twice as great in continuous-flow as in stop-flow instruments [10]. Therefore, the continuous-flow turbulent mixing has certainly reduced, if not eliminated, the stagnant layer found in stop-flow apparatus. The published spectrophotometric haemoglobin-ligand reaction records of Heidelberger and Reeves [12,13] and Reeves and Park [20] have an unusual initial shape. There is a very rapid (step) increase in the haemoglobin compound amounting to 5 to 10% of maximum, followed by an "S"-shaped curve, lasting as long as 10 milliseconds. It is possible that this contributes to an overestimate of the reaction velocity. We conclude that the continuous-flow instrument provides a reasonable measure of θ_{CO}.

LYSIS - Shear forces in the mixing chamber and turbulent flow in the observation tube produce lysis, which determines the maximal tolerable linear velocity of the reacting mixture and, therefore, sets a limit to the reaction time that can be resolved in the mixing apparatus. Another source of lysis was the stirring bar in the storage bottles which probably caused appreciable cell damage, particularly at the end of an experiment when the fluid level was low and the bar broke the surface of the liquid. To compensate for any possible increase of lysis with time, the sequence of measurements at the different reaction times and P_{O2} levels were randomized.

Haemolysis measured in the reactant mixtures at the end of experiments performed with blood of the last four pigs was negligible, but rose up to 8.5% for experiments on the blood of the first pig. This was likely caused by increased fragility because of the longer storage. During the experiments on the blood of the last four pigs, extra attention was paid to the above mentioned sources of lysis: blood was used as quickly as possible after it was drawn and the stirring of storage cell suspensions was minimized.

Haemolysis would add haemoglobin solution to the reacting cell suspension, accelerating the formation of HbCO. This would increase θ_{CO} erroneously. As the physico-chemical rate of the actual reaction of CO with haemoglobin to form HbCO slows down, that is as P_{O2} increases, the rate of CO uptake by red cells approaches the rate it combines with haemoglobin in solution. For example, the rate in cells is 0.46 times the rate in solution at P_{O2}=100 mmHg, but rises to 0.68 at P_{O2}=571 mmHg [22]. In the experiment at a P_{O2} of 566 mmHg (pig 1) which was done when the blood was more than two weeks old, lysis was 8.5% and the observed reaction velocity would have been raised by:

$$100\times\left(\frac{\textit{fractional lysis}}{\textit{rate in cells / rate in solution}}-\textit{fractional lysis}\right)=100\times\left(\frac{0.085}{0.68}-0.085\right)=4\% \quad (4)$$

This is the greatest amount of lysis seen and would not produce a significant error in our results as shown in FIGURE 2.

We conclude that the final outcome of this study, a steeper slope of the regression line of $1/\theta_{CO}$ on P_{O2} of pig blood compared to those previously obtained with dog and human blood under the same conditions, cannot be ascribed to lysis.

Variation of θ_{CO} between Species

Below we will consider to what extent differences in affinity of haemoglobin for O_2 and CO, cell size and intracellular haemoglobin concentration could explain the difference we found between θ_{CO} of pig blood and θ_{CO} of both human and dog blood. In TABLE 2 normal values are shown of the oxygen affinity of haemoglobin (P_{50}) [2,7]; the relative affinity for O_2 and CO of haemoglobin, i.e. equilibrium constant M of the Haldane equation [1,17]; red blood cell (RBC) volume and RBC haemoglobin content and concentration [7] for human, dog and pig.

TABLE 2. Normal values of haematological parameters for human, dog and pig

	human	dog	pig
P_{50} (mmHg) [2,7]	26.3	28	33.7
M* [1,17]	225	225	130
RBC volume (m^3) [7]	87	66	61.1
RBC Hb content (pg) [7]	29	23	21.5
RBC Hb concentration (pg/m^3 in %)† [7]	33.3	34.8	35.2

* M is defined by the relation $[HbCO]/[HbO_2] = M \times P_{CO}/P_{O2}$
† RBC haemoglobin content divided by RBC volume times 100

AFFINITY OF HAEMOGLOBIN - At a P_{O2} of 100 mmHg or more, in mammals at least, the concentration of reduced haemoglobin is small. Then, with regard to the displacement of O_2 from combination with Hb by CO, the only one of the four association reaction velocity constants left are k'_4 for HbO_2 and l'_4 for HbCO. The rate of the displacement reaction can then be approximately given by the following equation [22]:

$$d[HbCO]/dt = \frac{k_4 \times l'_4 \times [HbO_2] \times \alpha_{CO} P_{CO}}{4k'_4 \times \alpha_{O_2} P_{O_2} + 4l'_4 \times \alpha_{CO} P_{CO}} \qquad (5)$$

where k_4 is the velocity constant of the reaction $Hb_4(O_2)_4 \rightarrow Hb_4(O_2)_3 + O_2$; k'_4 is the velocity constant of the reaction $Hb_4(O_2)_3 + O_2 \rightarrow Hb_4(O_2)_4$; l'_4 is the velocity constant of the reaction $Hb_4(CO)_3 + CO \rightarrow Hb_4(CO)_4$; α_{CO} and α_{O2} are the solubilities of respectively CO and O_2 in blood. Substituting equation (5) into equation (3) leads to:

$$\frac{1}{\theta_{CO}} = \left(\frac{P_{CO}}{d[HbCO]/dt} \right) \times \frac{1}{0.2 \times 60} =$$
$$\left(\frac{k'_4 \times \alpha_{O_2} P_{O_2}}{k_4 \times l'_4 \times \alpha_{CO} \times [HbO_2]} + \frac{P_{CO}}{k_4 \times [HbO_2]} \right) \times \frac{4}{0.2 \times 60} \qquad (6)$$

The oxygen affinity of pig haemoglobin is less, i.e. P_{50} is higher than that of human and dog erythrocytes (TABLE 2). This would tend to increase the fraction of unliganded

haemoglobin and therefore increase the rate of formation of HbCO at a given P_{O2}. Experimentally Krawiec *et al.* [18] did find that an increase in acidity, i.e. a decrease in oxygen affinity, led to an increase in θ_{CO}. Heidelberger and Reeves [13], on the contrary, did not observe an effect of a change in oxygen affinity on the rates of O_2 uptake and release. In general a lower O_2 affinity would lead us to expect a lower O_2 overall association reaction velocity constant and/or a higher dissociation velocity constant, which would decrease, not increase the slope of $1/\theta_{CO}$ versus P_{O2}, the slope equalling $(k'_4 \cdot \alpha_{O2})/(k_4 \cdot l'_4 \cdot \alpha_{CO} \cdot [HbO_2])$. M, which is a measure of the competition of CO with O_2 for unoccupied haem binding sites (TABLE 2) and which equals $(l'_4/k'_4) \cdot (k_4/l_4)$, is less in pig blood than in human or dog blood. A lower k'_4/k_4 (i.e. higher k_4/k'_4) cannot explain the lower M, thus l'_4/l_4 must be considerably less in pig cells for M to be less. We are not aware of any measurements of the velocity constants for the reactions of CO with pig haemoglobin in solution, but a considerably lower l'_4 would explain the greater slope in the $1/\theta$ versus P_{O2} plot for pig blood when compared to human or dog blood.

CELL SIZE - The smaller the cells, the shorter the diffusion distances and naively the more rapid CO uptake should be. This is experimentally shown by Holland and Forster [16] and Holland [14,15] with use of a stop-flow apparatus. The effect of cell size was greatest for the reaction of oxygen with haemoglobin, and appeared to be much less for the considerably slower CO replacement reaction. These results could be an artefact of the stop-flow technique itself. After cessation of flow, a stagnant layer is established around each cell in microseconds [5] and diffusion through this layer slows the overall uptake rate of the gas, resulting in an underestimate of the latter.

Carlsen and Comroe [3] concluded from experiments with a constant-flow rapid-mixing apparatus, that the initial uptake rate of CO by reduced cells is not cell size dependent. They attributed the dramatic reduction of the initial overall-rate in the shrunken cells to a change in orientation and concentration of the intracellular haemoglobin.

Our results are not in concordance with either the results of Holland or those of Carlsen and Comroe. We would have expected the $1/\theta_{CO}$ versus P_{O2} line of both pig and dog, whose cells are smaller than human cells (TABLE 2), to lie parallel and below the line for human red cells according to the results of Holland and upon the line of man according to the results of Carlsen and Comroe.

INTRACELLULAR HAEMOGLOBIN - The results of Carlsen and Comroe [3] show an effect of the intracellular haemoglobin concentration on the initial overall-rate of gas uptake, but only above a critical level. Since the haemoglobin concentration in pig (and dog) cells is just a fraction higher than that in human cells (TABLE 2), it can hardly explain the difference we found. We are therefore left with no reasonable explanation for the greater slope of pig blood in FIGURE 2.

We therefore conclude, that the most reasonable explanation for the greater slope of pig blood in FIGURE 2 is that $1'_4$ is much less in pig blood than in human or dog blood.

Estimation of Q_c in Pigs Using Human or Dog θ_{CO} Values

It is informative to calculate the error which we would make if we used the available values of θ_{CO} for other species in measurements of Q_c in pigs. For this, we used previously published values for human [9,18] and dog [6] blood which were obtained with the same technique and under the same conditions. Q_c can be derived from measurements of DL_{CO} using at least two different alveolar P_{O2} levels, *I* and *II*, according to:

$$1/DL_{CO(x)} = 1/D_M + 1/(\theta_{CO(x)} \times Q_c) \qquad (7)$$

where the subscript (*x*) stands for (*I*) and (*II*) respectively, resulting in two equations. Combination of these two equations leads after rearranging to:

$$Q_c = (1/\theta_{CO(II)} - 1/\theta_{CO(I)})/\Delta R \qquad (8)$$

where $\Delta R = 1/DL_{CO(II)} - 1/DL_{CO(I)}$. Substituting $1/\theta_{CO} = a + b \times P_{O2}$ into equation (8) leads to:

$$Q_c = (b \times \Delta P_{O_2})/\Delta R \qquad (9)$$

where ΔP_{O2} is the difference in mmHg of the two P_{O2} levels. The use of θ_{CO}-values of human blood in the calculation of Q_c in pigs would result in an error of:

$$100 \times \frac{Q_{c,human} - Q_{c,pig}}{Q_{c,pig}} = 100 \times \frac{b_{human} - b_{pig}}{b_{pig}} = 100 \times \frac{0.0041 - 0.0084}{0.0084} \approx -51\% \qquad (10)$$

Using θ_{CO}-values of dog blood, the error would be about -50%. Thus, the derivation of the pulmonary capillary blood volume in pigs with use of θ_{CO}-values of human or dog blood would result in a considerable underestimate.

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CHAPTER 4

COMPONENTS OF CARBON MONOXIDE TRANSFER AT DIFFERENT ALVEOLAR VOLUMES DURING MECHANICAL VENTILATION IN PIGS

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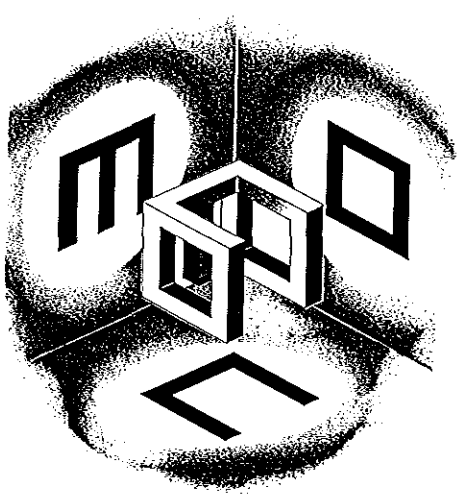

- submitted for publication -

INTRODUCTION

Mechanical ventilation has been applied in critically ill patients to improve gas transfer in the lungs. A measure of gas transfer is the diffusing capacity of the lungs for carbon monoxide (DL_{CO}). According to Roughton and Forster [21], DL_{CO} consists of two components, a membrane component (D_M), i.e. the diffusion of carbon monoxide across the alveolar capillary membrane to the surface of the red cells, and an intracapillary component which is a function of blood volume present in the pulmonary capillaries (Q_c) and the reaction rate of carbon monoxide with intracellular haemoglobin (θ_{CO}).

The effect of lung inflation on DL_{CO} and its components has been extensively studied in spontaneously breathing subjects. In general, increases in DL_{CO} and D_M have been associated with lung inflation [6,10,16,22,24]. Less consensus has been obtained for Q_c, either no change [16], an increase [22] or a decrease [10,24] have been reported.

The few results obtained during mechanical ventilation are not consistent. Macnaughton and co-workers [17] found in healthy volunteers using a rebreathing technique, that DL_{CO} and Q_c decreased and D_M did not change when lung volume was increased by changing the positive end-expiratory pressure (PEEP) from 0 to 10 cmH_2O. According to results of Hsia *et al.* [11] and Johnson *et al.* [13] obtained in anaesthetized and mechanically ventilated dogs, also with use of a rebreathing technique, DL_{CO} and D_M increased and Q_c did not change by increasing lung volume. In a previous study, we found no change in DL_{CO} by increasing alveolar volume using an inspiratory pause procedure (equivalent of the single-breath technique during spontaneous breathing) in anaesthetized, paralysed and mechanically ventilated pigs [18] (CHAPTER 2).

Because of these opposing results, we decided to evaluate the changes in DL_{CO} and its components under conditions of mechanical ventilation. An inspiratory pause procedure was used because changes in alveolar volume can be easily applied and more precisely controlled than with use of a rebreathing technique.

METHODS

Surgical Procedures

All experiments were performed according to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health [NIH publication 85-23, Revised 1985] and approved by the Animal Care Committee of the Erasmus University Rotterdam, the Netherlands.

Eight pigs (8-9 weeks old, 11.2 *(0.7)* kg; mean *(SD)* body weight), were anaesthetized with an intraperitoneal injection of pentobarbital sodium (30 $mg.kg^{-1}$), followed by a continuous intravenous infusion of 8.5 $mg.kg^{-1}.h^{-1}$. After tracheostomy, they were connected to a computer-controlled ventilator in supine position on a thermo-controlled operation table to maintain body temperature at about 38°C.

A catheter was inserted via the right common carotid artery into the aortic arch for measurement of arterial blood pressure and blood sampling. A Swan-Ganz catheter was placed in the left pulmonary artery via the right external jugular vein to measure pulmonary arterial blood pressure and core temperature and sample blood. Sampled blood was replaced by saline. A four lumen catheter, which was also inserted through the right external jugular vein, was used to measure central venous pressure and infuse pentobarbital and, after surgery, tubocurarine (0.2 $mg.kg^{-1}.h^{-1}$). Each pressure catheter was continuously flushed with saline, containing a low dose of heparin (10 I.U. per ml) to avoid clotting, at a rate of 3 $ml.h^{-1}$.

Experimental Procedures

The pigs received volume-controlled ventilation after tracheostomy. The ventilatory frequency was set at 10 breaths per minute (2.4 seconds inflation and 3.6 seconds expiration) and a positive end-expiratory pressure (PEEP) of 2 cmH_2O was applied. Tidal volume was adjusted to an arterial CO_2 tension of 5.1-5.6 kPa during ventilation with an O_2 fraction of 0.30 and was kept constant throughout the experiment. The animals were successively ventilated with gas mixtures containing O_2 fractions of 0.30, 0.60 and 0.94 in N_2.

For estimation of DL_{CO}, an inspiratory pause (IP) procedure (FIGURE 1), simulating the single-breath manoeuvre during spontaneous breathing, was used [23]. The IP procedure consisted of an inflation of 2.4 seconds, an inspiratory pause of 7.2 seconds and an expiration of 2.4 seconds. The flow rates of both inflation and expiration were

constant. Normal mechanical ventilation and the IP procedures were performed with a microcomputer-controlled ventilator [12]. The ventilator consisted of two independently functioning concertina bellows, one for continuous ventilation and the other for the IP procedures. The latter was filled, prior to the IP procedure, with a known volume of test gas containing labelled carbon monoxide ($C^{18}O$, fraction 0.003), He (fraction 0.05), and O_2 (fraction either 0.30, 0.60 or 0.94, depending on the O_2 fraction used during the normal ventilatory mode) in N_2.

At each inspiratory oxygen level, DL_{CO} was determined at four different alveolar volumes in each pig by inflating 15, 20, 25 or 30 $ml.kg^{-1}$ in random order. The IP procedures were performed at intervals of at least 5 minutes to allow for washing out of test gases and regaining haemodynamic stability.

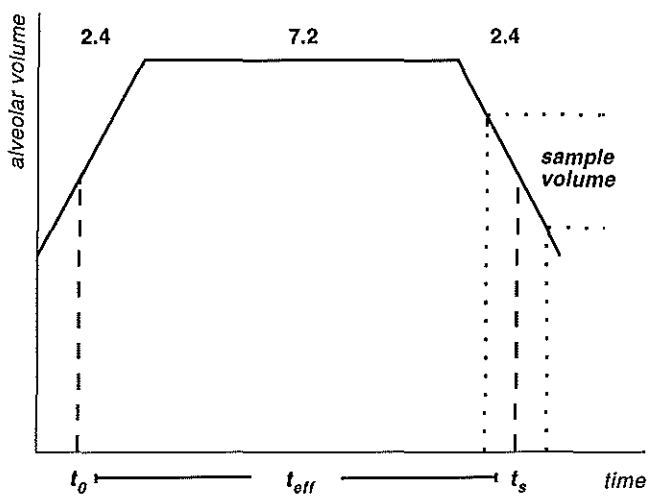

FIGURE 1. Inspiratory pause procedure for estimation of DL_{CO}
Alveolar volume is plotted on the Y-axis against time on the X-axis, both in arbitrary units. The inspiratory pause procedure consisted of an inflation of 2.4 seconds, a pause of 7.2 seconds and a controlled expiration of 2.4 seconds. t_s is the time at the midpoint of the sampled volume during expiration and t_0 is the time at the corresponding lung volume level during inflation. $C^{18}O$ was assumed to be taken up by the blood from time t_0 to t_s (= effective time, t_{eff}) and from an alveolar volume equal to the area under the volume-time curve from t_0 to t_s divided by t_{eff} (= effective or time-weighted mean alveolar volume, $V_{A,eff}$).

Measured and Calculated Data

Arterial and mixed-venous blood gas tensions for O_2 and CO_2, haemoglobin (Hb) concentration and arterial pH were determined with an automatic blood gas system (ABL510, Radiometer, Copenhagen, Denmark). Oxyhaemoglobin (HbO_2) and carboxy-haemoglobin (HbCO) were determined with a haemoximeter (OSM3, Radiometer, Copenhagen, Denmark). Blood pressures and tracheal pressure were monitored to control steady-state conditions. ECG as well as blood pressures were sampled by a computer at 250 Hz prior to and after a series of IP procedures for calculation of baseline values of heart rate and blood pressures (average values over one ventilatory cycle) and to check stability throughout the series.

Gas flow rates were measured with a Godart-Statham pneumotachograph (Fleisch no.0, Switzerland) attached to a differential pressure transducer. Volume changes during inflation and expiration were obtained by integration of the flow signal. All gas fractions ($C^{18}O$, He, O_2, CO_2 and N_2) were continuously measured during the IP procedures with a mass spectrometer (Perkin-Elmer, MGA 1100, Pomona, California USA) which sampled gas at 1 $ml.s^{-1}$. A correction was made for the delay time of 280 ms of the mass spectrometer relative to the flow signal. For each IP procedure the end-expiratory alveolar volume was calculated from the He-mass balance. In the calculation, both the Fowler respiratory dead space for CO_2 [8] and the apparatus dead space were taken into account.

Analysis

For each IP procedure, the $C^{18}O$ and He fractions of the expired gas volume, excluding the dead space wash out volume and the last part of the expired gas, were averaged. This expired gas volume is referred to as alveolar sample. An effective diffusion time (t_{eff}) and an effective alveolar volume ($V_{A,eff}$) from which $C^{18}O$ was taken up were estimated in a similar way as has been described by Cotton *et al.* [5] (FIGURE 1). Then DL_{CO} was calculated using the following formula:

$$DL_{CO} = ln\left(\frac{FA_{C^{18}O(t_0)}}{FA_{C^{18}O(t_s)}}\right) \times \frac{1}{t_{eff}} \times \frac{V_{A,eff}}{22.4 \times 10^{-6} \times P_A}$$

where DL_{CO} is in $mmol.s^{-1}.kPa^{-1}$; $FA_{C^{18}O}(t_0)$ is the $C^{18}O$ fraction at the start of $C^{18}O$ uptake estimated from the He dilution; $FA_{C^{18}O}(t_s)$ is the mean $C^{18}O$ fraction of the

alveolar sample; t_{eff} is in seconds; $V_{A,eff}$ in L STPD; $22.4x10^{-6}$ is the volume in ml STPD of 1 mmol of gas; P_A is the total dry pressure in the alveolar gas in kPa estimated as $P_B+P_T-P_{H2O,s}$ where P_B is the barometric pressure, P_T tracheal pressure and $P_{H2O,s}$ saturated water vapour pressure for the prevailing body temperature. DL_{CO}/V_A is expressed as $mmol.s^{-1}.kPa^{-1}$ per litre BTPS alveolar volume.

By measuring DL_{CO} at each lung volume level at three different oxygen levels, D_M and Q_c can be derived. According to the Roughton-Forster equation, DL_{CO}, D_M and Q_c are related as follows [21]:

$$\frac{1}{DL_{CO}} = \frac{1}{D_M} + \frac{1}{\theta_{CO} \times Q_c}$$

For derivation of θ_{CO}, we used the relationship of $1/\theta_{CO}$ versus (ideal) alveolar oxygen tension as has been found in pig blood with use of a continuous-flow technique, namely $1/\theta_{CO} = 0.113 + 0.011\ P_{AO2}$,[1] where θ_{CO} is in $\mu mol\ CO.s^{-1}.kPa^{-1}.ml\ blood^{-1}$ [19] (CHAPTER 3). P_{AO2} (in kPa) was derived from the alveolar gas equation. As the θ_{CO} equation is based on blood with a standard CO capacity of 0.2 ml per ml, i.e. Hb concentration of 8.9 $mmol.L^{-1}$ ($=14.4\ g.dL^{-1}$), the derived θ_{CO} was adjusted to the actual concentration by multiplying with $Hb_{actual}/8.9$. D_M and Q_c can be obtained from the Y-axis intercept and slope respectively using least-squares regression on the data by plotting $1/DL_{CO}$ against adjusted $1/\theta_{CO}$. All three alveolar oxygen levels were chosen above 20 kPa to ensure full saturation of haemoglobin. In healthy humans, the relationship of $1/\theta_{CO}$ versus P_{AO2} is not reliable at P_{AO2} values below 13-20 kPa since an increasing amount of reduced haemoglobin will be present [21].

Carbon Monoxide Back Pressure

Back pressure was estimated immediately prior to and after a series of IP procedures using the Haldane relationship [7]. For this purpose HbCO, HbO_2 and O_2 tension were determined in an arterial blood sample. The reported value of 130 for the Haldane equation's constant for pig blood was used [15]. A linear interpolation between the back pressures prior to and after a series of procedures was performed. Back pressure

[1] *SI units instead of Traditional Units which were used in CHAPTER 3.*

was modified to fraction and subtracted from both alveolar carbon monoxide fractions at t_0 and t_s.

Statistical Analysis

The Student's *t*-test for paired samples was used to compare baseline haemodynamic and gas exchange variables prior to and after each series of IP procedures, i.e. one O_2 level. The two way analysis of variance (ANOVA) was used to test for changes in those variables between the three series. When a significant change was found, further analysis was done by performing a multiple-comparisons procedure, the Bonferroni *t*-test. Multiple regression analysis was used to look at the variation, *within* subjects, in carbon monoxide gas transfer and its components (D_M and Q_c) by increasing V_A and O_2 fraction. Pig was treated as a categorical variable using dummy variables. Partial regression coefficients for $V_{A,eff}$, adjusted for subjects and/or O_2 fraction, with 95% confidence intervals (95%CI) are given. The statistical analysis was performed with the Statistical Package for Social Sciences 4.0; differences were considered statistically significant when $p < 0.05$.

RESULTS

Baseline Haemodynamic and Gas Exchange Variables

No significant changes were found in baseline haemodynamic and gas exchange values within each series, except for a small increase in P_{aCO2} in the series at an F_{IO2} of 0.30 (TABLE 1). Observation of baseline values between the three series revealed a higher P_{ao} and a slightly elevated P_{pa} and heart rate in the series at an F_{IO2} of 0.30 compared to both other series ($p < 0.05$).

In all pigs, the arterial haemoglobin concentration was about constant during successive tests throughout the experiment. For the whole group, Hb concentration ranged from 5.7 to 7.0 mmol.L^{-1} (mean 6.3 mmol.L^{-1}).

Pulmonary Carbon Monoxide Transfer

TOTAL PULMONARY DIFFUSING CAPACITY - In FIGURE 2, changes in DL_{CO} and DL_{CO}/V_A by increasing $V_{A,eff}$ are shown for each oxygen level. DL_{CO}/V_A as well as DL_{CO} consistently decreased by increasing $V_{A,eff}$ (TABLE 2). After statistical adjustment for

$V_{A,eff}$, DL_{CO} and DL_{CO}/V_A decreased on average 0.80 (95%CI: 0.70-0.89) $\mu mol.s^{-1}.kPa^{-1}$ and 1.64 (95%CI: 1.44-1.83) $\mu mol.s^{-1}.kPa^{-1}.L^{-1}$ respectively per 0.10 increase in F_{IO2}.

MEMBRANE DIFFUSING CAPACITY AND CAPILLARY BLOOD VOLUME - Since for each inflation volume, $V_{A,eff}$ never varied more than 5% between the three oxygen levels, its mean value was used in the analysis. On average, D_M increased slightly by 2.7 (95%CI: 0.2-5.2) $\mu mol.s^{-1}.kPa^{-1}$ and Q_c decreased by 241 (95%CI: 163-318) ml for each litre BTPS increase in $V_{A,eff}$ (FIGURE 3).

DISCUSSION

Baseline Haemodynamic and Gas Exchange Variables

Baseline values throughout all series of IP procedures were stable. The change in P_{aCO2} in the series at an F_{IO2} of 0.30 was regarded physiologically insignificant, because it was within the physiological range of 5.1-5.6 kPa and on average 0.1 kPa. With regard to differences between series, a higher P_{ao} was found in the series with an F_{IO2} of 0.30 compared to both other series. We expect that its effect on the DL_{CO} measurements will be insignificant, since P_{ao} only moderately decreased (7%), P_{pa} hardly changed and P_{aCO2} and pH were within the physiological range.

Pulmonary Carbon Monoxide Transfer

ABSOLUTE VALUES - So far no values have been reported on the pulmonary carbon monoxide transfer and its components, the capillary blood volume and membrane diffusing capacity, in pigs. Therefore we compared our data with those reported for other species during mechanical ventilation (TABLE 3). For this purpose, all values were expressed per kg body weight. Furthermore, the DL_{CO} values in pigs were corrected to a Hb concentration of 8.9 $mmol.L^{-1}$, i.e. oxygen capacity of 0.2 ml per ml, and an oxygen tension of 16 kPa with use of the Roughton-Forster equation by adjusting θ_{CO} to this Hb concentration and O_2 tension [4].

TABLE 1. Haemodynamic and gas exchange values prior to and after each series of IP procedures

	F_{IO2} = 0.30		F_{IO2} = 0.60		F_{IO2} = 0.94	
	prior	after	prior	after	prior	after
P_{ao} (mmHg)	89 (4)	90 (10)	84 (10)	82 (12)	79 (12)	80 (13)
P_{pa} (mmHg)	13.1 (1.5)	13.2 (1.8)	12.5 (1.7)	12.5 (1.4)	12.5 (1.6)	12.9 (1.7)
P_{cv} (mmHg)	2.0 (0.6)	2.1 (0.7)	2.0 (0.7)	2.1 (0.7)	2.0 (0.6)	2.0 (0.8)
HR (min^{-1})	140 (20)	147 (30)	135 (24)	132 (21)	131 (19)	131 (25)
pH_a	7.50 (0.02)	7.51 (0.03)	7.52 (0.03)	7.52 (0.03)	7.52 (0.03)	7.52 (0.03)
P_{aCO2} (kPa)	5.40 (0.16)	5.52* (0.20)	5.31 (0.25)	5.44 (0.17)	5.28 (0.18)	5.41 (0.25)
P_{aO2} (kPa)	19.4 (1.3)	19.7 (1.1)	43.8 (3.1)	43.5 (2.8)	72.4 (3.3)	72.7 (3.9)
S_{aO2} (%)	98.9 (0.3)	98.9 (0.3)	99.9 (0.1)	99.9 (0.1)	100 (0)	100 (0)

P_{ao} P_{pa} P_{cv} aortic, pulmonary artery and central venous pressure respectively; **HR** heart rate; **pH_a** arterial pH; **P_{aCO2}** and **P_{aO2}** arterial CO_2 and O_2 pressure respectively; S_{aO2} arterial O_2 saturation. Values are means *(SD)*; * $p < 0.05$ prior versus after, paired *t*-test

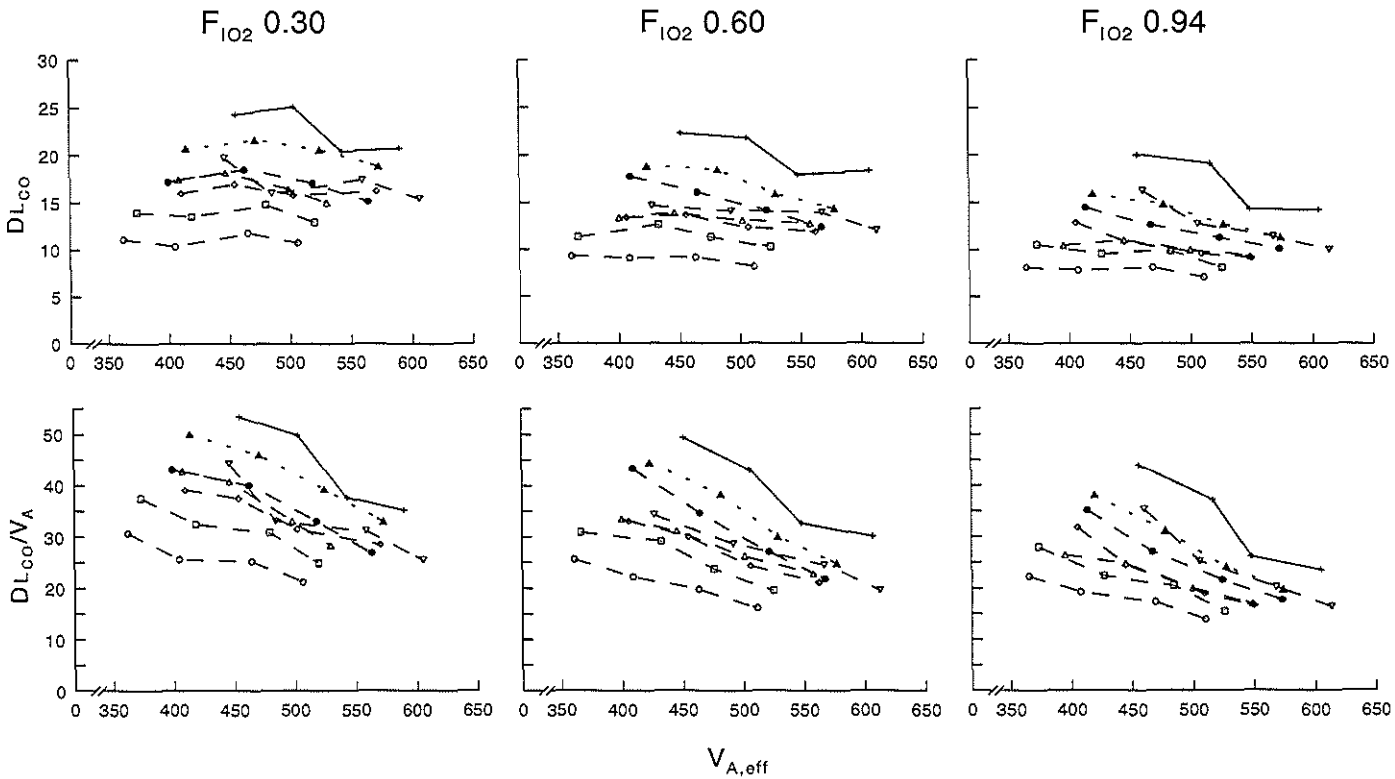

FIGURE 2. Pulmonary diffusing capacity versus effective alveolar volume

D_{LCO} in $\mu mol.s^{-1}.kPa^{-1}$ and D_{LCO}/V_A in $\mu mol.s^{-1}.kPa^{-1}.L(BTPS)^{-1}$ are plotted on the Y-axes against $V_{A,eff}$ in ml BTPS on the X-axes for all three F_{IO2} levels (0.30, 0.60 and 0.94). Each individual's responses are connected by straight lines (n=8). At all three F_{IO2} levels, both D_{LCO} and D_{LCO}/V_A decreased by increasing $V_{A,eff}$ ($p < 0.005$).

TABLE 2. Partial regression coefficients of DL_{CO} and DL_{CO}/V_A for each litre (BTPS) increase in $V_{A,eff}$ at each oxygen level

F_{IO2}	b(DL_{CO}) in $\mu mol.s^{-1}.kPa^{-1}.L^{-1}$	b(DL_{CO}/V_A) in $\mu mol.s^{-1}.kPa^{-1}.L^{-2}$
0.30	-11.8 (-19.6;-4.0)	-96 (-113;79)
0.60	-16.9 (-23.0;-10.7)	-94 (-108;79)
0.94	-23.8 (-30.9;-16.7)	-98 (-114;83)

b(DL_{CO}) and b(DL_{CO}/V_A) are partial regression coefficients of respectively DL_{CO} and DL_{CO}/V_A per litre increase in alveolar volume. Values between brackets are 95% confidence intervals.

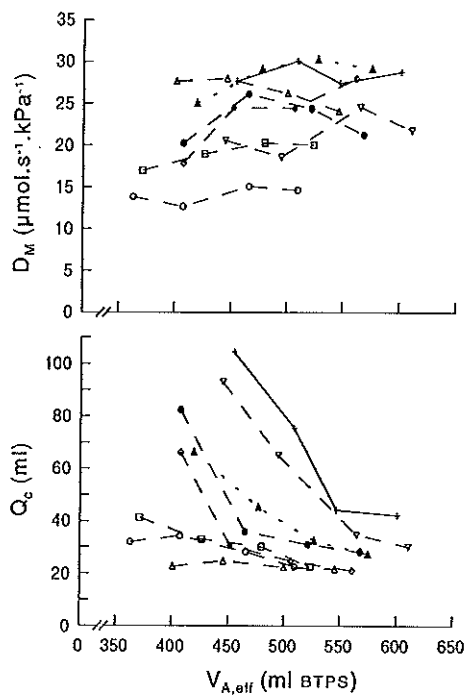

FIGURE 3. Membrane diffusing capacity and capillary blood volume versus effective alveolar volume

D_M in µmol.s^{-1}.kPa^{-1} and Q_c in ml are plotted on the Y-axes against $V_{A,eff}$ in ml BTPS on the X-axis. Each individual's responses are connected by straight lines (n=8). By increasing $V_{A,eff}$, D_M slightly but significantly increased ($p=0.03$) and Q_c decreased ($p<0.001$).

The values of Q_c and D_M depend on the value of theta (θ_{CO}). A number of different θ_{CO} values have been reported based on different measuring techniques, species, blood temperature and pH. Part of the differences in TABLE 3 may be ascribed to the uncertainty whether the "correct" theta has been used. In the study on humans [17], subjects were trained to tolerate positive pressure ventilation but were, in contrast to the subjects of the other studies, not anaesthetized. Furthermore, in humans, the change in lung volume was effected by imposing a positive end-expiratory pressure, whereas in the other studies different inflation volumes were applied. Although for these reasons, a proper comparison is hampered, the main differences in gas transfer between the studies will be considered.

DL_{CO} and D_M per kg body weight were lower in pigs than in dogs (TABLE 3). However, when expressed per litre lung volume, DL_{CO} was similar and D_M was lower in pigs. The lower D_M per litre lung volume may be explained by the lower blood Hb concentration in our pigs compared to the mean value in dogs. Burrows and Niden [2] hypothesized that the alveolar-capillary membrane may be most effective as a diffusing surface area near areas of red cell contact with the capillary wall. This idea was reinforced by the results of Jouasset-Strieder *et al.* [14]: a reduction in D_M which was proportional to the fall in Hb concentration after the induction of anaemia while Q_c did not change.

In pigs, the capillary blood volume is relatively high compared to both dogs and humans (TABLE 3). Could a higher Q_c be expected in pigs? Baseline cardiac output as measured by the direct Fick method for O_2 ranged from 1.9 to 2.9 $ml.s^{-1}.kg^{-1}$ (F_{IO2} of 0.30), this would be 8.5 to 13.1 $L.min^{-1}$ for a pig of 75 kg, which is relatively high compared to humans. Right-to-left shunt, calculated as decribed by Berggren [1], was low (range 1.0-3.6%) compared to values of about 10% reported for healthy humans during anaesthesia and mechanical ventilation [20]. Obviously, the lungs may have been more uniformly perfused and less of the capillary bed may have been collapsed.

Comparison of DL_{CO} values and its components between dogs [11,13] and humans [17] revealed much higher values in dogs. Carlin *et al.* also obtained relatively high values in dogs at rest and exercise during spontaneous breathing with use of a rebreathing technique as did Jouasset-Strieder *et al.* [14] during forced inflation procedures. Carlin *et al.* suggested that DL_{CO} may be more closely matched to metabolic capacity than to body size.

TABLE 3. Comparison of carbon monoxide gas transfer indices between previously reported studies and the present one

AUTHORS	species (number)	weight[a] kg	V_I ml.kg^{-1}	V_{lung}[a] ml.kg^{-1}	DL_{CO}[a] µmol.s^{-1}.kPa^{-1}.kg^{-1}	Q_c[a] ml.kg^{-1}	D_M[a] µmol.s^{-1}.kPa^{-1}.kg^{-1}	DL_{CO}/V_A µmol.s^{-1}.kPa^{-1}.L^{-1}
HSIA *et al.* [11][b]	dog (6)	25.3	15-30-45-60	79 (56-101)	3.46[e]	2.35	6.81	43.8[i]
JOHNSON *et al.* [13][c]	dog (6)	14.9	15	58	3.1[f]	2.2	6.4	53.4[i]
			30	80	4.2[f]	2.4	10.6	52.5[i]
			45	95	4.4[f]	2.4	10.0	46.3[i]
MACNAUGHTON *et al.* [17]	human (8)	75[d]	13.3 ($PEEP_0$)	44	1.31[g]	1.09	1.67	28.3
			13.3 ($PEEP_{10}$)	55	1.18[g]	0.75	1.60	18.3
TE NIJENHUIS *et al.*	pig (8)	11.2	15	36.5	1.68[h]	5.6	1.90	45.8[h]
				(34-40)	(1.40-2.27)	(2.1-9.2)	(1.34-2.49)	(33.8-56.4)
			30	50.3	1.66[h]	2.4	2.10	33.0[h]
				(48-54)	(1.19-2.12)	(1.9-3.7)	(1.42-2.72)	(24.3-40.6)

$\mathbf{V_I}$ inflation volume; $\mathbf{V_{lung}}$ lung volume; [a] mean values (range); [b] overall mean values; [c] values derived from graphs in this paper; [d] assumed mean body weight; [e] F_{IO2} of 0.30 and a mean haematocrit of 44%; [f] F_{IO2} of 0.23 and mean Hb of 8.0 mmol.L^{-1}; [g] F_{IO2} of 0.21 and corrected to a Hb of 9.1 mmol.L^{-1}; [h] corrected to an P_{AO2} of 16 kPa (i.e. $F_{IO2}\approx0.23$) and a Hb of 8.9 mmol.L^{-1}; [i] derived from data in paper

RELATIONSHIP WITH (ALVEOLAR) LUNG VOLUME - At all oxygen levels, we found a moderate decrease in DL_{CO} by increasing alveolar volume (TABLE 2). Macnaughton *et al.* [17] also found a decrease in DL_{CO} when lung volume was increased by changing the positive end-expiratory pressure from 0 to 10 cmH_2O. Hsia *et al.* [11] and Johnson *et al.* [13], on the contrary, observed an increase in DL_{CO} with increasing inflation volumes. Note that the changes in DL_{CO} of the different studies are moderate, except for the study of Johnson *et al.* (TABLE 3).

With increasing alveolar volume, the diffusing capacity of the pulmonary membrane slightly increased. We would have expected a bigger rise in D_M, if the total alveolar membrane area is involved in gas exchange. Provided that the lung expands as a sphere or any similar shape, i.e. isotropical expansion, or by recruitment of spheres, its surface should change by $V_A^{2/3}$. Based on a morphological study of Gil and Weibel [9] in rat lungs, evidence was provided that alveolar surface area even changes in direct proportion to air volume and that, above end-expiratory volume, the alveoli maintain a constant shape. However, since part of the alveolar-capillary membrane for gas transfer is lost by a decrease in capillary blood volume, the effective part of the surface area available for gas transfer will be smaller than might be expected from the increase in alveolar volume.

Q_c decreased by increasing alveolar volume: doubling of the inflation volume from 15 to 30 $ml.kg^{-1}$ led to a decrease in Q_c which was on average 3.2 (range 0.1-5.5) $ml.kg^{-1}$. This is much larger than the decrease found by Versprille *et al.* [23] in *total* pulmonary blood volume which was less than 1.0 $ml.kg^{-1}$. They also performed inspiratory pause procedures in pigs of about the same age and weight under similar experimental conditions; the main difference in experimental design was that these pigs had undergone thoracotomy. The decrease in total pulmonary blood volume resulted from a shift of blood from the pulmonary into the systemic circulation, derived from differences between right and left ventricular output, during inflation [23]. Both squeezing of the pulmonary vessels by the intrapulmonary pressure rise and stretching of lung tissue may have attributed to this. The larger decrease in Q_c we found compared to the reported decrease in total pulmonary blood volume under similar conditions may be explained by a shift of blood from the capillaries to the arterial or venous part of the pulmonary vascular bed or both, beside the shift to the systemic circulation. Since the transmural pressure of the pulmonary artery during inflation

either hardly changes or modestly decreases [23], a substantial shift to the arterial part is not to be expected.

We conclude that 1) during mechanical ventilation, DL_{CO} moderately decreases by increasing alveolar volume which results from a slightly increasing membrane diffusing capacity and a decreasing capillary blood volume; 2) the increase in D_M is less than may be expected from the increase in alveolar volume itself, which we explained by a loss of the alveolar-capillary membrane for gas transfer by the concomitant decrease in Q_c; 3) the larger decrease in Q_c compared to the reported decrease in total pulmonary blood volume may be explained by a shift of blood from the capillaries to the pulmonary veins by the squeezing effect of the intrapulmonary pressure rise during inflation. The data on carbon monoxide transfer in normal pigs presented in this study may be used as reference values for studies on carbon monoxide transfer in similar pigs with experimentally induced pathology.

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"Wij leren veel van onze successen,
maar leren meer van ons falen."

HENRY FORD

CHAPTER 5

THE EFFECT OF INCREASING ALVEOLAR VOLUME BY MECHANICAL VENTILATION ON THE PULMONARY CIRCULATION IN PIGS

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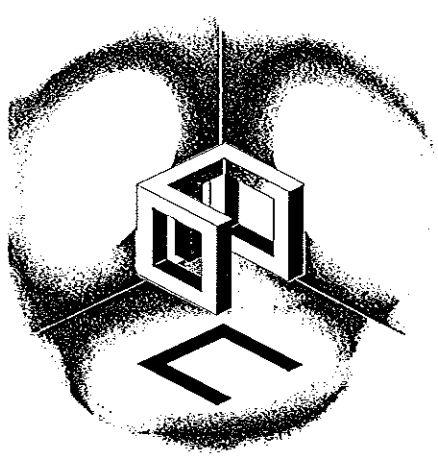

INTRODUCTION

The pulmonary capillary blood volume, a major determinant of gas transfer in the lungs, can be derived from estimates of carbon monoxide diffusing capacity of the lungs (DL_{CO}) at different alveolar oxygen tensions and using the Roughton-Forster relationship [29]. In a previous study, we found that the capillary blood volume (Q_c) decreased with increasing alveolar volume during mechanical ventilation, i.e. forced inflation, in anaesthetized and paralysed healthy pigs [24]. The estimates of Q_c and its decrease with alveolar volume were rather large compared to data obtained during forced inflation in other species [14,19,22], taking body weight and alveolar volume into consideration. Furthermore, we found that the decrease in capillary blood volume was larger than the decrease in total pulmonary blood volume as reported by Versprille *et al.* [33]. They observed a shift of blood from the pulmonary into the systemic circulation during forced inflation, which they ascribed to squeezing of the pulmonary vessels by the rise in intrapulmonary pressure and stretching of lung tissue.

Lung inflation may affect total and capillary pulmonary blood volume differently. For instance, squeezing of the (alveolar) capillaries may result in a shift of blood not only into the systemic circulation but also into the larger pulmonary vessels. Howell *et al.* [13] reported a decrease in volume of the smaller vessels (including the alveolar capillaries) and an increase in volume of the larger vessels of the pulmonary circulation during lung inflation. Their finding was based on observations in excised dog lungs. We, however, are interested in the effect of forced inflation on the pulmonary vascular bed under normal circulatory conditions (i.e. in vivo) in anaesthetized and paralysed subjects.

The aim of this study was to relate changes in the capillary blood volume to changes in total pulmonary blood volume. For this purpose, independent estimates of total pulmonary blood volume with use of a double-indicator-dilution technique (cold and conductivity) were obtained in addition to capillary blood volume estimates derived from DL_{CO} measurements at two different O_2 fractions. Both blood volumes were estimated at the same inflation volumes within the same pigs. A comparison was made between capillary and total pulmonary blood volume with respect to 1) their absolute values, and 2) their relationship with alveolar volume. In our previous study Q_c was derived from DL_{CO} determinations at three different inspiratory O_2 fractions, 0.30, 0.60 and 0.94 [24]. In the present study, DL_{CO} was determined at only two O_2 fractions

to minimize carbon monoxide back pressure. The effect of such a reduction in the number of DL_{CO} determinations on capillary blood volume estimates was additionally examined with use of data from our previous study.

METHODS

Surgical Procedures and Ventilatory Conditions

All experiments were performed according to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health [NIH publication 85-23, Revised 1985] and approved by the Animal Care Committee of the Erasmus University Rotterdam, the Netherlands.

Ten healthy pigs (8-9 weeks old; 10.5 *(0.5)* kg, mean *(SD)* body weight) were anaesthetized with an intraperitoneal injection of pentobarbital sodium (30 $mg.kg^{-1}$), followed by a continuous intravenous infusion of 8.5 $mg.kg^{-1}.h^{-1}$. They were placed, in supine position, on a thermo-controlled operation table to maintain body temperature at about 38.5°C and connected, after tracheostomy, to a computer-controlled ventilator.

A catheter was inserted via the right common carotid artery into the aortic arch for measurement of arterial blood pressure and blood sampling. Three catheters were inserted via the right external jugular vein: a Swan-Ganz catheter (Baxter; model 93-132-5F) was placed in the left pulmonary artery to sample blood and measure pulmonary arterial blood pressure and temperature; a double-walled injection catheter in the right atrium for injections of cold saline; a four lumen catheter in the superior vena cava to measure central venous pressure and infuse pentobarbital sodium and, after surgery, tubocurarine (0.2 $mg.kg^{-1}.h^{-1}$). Via the femoral artery, a sensing catheter for blood electrical conductivity was inserted into the aortic arch (near the tip of the aortic pressure catheter). Each pressure catheter was continuously flushed at a rate of 3 $ml.h^{-1}$ with saline containing a low dose of heparin (10 I.U. per ml) to avoid clotting. Sampled blood was replaced by normal saline.

After tracheostomy, the pigs received volume-controlled ventilation using a microcomputer-controlled ventilator [16]. The ventilatory frequency was set at 10 breaths per minute (2.4 sec inflation and 3.6 sec expiration) and a positive end-expiratory pressure (PEEP) of 2 cmH_2O was applied. Tidal volume was adjusted to an arterial CO_2 tension of 5.1-5.6 kPa and kept constant throughout the experiment.

Measurements and Analysis

BASELINE CARDIAC OUTPUT - At the beginning and end of each series of observations, cardiac output was determined during the normal ventilatory mode by the thermodilution method. For this purpose, 2.5 ml normal saline at room temperature were injected via the double-walled injection catheter into the right atrium by a pneumatically driven syringe [17]. All injections were computer controlled. Four measurements equally spread over the ventilatory cycle were averaged and this value was considered to be an accurate estimate of mean cardiac output [17,18].

PULMONARY CAPILLARY BLOOD VOLUME - The pulmonary capillary blood volume (Q_c) was derived from two determinations of the diffusing capacity of the lungs for carbon monoxide (DL_{CO}), at two different O_2 fractions (0.30 and 0.94), using the Roughton-Forster relationship:

$$\frac{1}{DL_{CO}} = \frac{1}{D_M} + \frac{1}{\theta_{CO} \times F_{Hb} \times Q_c} \qquad (1)$$

where D_M is the diffusing capacity of the pulmonary membrane for carbon monoxide; θ_{CO} (theta for CO) is the reaction rate of carbon monoxide with intracellular haemoglobin per unit of blood volume with a standard haemoglobin concentration of 8.9 $mmol.L^{-1}$ (=14.4 $g.dl^{-1}$); and F_{Hb} is the actual haemoglobin concentration as a fraction of the standard concentration. θ_{CO} values were derived from the relationship of $1/\theta_{CO}$ versus (ideal) alveolar oxygen tension reported for pig blood obtained with a continuous-flow technique: $1/\theta_{CO} = 0.113 + 0.011\ P_{AO2}$, where θ_{CO} is in µmol $CO.s^{-1}.kPa^{-1}.ml\ blood^{-1}$ [25]. P_{AO2} (in kPa) was derived from the alveolar gas equation. The θ_{CO} equation was based on blood with a standard CO capacity of 0.2 ml per ml, i.e. Hb concentration of 8.9 $mmol.L^{-1}$.

DL_{CO} was determined with use of an inspiratory pause procedure, hereafter referred to as $C^{18}O$-IP procedure, which has been previously reported in detail [23]. A $C^{18}O$-IP procedure consisted of an inflation of 2.4 sec, an inspiratory pause of 7.2 sec and an expiration of 2.4 sec. The flow rates of both inflation and expiration were constant. These manoeuvres were also performed with the micro-computer controlled ventilator which consisted of two independently functioning concertina bellows, one for the volume-controlled ventilation and the pause procedures for determination of total pulmonary blood volume and the other for the $C^{18}O$-IP procedures [23]. During the

$C^{18}O$-IP procedure a test gas was inflated containing 0.3% $C^{18}O$, 5% He, 30 or 94% O_2 and balance N_2. In the calculation of DL_{CO}, corrections were made for the time and volume from which carbon monoxide was taken up by blood: an effective time (t_{eff}) and a time-weighted mean alveolar volume, i.e. effective alveolar volume ($V_{A,eff}$), were determined [8,23]. Then DL_{CO} was calculated using the following formula:

$$DL_{CO} = ln\left(\frac{FA_{C^{18}O(t_0)}}{FA_{C^{18}O(t_s)}}\right) \times \frac{1}{t_{eff}} \times \frac{V_{A,eff}}{22.4 \times 10^{-6} \times P_A} \qquad (2)$$

where DL_{CO} is in $\mu mol.s^{-1}.kPa^{-1}$; $FA_{C^{18}O}(t_0)$ is the $C^{18}O$ fraction at the start of $C^{18}O$ uptake estimated from the He dilution; $FA_{C^{18}O}(t_s)$ is the mean $C^{18}O$ fraction of an alveolar sample; t_{eff} is in seconds; $V_{A,eff}$ is in ml STPD; $22.4x10^{-6}$ is the volume in L STPD of 1 µmol of gas; P_A is the total dry pressure in the alveolar gas in kPa estimated as $P_B+P_T-P_{H2O,s}$ where P_B is the barometric pressure, P_T tracheal pressure and $P_{H2O,s}$ saturated water vapour pressure for the prevailing body temperature. The alveolar sample consisted of the expired gas volume, excluding the dead space wash out volume and the last part of the expired gas.

Carbon monoxide back pressure was estimated immediately prior to and after a series of $C^{18}O$-IP procedures using the Haldane relationship [9]. For this purpose HbCO, HbO_2 and O_2 tension were determined in an arterial blood sample. The reported value of 130 for the Haldane's equation constant of pig blood was used [20]. A linear interpolation between the back pressures prior to and after a series of procedures was performed. Back pressure was modified to fraction and subtracted from both alveolar carbon monoxide fractions at t_0 and t_s.

In our previous study [24], DL_{CO} was determined at three inspiratory O_2 fractions (F_{IO2}): 0.30, 0.60 and 0.94. In the present study, DL_{CO} determinations were made at only two F_{IO2}'s: 0.30 and 0.94. We therefore examined, using our previous results, the effect of leaving out the DL_{CO} determination at F_{IO2}=0.60 on final estimates of both capillary blood volume and membrane diffusing capacity.

TOTAL PULMONARY BLOOD VOLUME - The total pulmonary blood volume was estimated with use of a double-indicator-dilution technique: cold and conductivity (FIGURE 1). Hypertonic saline (3 $mol.L^{-1}$; 2.5 ml) at room temperature was injected via the double-walled injection catheter into the right atrium using the same automatic injection system as for the baseline cardiac output measurements. When switching from normal

(isotonic) to hypertonic saline injections or vice versa, the system and the injection catheter were flushed.

Injections were applied during *i*nspiratory *p*ause procedures (saline-IP procedure) as well as during prolonged *e*xpiratory *p*auses (saline-EP procedure), one second after the onset of the pause when constant haemodynamic conditions were present. Pause times of 18 seconds were chosen to ensure that, at least under normal conditions, the decay of a dilution curve to its initial base-line value was completed within the pause time. Dilution curves of temperature were detected in the pulmonary artery and those of electrical conductivity in the aortic arch and both were sampled at 250 Hz.

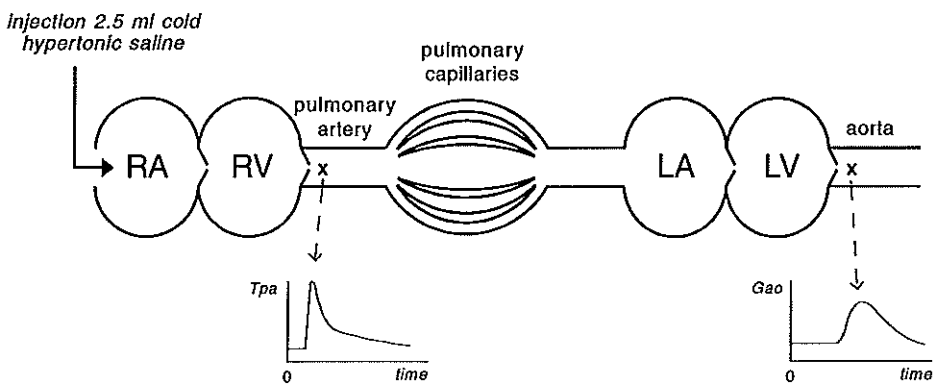

FIGURE 1. Schematic diagram of double-indicator-dilution technique
Hypertonic saline (3 mol.L^{-1}; 2.5 ml) at room temperature is injected into the right atrium. Subsequently, changes in blood temperature in the pulmonary artery (sensed by the Swan-Ganz catheter) and blood conductivity in the aortic arch (sensed by the conductance catheter) are recorded. The blood volume between these two detection points is obtained by multiplying the blood flow as obtained from the thermo-dilution signal in the pulmonary artery by the difference in mean transit times between the thermal and conductivity signal. **RA RV** right atrium and ventricle respectively; **LA LV** left atrium and ventricle respectively; T_{pa} temperature of blood in pulmonary artery; G_{ao} conductivity of blood in aortic arch

The conductivity sensing catheter had four electrodes equidistantly (5 mm) placed at its distal end. An alternating current of 70 μA (RMS) and 20 kHz was applied to the

two outer electrodes and the induced voltage was measured at the two inner electrodes (model Sigma 5, Leycom, Cardiodynamics, Rijnsburg, the Netherlands).

Thermodilution cardiac output (Q'_{TD}) was calculated according to the modified Stewart-Hamilton formula [15]. The temperature-time curves sensed in the pulmonary artery were digitally integrated, accounting for base-line drift and heat capacity of the injection catheter [17,18]. Mean transit times for both cold and conductivity dilution curves were derived as follows [34]:

$$\bar{t} = \int_{t1}^{t2} t \times c(t)dt \; / \; \int_{t1}^{t2} c(t)dt \qquad (3)$$

where $\bar{t}$ is mean transit time, t1 is injection time in seconds, t1 to t2 is the integration interval, c is concentration of indicator (either cold or conductivity) in arbitrary units. For an accurate analysis of $\bar{t}$, a log-normal distribution model was fitted to the (undisturbed part of the) curves [15]. Total pulmonary blood volume including the volume of the left heart (Q_{P+LH}) could then be computed by:

$$Q_{P+LH} = Q'_{TD}\,(\bar{t}_{cond} - \bar{t}_{temp}) \qquad (4)$$

where $\bar{t}_{cond}$ is mean transit time of conductivity change and $\bar{t}_{temp}$ mean transit time of temperature change from the injection catheter to the sensing electrodes in respectively the aortic arch and the pulmonary artery.

Data Acquisition

Arterial and mixed-venous blood gas tensions for O_2 and CO_2, haemoglobin (Hb) concentration and arterial pH were determined with an automatic blood gas system (ABL510, Radiometer, Copenhagen, Denmark). Oxyhaemoglobin (HbO_2) and carboxy-haemoglobin (HbCO) were determined with a haemoximeter (OSM3, Radiometer, Copenhagen, Denmark). At the beginning and end of each series, ECG and blood pressures were sampled by a computer at 250 Hz for calculation of baseline values of heart rate and blood pressures (average values over one ventilatory cycle).

Gas flow rates were measured with a Godart-Statham pneumotachograph (Fleisch no.0, Switzerland) attached to a differential pressure transducer. Volume changes during inflation and expiration were obtained by integration of the flow signal. All gas fractions ($C^{18}O$, He, O_2, CO_2 and N_2) were continuously measured during the $C^{18}O$-IP

procedures with a mass spectrometer (Perkin-Elmer, MGA 1100, Pomona, California USA) which sampled gas at a rate of 1 $ml.s^{-1}$. A correction was made for the delay time of 260 ms of the mass spectrometer relative to the flow signal. For each $C^{18}O$-IP procedure, the end-expiratory alveolar volume was calculated from the He-mass balance. In the calculation, both the Fowler respiratory dead space for CO_2 [11] and the apparatus dead space were taken into account.

Protocol

After a stabilization period of about 30 minutes, two similar series of observations were performed, one ventilating with 30% O_2 in N_2 and the other with 94% O_2 in N_2. Blood pressures and tracheal pressure were monitored throughout the experiments to check steady-state conditions. At the beginning of each series, baseline haemodynamic and gas exchange values were obtained. Subsequently, a $C^{18}O$-IP procedure followed by a saline-IP procedure were performed at three different inflation volumes: 15, 20 and 25 $ml.kg^{-1}$ respectively. Next, a prolonged expiratory pause (saline-EP procedure) was done during which only total pulmonary blood volume was estimated. The series was ended again with sampling of baseline haemodynamic and gas exchange values.

The two series with different inspiratory oxygen fractions and the inflation volumes within the two series were randomized. The pause procedures were performed at intervals of at least 5 minutes to allow for washing out of test gases ($C^{18}O$ and He) or saline and regaining haemodynamic stability. At the end of the experiments, the position of each catheter was confirmed at autopsy.

Statistical Analysis

The Student's *t*-test for paired samples was used to compare baseline haemodynamic and gas exchange variables at the beginning and end of each series of observations, and between the two series ($F_{IO2}=0.30$ vs $F_{IO2}=0.94$).

Multiple regression analysis [2] was used to look at the change *within* subjects in carbon monoxide gas transfer and its components, and pulmonary blood volume by increasing lung volume and O_2 fraction. Differences *between* pigs were adjusted for by treating them as a categorical variable using dummy variables. Partial regression coefficients with 95% confidence intervals (95%CI) and partial correlation coefficients [7] are given. Differences were considered statistically significant when $p<0.05$. The statistical analysis was performed with the Statistical Package for Social Sciences 4.0.

RESULTS

Baseline Haemodynamic and Gas Exchange Variables

Within each series, almost all baseline haemodynamic and gas exchange variables were stable, except for the following slight changes: a decline of about 2% of Hb concentration in each series; an increase (on average 5%) in pulmonary artery pressure and a decrease (on average 3.8%) in thermodilution cardiac output in the series at $F_{IO2}=0.94$ ($p<0.05$).

For comparison between the series, values at the beginning and end of each series were averaged; these values are presented in TABLE 1. Aortic as well as pulmonary artery pressure were significantly lower and arterial O_2 tension and saturation were significantly higher at $F_{IO2}=0.94$ compared to $F_{IO2}=0.30$ ($p<0.05$).

TABLE 1 Baseline haemodynamic and gas exchange values

	F_{IO2}=0.30		F_{IO2}=0.94	
	mean	(SD)	mean	(SD)
P_{ao} (mmHg)	94.1*	(9.8)	86.9	(10.7)
P_{pa} (mmHg)	16.1*	(1.2)	14.7	(1.1)
P_{cv} (mmHg)	3.31	(0.74)	3.39	(0.63)
HR (min^{-1})	141	(23)	133	(26)
Q'_{TD} ($ml.s^{-1}$)	26.4	(3.2)	26.6	(4.4)
Hb ($mmol.L^{-1}$)	6.3	(0.6)	6.2	(0.6)
P_{aO2} (kPa)	17.4*	(1.3)	66.4	(4.5)
S_{aO2} (%)	98.5*	(0.4)	99.9	(0.1)
P_{aCO2} (kPa)	5.6	(0.2)	5.6	(0.1)
pH_a	7.48	(0.02)	7.48	(0.03)

F_{IO2} inspiratory O_2 fraction; **P_{ao} P_{pa} P_{cv}** aortic, pulmonary artery and central venous pressure respectively; **HR** heart rate; **Q'_{TD}** thermal dilution cardiac output; **Hb** haemoglobin concentration; **P_{aO2} S_{aO2}** arterial O_2 pressure and saturation respectively; **P_{aCO2}** arterial CO_2 pressure; **pH_a** arterial pH. * $p<0.05$, $F_{IO2}=0.30$ vs $F_{IO2}=0.94$, paired *t*-test (n=10)

Number of DL_{CO} determinations

The derivation of capillary blood volume as well as membrane diffusing capacity from three DL_{CO} determinations at inspiratory O_2 fractions of 0.30, 0.60 and 0.94, and from only two, at the lowest and highest O_2 fraction, resulted in the same values (FIGURE 2).

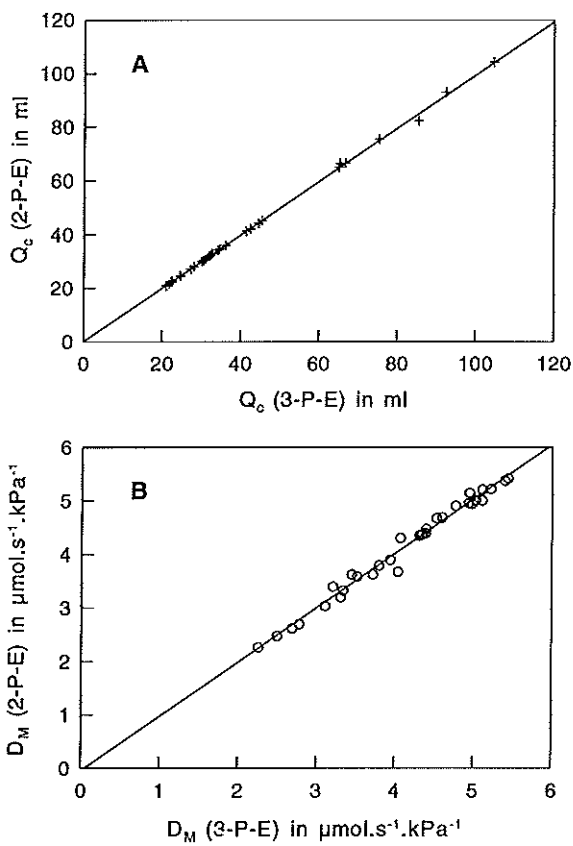

FIGURE 2. Values of Q_c (A) and D_M (B) derived from two or three DL_{CO} estimates
Individual values of capillary blood volume (Q_c) and membrane diffusing capacity (D_M) derived from DL_{CO} estimates at three different F_{IO2}'s (0.30, 0.60 and 0.94; **3-Point-Estimates**) are plotted versus values derived from two out of three DL_{CO} estimates (F_{IO2}: 0.30 and 0.94; **2-Point-Estimates**). Solid lines are linear regression lines, respectively $Q_{c(2\text{-}P\text{-}E)} = -0.1 + 1.0 \times Q_{c(3\text{-}P\text{-}E)}$ (r=1.0) and $D_{M(2\text{-}P\text{-}E)} = 0.1 + 0.97 \times D_{M(3\text{-}P\text{-}E)}$ (r=0.992). r is Pearson correlation coefficient.

Capillary versus Total Pulmonary Blood Volume

ABSOLUTE VALUES - Since no significant difference was found between total pulmonary blood volume values derived at an $F_{IO2}=0.30$ and those at $F_{IO2}=0.94$ ($p=0.49$; paired *t*-test), the mean values were used in the analysis. In FIGURE 3, the relation between capillary blood volume and mean total pulmonary blood volume is shown. All Q_c values lie below the line of identity, i.e. $Q_c < Q_{P+LH}$. The mean ratio between the two blood volumes is 0.5. Note that left heart volume is included in the total pulmonary blood volume estimates.

After removal of the variation due to differences between subjects, the following relationship between Q_{P+LH} and Q_c was found: each ml increase in total pulmonary blood volume was associated with an increase in capillary blood volume of 0.78 (95%CI: 0.38-1.18) ml. The correlation coefficient within subjects between Q_c and Q_{P+LH} was 0.42 ($p<0.001$).

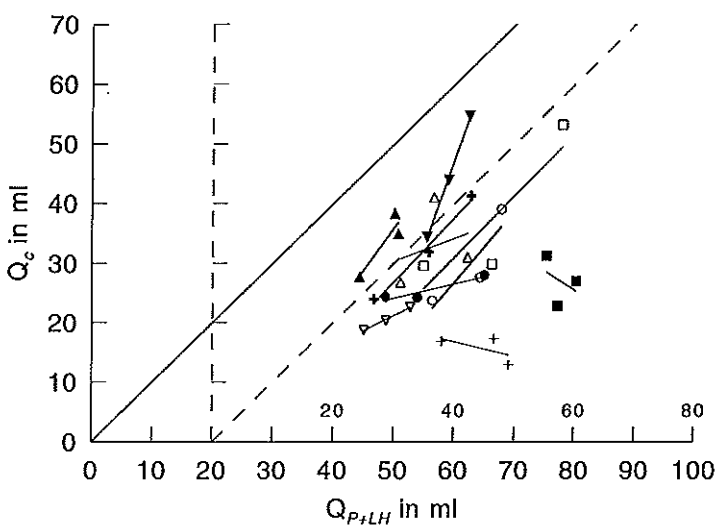

FIGURE 3. Capillary versus total pulmonary blood volume
Capillary blood volume (Q_c) in ml is plotted on the Y-axis against total pulmonary blood volume including left heart volume (Q_{P+LH}) in ml on the X-axis. Data obtained in each of the pigs (n=10) are represented by different symbols. Individual linear regression lines are shown. Solid line is line of identity. Broken lines represent Y-axis and line of identity respectively, if left heart blood volume is assumed to be about 20 ml.

TABLE 2. Partial regression coefficients of gas transfer indices and blood volumes for each litre increase in $V_{A,eff}$

	F_{IO2}=0.30	F_{IO2}=0.94	combined
	PRESENT STUDY		
b(DL_{CO})	-7.8 (-17.9;2.3)	-14.3 (-21.3;-7.3)	---
b(DL_{CO}/V_A)	-106 (-129;-83)	-92 (-111;-74)	---
b(Q_c)	---	---	-106 (-158;-53)
b(Q_{P+LH})	-115 (-167;-63)	-108 (-147;-69)	-111 (-146;-75)
	PREVIOUS STUDY [24]		
b(DL_{CO})	-11.8 (-19.6;-4.0)	-23.8 (-30.9;-16.7)	---
b(DL_{CO}/V_A)	-96 (-113;-79)	-98 (-114;-83)	---
b(Q_c)	---	---	-241 (-318;-163)

b(...) partial regression coefficients, i.e. adjusted for differences between pigs, representing the change in the corresponding parameter per L increase in effective alveolar volume ($V_{A,eff}$). Values between brackets are 95%CI. DL_{CO} diffusing capacity of the lungs (in $\mu mol.s^{-1}.kPa^{-1}$); V_A alveolar volume (in L BTPS); D_M diffusing capacity of the pulmonary membrane (in $\mu mol.s^{-1}.kPa^{-1}.L^{-1}$); Q_c capillary blood volume (in ml); Q_{P+LH} total pulmonary blood volume including left heart volume (in ml); F_{IO2} inspiratory oxygen fraction; **combined** values derived from both series (F_{IO2}=0.30 and F_{IO2}=0.94)

RELATIONSHIP WITH ALVEOLAR VOLUME - At each inflation volume, capillary blood volume was derived from DL_{CO} measurements at two different inspiratory oxygen levels, yielding two independent values of $V_{A,eff}$. These never differed more than 4%, except for one pig in which differences were found of 5, 7 and 10% at inflation volumes of 15, 20 and 25 $ml.kg^{-1}$ respectively. The mean value of $V_{A,eff}$ per inflation volume was used in the analysis. The partial regression coefficients (adjusted for differences between pigs) of capillary and total pulmonary blood volume for litre BTPS increase in $V_{A,eff}$ are given in TABLE 2. Q_c and Q_{P+LH} decreased with alveolar volume to a similar degree. In FIGURE 4, mean *(SD)* values of capillary and total pulmonary blood volume expressed per kg body weight are plotted versus the different inflation volumes. Mean

end-expiratory alveolar volume for the whole group was 16.7 *(SD 1.5)* ml STPD per kg, reflecting differences in $V_{A,eff}$ between the pigs.

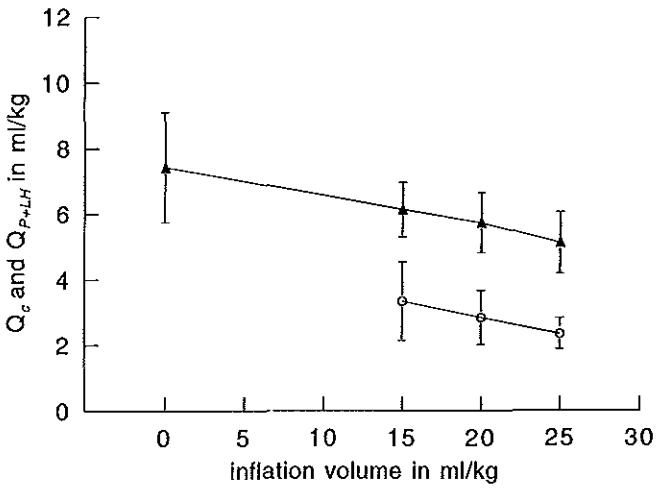

FIGURE 4. Relationship of capillary and total pulmonary blood volume with lung volume by forced inflation

Capillary blood volume (Q_c; ○) in ml.kg^{-1} and total pulmonary blood volume including left heart volume (Q_{P+LH}; ▲) in ml.kg^{-1} are plotted on the Y-axis against inflation volume in ml.kg^{-1} on the X-axis. Values are mean *(SD)*; n=10. Since no significant difference was found between Q_{P+LH} values derived at F_{IO2}=0.30 and those at F_{IO2}=0.94, mean Q_{P+LH} values are shown.

DISCUSSION

Baseline Haemodynamic and Gas Exchange Variables

Within both series, baseline values were stable. The few observed minimal changes were regarded physiologically insignificant. Between both series, significantly lower aortic and pulmonary artery pressures were found at F_{IO2}=0.94 compared to F_{IO2}=0.30, whereas central venous pressure and cardiac output did not change. Asher *et al.* [5] also reported a decrease in pulmonary artery pressure without a significant change in cardiac output when studying the effect of increasing inspired O_2 fraction on haemodynamic variables under mechanical ventilatory conditions in anaesthetized and paralysed Yorkshire pigs (10-15 kg).

Capillary Blood Volume Estimation from DL_{CO} Values

When plotting $1/DL_{CO}$ against $1/\theta_{CO}$ for different alveolar O_2 tensions and calculating the least-squares regression line, the slope of this line represents $1/Q_c$ and the Y-intercept $1/D_M$. That is, if we assume similar pulmonary haemodynamic conditions for the different DL_{CO} determinations and no effect of alveolar O_2 tension on capillary blood volume and membrane diffusing capacity. Could the decrease in pulmonary artery pressure with increasing inspiratory O_2 fraction have affected the estimates of capillary blood volume, since they were derived from DL_{CO} measurements at a low and high O_2 fraction? During the pause procedures at equal inflation volume, total pulmonary blood volume was the same at normal and high inspiratory O_2 fraction. Furthermore, Siegel *et al.* [30] showed that hypoxia (i.e. low alveolar O_2 tension resulting in an increase in pulmonary artery pressure and vascular resistance) primarily produced an arterial pulmonary vasoconstriction and did not affect pulmonary capillary pressure. They compared the effect of hypoxic ventilation, inspiratory O_2 fraction of 0.14, with baseline observations during ventilation with an inspiratory O_2 fraction of 0.99 in (intact) anaesthetized sheep. Based on our and their results, we assume that the change in the inspiratory O_2 fraction from 0.30 to 0.94 or vice versa has not affected pulmonary capillary blood volume.

The accuracy of estimation of capillary blood volume and membrane diffusing capacity [29] may be improved by determining DL_{CO} at more different alveolar O_2 tensions. However, from FIGURE 2 it can be concluded that a reduction in the number of DL_{CO} determinations, using only two values (at a low and high O_2 fraction), had no effect on the final estimates of both capillary blood volume and membrane diffusing capacity.

Total Pulmonary Blood Volume Estimation

NACL AS NONDIFFUSIBLE INDICATOR - Sodium chloride has been reported to be a useful nondiffusible, i.e. intravascular, indicator in the first passage through normal lungs since no evidence was found of sodium leak across the normal capillary endothelium [4,26,27].

CONTRIBUTION OF LEFT HEART - The volume we measured with the double-indicator-dilution technique cannot be considered a measure of "true" pulmonary blood volume, since it included the blood volume of the left heart. In our pigs (10.0-11.3 kg), mean baseline cardiac output and heart rate were 26.3 $ml.s^{-1}$ and 141 $beats.min^{-1}$ (i.e. 2.35

Hz) respectively, resulting in a mean stroke volume of 11.2 ml. Assuming an ejection fraction of about 50%, which has been reported for mechanically ventilated subjects under steady-state conditions [6,32], the mid-value of ventricular (LV) volume over a heart cycle (between diastole and systole) will be about 16.8 ml in our study. This value is similar to measurements of left ventricular volume obtained with the conductance catheter in mini pigs (10-13 kg BW) by Szwarc *et al.* [32]. They reported mean values at end-diastole and end-systole of 25.8 and 13.8 ml respectively. We therefore assume 20 ml to be an approximate value of left heart volume (ventricle plus atrium) averaged over a total cardiac cycle in our pigs.

Versprille *et al.* [33] concluded, based on data reported by Lorell *et al.* [21], that left heart would contribute for about 7% to the total change in blood volume of the pulmonary circulation including the left heart during a forced inflation. This percentage was calculated from the decrease in left ventricular transmural end-diastolic pressure during inflation which was on average 0.27 kPa (2 mmHg). The observations were done in pigs which were of about the same age and weight as our pigs. We therefore regard the changes we found in total pulmonary plus left heart blood volume a close approximation of the changes in total pulmonary blood volume.

Capillary versus Total Pulmonary Blood Volume

ABSOLUTE VALUES - The line of identity in FIGURE 3 will shift 20 ml to the right along the X-axis, when the assumed left heart volume of 20 ml is taken into consideration. Then six out of 30 capillary blood volume estimates will be larger than total pulmonary blood volume (Q_P), and the overall mean Q_c/Q_P ratio will be 0.75 instead of 0.50. From this, we may conclude that either the capillary blood volume values derived with use of the Roughton-Forster equation are overestimated or total pulmonary blood volume values derived from the double-indicator dilution technique are underestimated. The latter seems less likely since the overall mean pulmonary blood volume value of 58.2 ml (i.e. 5.5 $ml.kg^{-1}$), as obtained during the prolonged expiratory pauses (saline-EP procedure) and corrected for left heart volume, is comparable to estimates obtained in humans, 425 ml for adults of about 75 kg (i.e. 5.7 $ml.kg^{-1}$) [12]. If we assume 75-100 ml to be a representative value of capillary blood volume [12,31], then in human a Q_c/Q_P ratio of about 0.20 will be found. The high Q_c/Q_P ratio in pigs seems therefore unlikely.

The capillary blood volume varies in direct proportion to the slope of the $1/\theta_{CO}$ versus P_{AO2} relationship [25]. For instance, if we had used the $1/\theta_{CO}$ versus P_{O2} relationship as found for human blood, capillary blood volume estimates would have been reduced by half. We, however, used values derived for pig blood at 37°C and pH 7.40. The relationship of $1/\theta$ versus P_{AO2} can be affected by changes in temperature and pH [10]. In our pigs, mean *(SD)* pH and body temperature were respectively 7.48 *(0.02)* and 39.0 *(0.5)* °C. Theta, and consequently capillary blood volume, would have hardly been affected by these minor differences in pH and temperature. Although, it remains uncertain whether the reported theta of pig blood will reflect the true reaction rate of CO with intracellular haemoglobin in vivo, we have no other option but to rely upon the in vitro estimates.

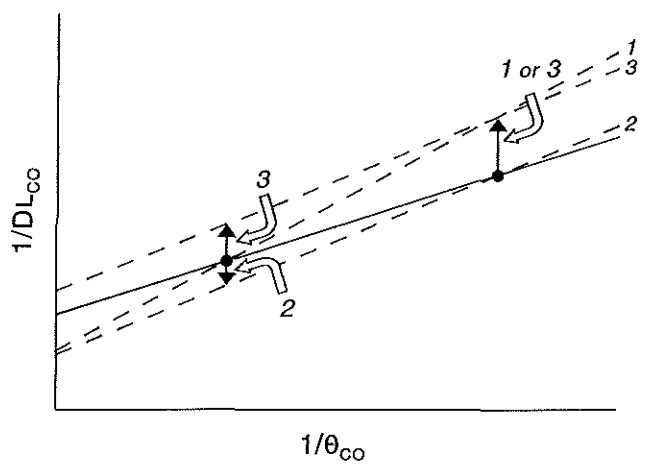

FIGURE 5. Diagram to show the effect of changes in DL_{CO} on Q_c estimates
DL_{CO} is related to θ_{CO} according to: $1/DL_{CO} = a + b \times 1/\theta_{CO}$, where a and b represent $1/D_M$ and $1/Q_c$ respectively. Thus Q_c varies in inverse proportion to the slope of $1/DL_{CO}$ versus $1/\theta_{CO}$.
Closed circles: determinations of $1/DL_{CO}$ at a low and high alveolar O_2 tension.
Arrows: corrections of $1/DL_{CO}$ when *1)* DL_{CO} is overestimated at high alveolar O_2 tension, *2)* DL_{CO} is underestimated at low alveolar O_2 tension, *3)* DL_{CO} is proportionally overestimated at both alveolar O_2 tensions. Slope of *solid line* represents actual calculated $1/Q_c$. Slopes of *broken lines* represent corrected $1/Q_c$. All corrections shown result in a steeper slope of the relationship of $1/DL_{CO}$ versus $1/Q_c$, and thus in lower Q_c values.

It can be derived from the Roughton-Forster equation, see formula (1), that the capillary blood volume will be overestimated, i.e. $1/Q_c$ underestimated, if DL_{CO} is:

1) CO overestimated (i.e. $1/DL_{CO}$ underestimated) at $F_{IO2}=0.94$; or

2) underestimated at $F_{IO2}=0.30$; or

3) proportionally overestimated at both inspiratory O_2 fractions.

This is also illustrated in FIGURE 5. DL_{CO} will be affected by CO back pressure, i.e. tension in pulmonary capillary blood. If back pressure is erroneously ignored, DL_{CO} will be underestimated. Correction of the diffusion gradient for back pressure will result in a smaller pressure difference between alveolar gas and capillary blood. DL_{CO} and consequently, capillary blood volume estimates will be corrected upward. In the present study, back pressure was considerable and thus could not be ignored, certainly not at the high alveolar O_2 tension as equilibrated CO tension is proportional to O_2 tension according to the Haldane relationship [9]. Mean *(SD)* carboxyhaemoglobin, HbCO, was 2.4% *(0.3)* before any determination of DL_{CO} was made. In non-smoking not professionally CO exposed healthy humans, normal values of 0.9% [28] and 1.8% [3] have been reported. In humans, the rate of reaction of CO with haemoglobin in the red cell, theta, is generally regarded to be so fast that plasma CO tension will be negligible. However, in pigs, theta is much lower than in humans [25]. Furthermore, Haldane's affinity constant M[1], which is a measure of the relative affinity of haemoglobin for CO and O_2, is almost twice as low in pigs compared to humans. In FIGURE 6, effects of changes in M and O_2 tensions on equilibrating CO tension are shown using pig and human blood as an example. Even though we corrected our data for back pressure, large errors may have crept into the estimate of the CO diffusing gradient (and consequently DL_{CO}), because of numerous approximations in the calculations. Back pressure was determined with use of the Haldane's relationship which is only valid when CO, O_2 and haemoglobin are in equilibrium. This may not have been the case during the in vivo conditions. The time necessary to reach equilibrium will have been affected by the ongoing ventilation during which CO is washed out and the large changes in inspiratory O_2 fraction. It is not inconceivable that back pressure has been overestimated, especially at the high inspiratory O_2 fraction, i.e. line 1 in FIGURE 5 would then apply to our results.

[1] $[HbCO]/[HbO_2] = M \times P_{CO}/P_{O2}$

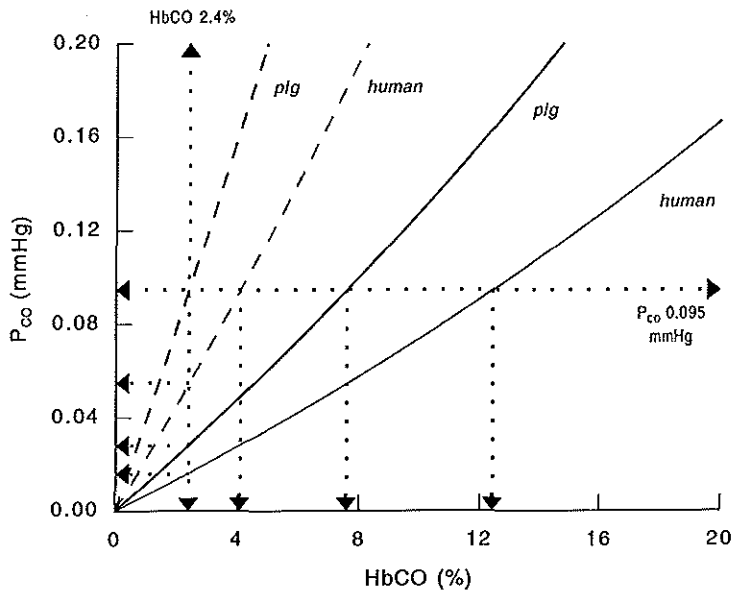

FIGURE 6. Diagram to show the effect of changes in M and O_2 tension on CO back pressure
Carbon monoxide back pressure (P_{CO}) is plotted on the Y-axis against carboxyhaemoglobin (HbCO) in % on the X-axis. Lines are constructed from Haldane's equation [9] for pig and human blood, assuming a P_{O2} of respectively 150 mmHg (solid lines) and 500 mmHg (broken lines) and using M=130 for pig blood [20] and M=225 for human blood [1]. In this graph, the equilibrating P_{CO} at the two different O_2 tension are shown for both species when HbCO is 2.4%. Also, differences in HbCO content in blood are shown for a prevailing P_{CO} of 0.095 mmHg.

RELATIONSHIP WITH ALVEOLAR VOLUME - In the present study, the decrease in capillary blood volume with alveolar volume was twice as small as compared to the decrease obtained in our previous study (TABLE 2) [24]. This is reflected in the differences between the two studies in partial regression coefficients of DL_{CO} at the two O_2 levels: the partial regression coefficients at $F_{IO2}=0.30$ are similar, but in the present study the coefficient at $F_{IO2}=0.94$ is lower (TABLE 2). Experimental conditions in the former and the present study were similar and pigs of the same breed, weight and age were used. CO back pressure was somewhat higher in the former study, particularly during

ventilation with 94% O_2 where back pressure ranged from 0.11 to 0.17 mmHg compared to 0.08 to 0.12 mmHg in the present study. This was probably due to the number of DL_{CO} determinations which was larger in the former study.

During inflation of the lungs with 25 $ml.kg^{-1}$, Q_{P+LH} decreased on average 2.3 $ml.kg^{-1}$ (95%CI: 1.7-2.8) compared to its value at the end of expiration (FIGURE 4). Versprille *et al.* [33] found a maximum decrease in total pulmonary blood volume of about 1.5 $ml.kg^{-1}$ at inflation volumes above 20 $ml.kg^{-1}$. In both studies, changes in pulmonary blood volume included changes in left heart volume. The pigs in the study of Versprille *et al.* had undergone thoracotomy, whereas our observations were done in intact pigs. It is our experience that cardiac output is about 10 to 20% lower after thoracotomy, and that in spite of re-establishment of negative intra-thoracic pressure at end-expiration, a complete recovery of cardiac output to pre-thoracotomy values does not occur. We cannot exclude that the difference in pulmonary blood volume changes between both studies results from the difference in experimental conditions.

In conclusion: Two DL_{CO} determinations were adequate to estimate capillary blood volume. The interpretation of changes in capillary blood volume with increasing alveolar volume with respect to changes in total pulmonary blood volume is complicated as the derivation of capillary blood volume may have been less accurate due to considerable CO back pressure. However, the data as presented in FIGURE 3 indicate that forced inflation of the lungs causes a considerable shift of blood from the capillary part of the pulmonary circulation into the systemic circulation (and not into the larger vessels of the pulmonary circulation). Such a shift would be disadvantageous to gas transfer during mechanical ventilation if the lungs are deeply inflated. The considerable CO back pressure in blood of our pigs could be ascribed to differences in theta (rate of uptake of CO by intracellular haemoglobin) and Haldane's affinity constant M which are both much lower in pigs than in humans.

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CHAPTER 6

FINAL CONSIDERATIONS

The Inspiratory Pause procedure has appeared to be useful in the determination of the diffusing capacity of the lungs for carbon monoxide (DL_{CO}) under conditions of mechanical ventilation. A study in which different inspiratory pause times were applied (CHAPTER 2) revealed an exponential decay of carbon monoxide which is a prerequisite for application of the conventional formula to calculate DL_{CO}. In this final chapter some aspects ensuing from our studies on gas transfer will be considered: firstly some difficulties encountered in the interpretation of DL_{CO} values between different studies, followed by uncertainties in the estimation of capillary blood volume, the pig as a model in gas transfer studies, and finally some suggestions for future research.

COMPARISON OF DL_{CO} VALUES BETWEEN DIFFERENT STUDIES

CO transfer values between different studies cannot easily be interpreted, because of differences in measuring and subject's conditions and in calculation procedure (TABLE 1). To overcome some of these differences, attempts have been made to standardize the procedure for estimation of DL_{CO} [2,8,14]. The resulting standardization reports were mainly focused on (breath-holding) DL_{CO} determinations at total lung capacity during spontaneous breathing. The guidelines presented by the different standardization reports are lacking consensus at some points, such as recommended inspiratory O_2 fraction and corrections for time of CO uptake. With regard to the mechanical ventilatory conditions, no standardization guidelines have been reported so far.

Below some factors which should be taken into account when calculating, interpreting and comparing DL_{CO} values will be considered: alveolar O_2 tension, haemoglobin concentration, CO back pressure and lung volume.

Alveolar O_2 Tension

Because of the competition between CO and O_2 for binding sites of haemoglobin, differences in alveolar O_2 tension will affect CO transfer in the lungs. For mutual comparison, it should either be standardized or a correction made. Standardization reports recommend an alveolar O_2 tension which should be in the normal range of 110-120 mmHg (=14.5-16 kPa). However, no consensus has been reached about the required inspiratory O_2 fraction: 0.21 [2,14] in the United States versus 0.17-0.18 [8] in the European community.

TABLE 1. Sources of variability in the estimation of pulmonary CO diffusing capacity

A. FACTORS RELATED TO MEASURING CONDITIONS

- ⇨ spontaneous breathing vs mechanical ventilation
- ⇨ technique used
  - *single-breath (SB)*
  - *multiple-breath: rebreathing (RB) or steady-state (SS)*
- ⇨ pattern of manoeuvre
  - *SB: time of inspiration, breath-hold and expiration; breath-hold lung volume; preceding lung volume (lung volume history)*
  - *RB: rebreathing volume; rebreathing frequency; mean lung volume*
- ⇨ test gas mixture: F_{ICO}, F_{IO2}, inert tracer gas for estimation lung volume by dilution
- ⇨ equipment (like gas analyzer and flow measurement)
- ⇨ position: lying (supine or prone), upright (sitting or standing)
- ⇨ number of and time between successive tests

B. CALCULATION

- ⇨ alveolar sample: timing and size of sample
- ⇨ washout volume: instrument and anatomical (Fowler) dead space
- ⇨ alveolar volume estimation: independent measure or included in D_{LCO} determination
- ⇨ applied corrections
  - *time during which CO is taken up: uptake during inspiration and expiration (alveolar sampling)*
  - *volume of which CO is taken up (dead space correction; maximum or effective alveolar volume)*
  - *CO back pressure*

C. FACTORS RELATED TO SUBJECT

- ⇨ species
- ⇨ gender
- ⇨ body weight, age, maturation
- ⇨ body temperature
- ⇨ pH blood
- ⇨ haemoglobin concentration
- ⇨ pre-exposure to CO (concentration and length of exposure)
  - *smoking, occupational exposure, industrial environment*

Correction to a standard alveolar O_2 tension can be done by adjusting theta for CO (θ_{CO}) to the standard alveolar O_2 tension and then recalculating DL_{CO}. However, this requires estimation of capillary blood volume and membrane diffusing capacity, thus DL_{CO} determinations at different inspiratory O_2 fractions. The correction can also be done arithmetically. Empirically derived factors have been reported to account for changes in alveolar O_2 tension [16,26]. As these factors were obtained and verified in a select group of humans, it is uncertain whether they also hold under varying conditions and in different species.

Haemoglobin Concentration

In literature, 14.4-14.6 $g.dl^{-1}$ (=8.9-9.1 $mmol.L^{-1}$) has been considered an average normal haemoglobin (Hb) concentration in humans. As DL_{CO} depends on the Hb concentration, it has been recommended to correct DL_{CO} values to this (standard) value to enable comparison [2,8]. However, all current methods of adjusting for Hb involve unproved assumptions. Two main methods (see TABLE 2) can be distinguished to correct for differences in Hb concentration: 1) via measurement of capillary blood volume and membrane diffusing capacity [8,9]; and 2) arithmetically [3,8,9,11].

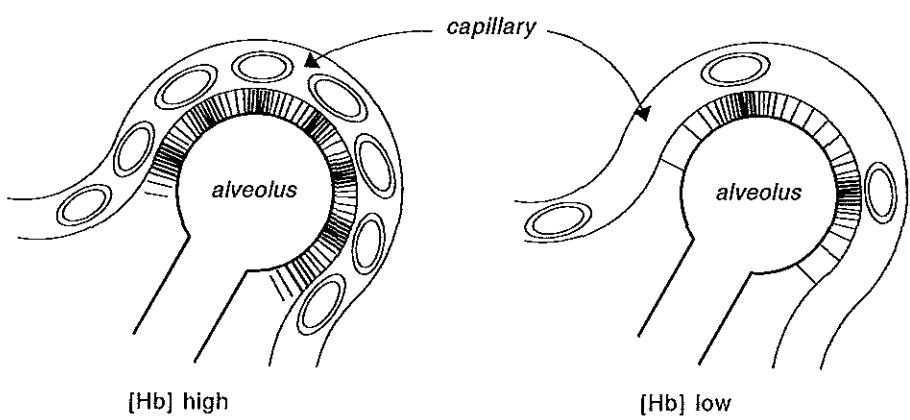

FIGURE 1. Schematic representation of the alveolar-capillary membrane
The shaded part represents the effective area for gas transfer from alveolar gas to haemoglobin in the red cells. The figure demonstrates that the effective membrane area may depend upon the haematocrit, i.e. haemoglobin concentration [Hb], of the pulmonary capillary blood.

The first method assumes that the capillary blood volume (Q_c) and membrane diffusing capacity (D_M) are unaffected by changes in Hb and that only θ_{CO} varies in direct proportion to Hb. However, FIGURE 1 demonstrates that the *effective* pulmonary membrane area (i.e. D_M) may be dependent upon the haemoglobin concentration in the capillary blood. Burrows and Niden [4] already suggested that gas diffusion across the alveolar-capillary membrane occurs largely in areas where this membrane is in direct contact with red blood cells. Data obtained by Jouasset-Strieder *et al.* [25] lent support to this concept. They demonstrated a proportional relationship between DL_{CO} and blood Hb in the absence of any significant change in capillary blood volume during experimentally induced anaemia in dogs.

TABLE 2. Equations to adjust the observed DL_{CO} to a standard Hb concentration

ADJUSTMENT OF DL_{CO} WHEN Q_c AND D_M ARE KNOWN	REFERENCES
$1/DL_{CO} = 1/D_M + 1/(\theta_{CO} \times F_{Hb} \times Q_c)$	*Cotes et al.* [8,9]
ADJUSTMENT OF DL_{CO} WHEN Q_c AND D_M ARE UNKNOWN - EMPIRICALLY DERIVED EQUATIONS (ARITHMETICAL) -	
DL_{CO} (*adjusted*)^a = DL_{CO} (*observed*) × ([10.22 + Hb]/1.7 Hb)	*Cotes et al.* [8,9]
DL_{CO} (*adjusted*)^b = DL_{CO} (*observed*) / (0.06965 × Hb)	*Dinakara et al.* [11]
DL_{CO} (*adjusted*)^c = DL_{CO} (*observed*) × (0.5 + [(15/Hb) × 0.5]	*Burgess et al.* [3]

F_{Hb} is the Hb concentration as a fraction of the assumed standard concentration; Hb is actual haemoglobin concentration; $^{a\ b\ c}$ adjusted to Hb of respectively 14.6, 14.4 and 15 $g.dl^{-1}$. See text for further explanation.

The arithmetical approaches to correct DL_{CO} for changes in Hb have resulted in different equations (see TABLE 2). The equation offered by Cotes *et al.* [8,9] was based on the Roughton-Forster equation assuming a D_M/Q_c ratio of 0.7. The empirically derived equation of Dinakara *et al.* [11] was based on DL_{CO} determinations in a group of patients with Hb concentrations ranging from 6.7 to 16.8 $g.dl^{-1}$. Burgess and associates [3] corrected their DL_{CO} estimates in dogs to a Hb concentration of 15 $g.dl^{-1}$

assuming that the membrane and cellular resistances, i.e. $1/D_M$ and $1/(\theta \cdot Q_c)$ respectively, to gas transfer are equal. In the pigs, we found a mean ("normal") Hb concentration of 10.2 $g.dl^{-1}$ (=6.3 $mmol.L^{-1}$), which is lower than in humans. Should the DL_{CO} values as observed in pigs be corrected to a Hb concentration which is regarded normal in humans? If such a correction would be meaningful, which method or equation would then be most appropriate to use? The first method (i.e. via measurement of capillary blood volume and membrane diffusing capacity) which we used for want of anything better available, would probably underestimate the correction of DL_{CO}. The different arithmetically derived equations, which may be inappropriate to use in pigs, would produce widely divergent adjusted DL_{CO} values as the change in Hb is rather large.

CO Back Pressure

In the introduction of this thesis (CHAPTER 1), emphasis was placed upon the advantage of the use of low concentrations CO instead of O_2 for the estimation of gas transfer in the lungs, namely that the capillary tension of this gas (CO) can be ignored. Although in (human) practice, CO tension in blood is often considered zero in calculating the diffusion gradient for CO, this can be criticized on two counts:

1) the rate of reaction of CO with haemoglobin inside the red cell (θ_{CO}), although extremely fast, is still not fast enough to maintain plasma P_{CO} equal to zero;

2) there is apparently always some carboxyhaemoglobin (HbCO) in blood, its percentage being dependent on endogenous CO production caused by haem breakdown (haemoglobin turnover) and exogenous CO exposure (such as smoking, profession, industrial environment, successive DL_{CO} determinations within a short period of time), which both will produce CO back pressure.

To estimate CO back pressure, two main techniques can be used:

1) calculation of CO back pressure for any given O_2 tension from measured HbCO and HbO_2 in peripheral blood with use of the Haldane's relationship [12];

2) measurement of the CO concentration in an alveolar gas sample after equilibration with pulmonary capillary blood, either by the subject's rebreathing in a closed system (in which the O_2 concentration is kept constant and from which CO_2 is absorbed) or by breath-holding for 1 to 2 minutes [24].

We found that CO back pressure in our pigs, as measured with the first method, became an important fraction of alveolar P_{CO} (see TABLE 3). The pigs were bred at a

farm which was located nearby a motorway. Another source of CO might have been a higher red cell (i.e. haemoglobin) turnover, however, we could not provide evidence for this from literature. Factors contributing to the development of CO back pressure were: the lower θ_{CO} in pig blood compared to that in human blood (given in CHAPTER 3), and the much lower affinity of pig haemoglobin for CO in competition with O_2 compared to human haemoglobin, especially at high O_2 concentrations as P_{CO} varies in proportion to P_{O2} (see also FIGURE 6 of CHAPTER 5).

It should be noted that, when back pressure is considerable, the final DL_{CO} results may be unreliable, even when the CO gradient has been corrected for back pressure (which we did), because of the numerous approximations in the calculation.

*TABLE 3. Minimum and maximum values of CO back pressure**

F_{IO2}	initial (%)	final (%)
0.30	3.5-5.0	7.9-14.3
0.94	11.9-17.0	18.7-31

Values are back pressures expressed as a percentage of respectively initial (t_0) and final (t_s) alveolar carbon monoxide tensions; averaged minimum and maximum values are given. F_{IO2}, inspiratory oxygen fraction.

** data are from DL_{CO} determinations of the study presented in CHAPTER 5*

Alveolar Volume

In normal humans, an increase in DL_{CO} has been associated with expansion of the lungs by spontaneous inhalation, i.e. increase in alveolar volume [35]. Data presented in this thesis, which were obtained during mechanical ventilation, demonstrated that DL_{CO} measured at "low" inspiratory O_2 fractions (0.20-0.30) hardly changed, with a tendency to decrease, with increasing alveolar volume. DL_{CO} decreased significantly when measured at high inspiratory O_2 fractions (0.60 and 0.94). For interpretation of DL_{CO} values, it is important to know at which O_2 fraction these were obtained and how the lungs were inflated: either voluntarily (spontaneously) or forced. To enable comparison between different species, DL_{CO} has been expressed per kg body weight or per litre alveolar volume (i.e. DL_{CO}/V_A), although DL_{CO} may be more closely matched

to other variables, e.g. metabolic capacity as suggested by Carlin *et al.* [5], than those related to body size. Instead of relating DL_{CO} to absolute values of alveolar volume, the degree of lung inflation expressed as a percentage of total lung capacity may be a better variable. However, unlike the estimation of alveolar volume (sum of end-expiratory and tidal volume), total lung capacity can only be roughly estimated during mechanical ventilation.

UNCERTAINTIES IN THE ESTIMATION OF Q_c

Capillary blood volume estimates may be inaccurate because of uncertainties and approximations in the determination of CO transfer in the lungs (DL_{CO}), theta or both.

CO Transfer in the Lungs

The capillary blood volume, as derived from the Roughton-Forster equation [33], depends on the accuracy of the DL_{CO} determinations at the different inspiratory O_2 fractions. DL_{CO} in turn depends on many parameters, see TABLE 1. Errors related to calculation of DL_{CO} (heading B in TABLE 1) will result in inaccurate values of DL_{CO} for a particular subject and condition. For example, when DL_{CO} is estimated at two different O_2 fractions, and is overestimated at the highest one, the capillary blood volume will be overestimated. This and other examples are shown in FIGURE 5 of CHAPTER 5.

Theta

Different techniques (stop-flow vs continuous-flow vs thin film) have provided different θ_{CO} values. The results of the stop-flow technique are regarded less reliable compared to the other techniques, because of the presence of a stagnant layer around the red cells which is rapidly formed after cessation of flow and acts as an additional diffusion barrier [7]. Such a stagnant layer will result in an underestimation of θ_{CO}, most likely at low O_2 tensions (i.e. when the competition between O_2 and CO is lowest). Reeves and Park [32] claimed that in the thin film technique, developed by Heidelberger and Reeves [21], the unstirred extra-cellular, i.e. stagnant, layers are considerably reduced. According to them this would explain the higher θ_{CO} as found with this technique compared to the continuous-flow estimates [15]. No real evidence

was provided to support their assumption of the existence of considerable stagnant layers in the continuous-flow apparatus, and this should therefore be further investigated. All three techniques have in common that they provide *in vitro* estimates of θ_{CO}. Many uncertainties exist whether the in vitro obtained θ_{CO} values will approximate the rate of uptake of CO by the red blood cells in vivo, as the in vitro and in vivo conditions are quite different. During in vivo conditions, blood will take up CO while flowing, which is not the case in the stop-flow and thin film technique. Furthermore, red blood cells will be "deformed" to some extent while passing the capillaries, whereas we are unsure about the shape of red blood cells during in vitro conditions. The red cells may be affected by the turbulent flow in the stop-flow and continuous-flow rapid mixing techniques or by the buffer solutions used. It also remains questionable whether the θ_{CO} values derived from diluted cell suspensions, as is the case in stop-flow and continuous-flow techniques, can be extrapolated to whole blood.

CHAPTER 3 provides evidence that θ_{CO} is species dependent. Also Holland [23] and Lawson [29] have reported species dependency, however, their results were obtained with the stop-flow technique. They both obtained higher θ_{CO} values for dog than for human blood, whereas the continuous-flow technique did not reveal any difference between the two species [10]. When studying gas transfer in the lungs of species other than human, one should be aware of differences in theta between species.

The rate of CO uptake by red blood cells may be affected by blood temperature and pH, which has been scarcely documented in literature. Most in vitro estimates of θ_{CO} were obtained at 37°C and physiological pH. Carlsen and Comroe [6] reported an increase of about 25% in the overall rate of CO uptake by red cells when changing the temperature from 38 to 48°C with use of a continuous-flow technique. Other available data on the effect of temperature on θ_{CO} were based on stop-flow measurements [23,29]. With regard to pH, continuous-flow measurements at pH 7.4 revealed a different relationship of $1/\theta$ versus P_{O2} than that obtained at pH 8.0 [15]. Krawiec *et al.* [28] showed that when pH was increased, θ_{CO} decreased by about 8.8% per pH unit, which can be regarded insignificant.

PIG AS A MODEL IN CO TRANSFER STUDIES

Why did we use pigs as a model in our studies of gas transfer? Initially, we had planned to study the effect of mechanical ventilation on gas transfer in the lungs and the pulmonary vascular bed in respiratory distress (i.e. acute lung failure). At this laboratory, two different respiratory distress models had successfully been developed in pigs [17,18]. For this reason, we chose pigs as a model in our studies. The effect of mechanical ventilation on carbon monoxide transfer in the lungs has only scarcely been documented in the literature. Therefore, the studies described in this thesis were restricted to its effect in healthy lungs in order to create a solid base for studies on the effect of mechanical ventilation on gas transfer in diseased lungs.

The interest in pigs as an animal model for human diseases has been growing over the last few decades, mainly based on similarities between the two species. Studies have proven that pigs constitute a valuable species in a wide variety of scientific areas, like circulation physiology, immunology, surgery and pharmacology [36]. However, for studies on pulmonary gas transfer and its components with use of carbon monoxide, pigs may not be the ideal model. Pulmonary CO transfer determinations may be less reliable as CO back pressure in pig blood is considerable according to our data which were derived with use of the Haldane's equation (see CHAPTER 5).

SUGGESTIONS FOR FUTURE RESEARCH

The high CO back pressure estimates found in pig blood intrigue us when compared to values reported in non-smoking humans. We reasoned that it could be ascribed to differences in the rate of uptake of CO by haemoglobin in red blood cells and in the affinity of haemoglobin for CO between pigs and humans. The derivation of back pressure via the Haldane relationship, the method we used, relies on an accurate estimate of the affinity constant M, an in vitro estimate, which in turn may depend on temperature and pH. Furthermore, the Haldane equation is only valid when CO, O_2 and haemoglobin are in equilibrium, which may not have been the case during the in vivo conditions. Because of these uncertainties, it would be valuable to determine the back pressures during ventilation with different O_2 fractions in a group of pigs of the same breed with another, probably more reliable, method. This could be done by

rebreathing into a closed system in which the O_2 concentration is kept constant and from which CO_2 is absorbed.

The problem of elevated back pressures has drawn our attention to (known and unknown) differences between species. Is it reasonable to assume that the constitution and/or configuration of haemoglobin varies between species and even between different subjects or breeds of the same species? With regard to the Haldane's affinity constant, wide interspecies variation have been reported in literature (see TABLE 4). The rate of uptake of CO by red cells also seems species dependent although no consensus about absolute values has been reached so far. Such differences would have important consequences with regard to the interpretation of data obtained on gas transfer in animal models and in the extrapolation of these data to the human situation. It may therefore be worthwhile to set up an investigation in which the uptake of CO by red cells and free Hb, velocity constants of binding and unbinding of CO, and affinity of Hb for CO are estimated at varying O_2 tensions and for different species. Doing this, one must have confidence in the in vitro estimates. Furthermore, the measurements would be laborious and time-consuming. It may, however, be helpful in the decision-making of an appropriate animal model for studies of gas transfer.

TABLE 4. Values of Haldane's affinity constant (M) for several mammalian species

species	M value
human	*225* (Allen & Root [1]), *210* (Sendroy & O'Neal [34]), *224/290* (Douglas et al. [12])
dog	*225* (Allen & Root [1]), *228* (Sendroy & O'Neal [34])
pig	*130* (Klimisch et al. [27])
rat	*192* (Allen & Root [1]), *141* (Klimisch et al. [27])
mouse	*148* (Douglas et al. [12])
rabbit	*109* (Klimisch et al. [27])
hamster	*181* (Klimisch et al. [27])
opossum	*247* (Sendroy & O'Neal [34])
monkey	*195* (Sendroy & O'Neal [34])
sheep	*162* (Sendroy & O'Neal [34])

M is defined by the relation $[HbCO]/HbO_2] = M \times P_{CO}/P_{O2}$

Studies of CO Transfer in Respiratory Distress

Compared to studies of carbon monoxide transfer in ventilated subjects with healthy lungs, studies in ventilated subjects with respiratory distress would involve even more problems. For instance, the choice of measuring technique: with regard to DL_{CO} determinations in these subjects with marked inhomogeneity of gas distribution, the rebreathing technique is generally regarded superior to the single-breath technique. However, the single-breath technique would interfere less with the normal ventilatory mode. Also, the determination of gas transfer at different alveolar volumes is much easier to perform with use of the single-breath technique.

Furthermore, the use of the helium dilution in diseased lungs as a measure of the initial alveolar CO concentration before CO is taken up, and as a measure of lung volume from which carbon monoxide is taken up, may lead to errors. Helium is much lighter than CO (molecular weight respectively 4 and 28), and will therefore diffuse more rapidly than CO. Compared to healthy lungs, the time required for optimal mixing of the inspired gas with the alveolar gas will be prolonged in respiratory distress. Then, the assumption that helium and CO dilute in a comparable way may be incorrect.

Capillary blood volume, from DL_{CO} determinations at a low and high O_2 fraction, can only be estimated when the patient's respiratory failure is of such a severity that it is allowed to ventilate with an O_2 fraction as low as 0.30. The only study we could find in which capillary blood volumes were obtained in patients with respiratory failure (only in the mild cases) was that of Macnaughton *et al.* [31]. In severe respiratory distress, subjects are usually ventilated with O_2 fractions of 0.60 or even higher. In addition, the pulmonary vascular bed is not uniformly affected in respiratory distress; active vasoconstriction, oedema and vascular clotting may be inhomogeneously distributed throughout the lungs [19]. An effect of large changes in O_2 fraction on the pulmonary vascular bed can therefore not be excluded.

Pulmonary vascular obstruction (e.g. pulmonary emboli) and haemorrhagic oedema are features which may occur in respiratory distress. Such stagnant intravascular (or slowly flowing) blood distal to vascular obstructions and extravasated blood will also take up CO when in contact with alveolar gas, however, will not clear CO. The presence of stagnant blood was shown to cause an apparent increase in CO diffusing capacity of the lung during breath-holding [13,30]. On the other hand, the CO uptake in the lungs was shown to diminish progressively on repeated exposure to CO [20,22].

Thus, DL_{CO} estimates in the presence of stagnant blood, as may be the case in respiratory distress, should be carefully interpreted.

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"Een proefneming kan zó mislukken,
dat zij voert tot gewichtige
ontdekkingen."

OTTO WEISS

SUMMARY

During forced inflation, i.e. mechanical ventilation, total pulmonary blood volume was found to decrease [Versprille *et al.*, 1990]. As we have been interested in gas transfer from the alveolar gas to capillary blood during mechanical ventilation, we wondered whether the capillary part of the pulmonary circulation, which is a main determinant of gas transfer, would be similarly affected. This would be disadvantageous to gas transfer whereas mechanical ventilation is meant to improve it.

This thesis comprises studies on gas transfer and blood volume in the lungs during mechanical ventilation in healthy pigs. A procedure was described for determination of pulmonary gas transfer during forced inflation with use of carbon monoxide (CO). From such measurements, also the capillary blood volume could be derived.

In ***CHAPTER 1*** some general aspects were considered against the background of this thesis. First, a review was given of the historical aspects of the determination of pulmonary carbon monoxide transfer, also called diffusing capacity (DL_{CO}). Next, we explained the derivation of the capillary blood volume from DL_{CO} determinations at different alveolar O_2 tensions using the Roughton-Forster relationship (see FIGURE 1 of CHAPTER 1). Roughton and Forster showed in 1957 that the reciprocal of DL_{CO}, i.e. the resistance of the lungs to diffusion of carbon monoxide equals the sum of two resistances connected in series: 1) the resistance to diffusion of CO of the alveolar-capillary membrane; and 2) the resistance to passage of CO into the red cell and chemical binding to haemoglobin. For a better understanding and interpretation of gas transfer determinations obtained during forced inflation, the circulatory responses to changes in intrapulmonary pressure during mechanical ventilation were considered and compared with those during spontaneous breathing. Finally, an outline of this thesis was given.

In ***CHAPTER 2***, the effect of forced inflation on carbon monoxide diffusing capacity (DL_{CO}) was studied at different alveolar volumes in anaesthetized and paralysed healthy pigs. For this, an inspiratory pause procedure (equivalent of the single-breath technique during spontaneous breathing) was developed which consisted of a pause in between an inspiration and expiration. Both inspiration and expiration were applied at a constant flow rate. The inspiratory pause procedures, as well as the normal ventilation in between those procedures, were computer-controlled and could thus be well standardized. During the inspiratory pause a test gas mixture containing a low concentration labelled CO (0.3%), helium (5-10%) and balance air was inflated.

Labelled CO ($C^{18}O$) was used so that all gas concentrations could be measured with use of a mass spectrometer. Lung volume was derived from the helium dilution. In the calculation of DL_{CO}, corrections were made for time and volume from which carbon monoxide was taken up (see FIGURE 2 of CHAPTER 2), apparatus and anatomical dead space and CO back pressure.

In five pigs (mean body weight 12.7 kg), alveolar volume was varied at constant inflation volume by increasing the positive end-expiratory pressure (PEEP) from 2 to 10 cmH_2O. Also the (post)inspiratory pause time was varied from 1 to 8 seconds to verify whether the decay of CO was exponential which is a precondition for application of the conventional formula to calculate DL_{CO}. In another group of nine pigs (mean body weight 11.2 kg), alveolar volume was varied at constant PEEP (2 cmH_2O) by changing inflation volume from 15 to 30 $ml.kg^{-1}$ in steps of 5 $ml.kg^{-1}$.

An exponential decay of CO was always obtained. DL_{CO} did not change significantly when alveolar volume was increased by either changing PEEP or inflation volume. Since the diffusing capacity of the pulmonary membrane is expected to increase with increasing alveolar volume, the constant DL_{CO} may be attributed to a decrease in capillary blood volume.

In order to test this hypothesis, the capillary blood volume and membrane diffusing capacity have to be known at increasing alveolar volume. Both variables can be estimated from the Roughton-Forster equation, if the rate of uptake of carbon monoxide by haemoglobin in the red cells (θ_{CO}) is known. As no θ_{CO} values were available for pig blood, a study was initiated to measure theta in pig blood of which the results are presented in ***CHAPTER 3.***

The measurements on θ_{CO} were performed on blood of five different female pigs. θ_{CO}, the ml CO taken up by one ml of whole blood per minute per mmHg CO tension (traditional units), was determined on each blood sample at 37°C and pH 7.4, at four to five different oxygen tensions. We used a continuous-flow rapid-mixing technique. The method is based on rapid mixing of an erythrocyte suspension containing no carboxyhaemoglobin and a buffer solution containing carbon monoxide, both of which have been equilibrated at the same pre-set O_2 tension. The extent of the reaction of haemoglobin with CO was determined with use of double-beam spectrophotometry.

For each pig blood sample, a regression line of $1/\theta_{CO}$ versus P_{O2} was obtained. These lines (five in total) were not significantly different with respect to slope and

elevation. Therefore, a common regression equation was calculated: $1/\theta_{CO} = 0.0084 \times P_{O2} + 0.63$. The slope of this regression line was significantly steeper than the slopes of the regression lines for human [Krawiec *et al.*, 1983] and dog [Crapo *et al.*, 1989] erythrocytes which had been obtained at the same laboratory under the same conditions. We calculated the error which we would make in the estimation of capillary blood volume from diffusing capacity determinations in pigs, if θ_{CO} values for human or dog erythrocytes are used. This would result in a considerable underestimation of about 50%.

The equation derived in CHAPTER 3 was used in the study described in ***CHAPTER 4*** in which capillary blood volume (Q_c) and membrane diffusing capacity (D_M) were derived at increasing alveolar volume in eight anaesthetized and paralysed healthy pigs (mean body weight 11.2 kg). DL_{CO} was determined with use of the inspiratory pause procedure (see CHAPTER 2); for each alveolar volume at three different alveolar O_2 tensions. Alveolar volume was changed at constant PEEP by inflating 15, 20, 25 and 30 $ml.kg^{-1}$ in random order. As the θ_{CO} equation in CHAPTER 3 was based on whole blood with a standard CO capacity of 0.2 ml per ml, i.e. haemoglobin concentration of 8.9 $mmol.L^{-1}$ (=14.4 $g.dl^{-1}$), θ_{CO} was adjusted to the actual haemoglobin concentration before calculating Q_c and D_M.

DL_{CO}, at an inspiratory oxygen fraction of 0.30, decreased on average by 11.8 $\mu mol.s^{-1}.kPa^{-1}$ for each litre BTPS increase in effective alveolar volume. Capillary blood volume also decreased, on average 241 ml. The membrane diffusing capacity slightly increased by 2.7 $\mu mol.s^{-1}.kPa^{-1}$. The increase in membrane diffusing capacity was much smaller than might be expected from the increase in alveolar volume. This, we ascribed to a loss of the alveolar-capillary membrane for gas transfer due to the concomitant decrease in capillary blood volume. The decrease in capillary blood volume may be explained by a squeezing effect of the intrapulmonary pressure rise on the alveolar wall during forced inflation and by stretching of lung tissue.

The absolute values of capillary blood volume presented in CHAPTER 4, were rather large when compared to data obtained during forced inflation in other species (human and dog) taking differences in body weight and alveolar volume into account. Furthermore, its decrease with increasing alveolar volume appeared to be larger than the decrease observed for total pulmonary blood volume during mechanical ventilation

in pigs [Versprille *et al.*, 1990]. In an additional study, presented in ***CHAPTER 5***, the relationship between changes in capillary and total pulmonary blood volume was studied in ten pigs (mean body weight 10.5 kg) at different inflation volumes, as lung inflation may affect both blood volumes differently.

Capillary blood volume was derived from DL_{CO} determinations at two different alveolar O_2 tensions and using the Roughton-Forster relationship (see also CHAPTER 2 to 4). Total pulmonary blood volume including left heart volume (Q_{P+LH}) was derived with use of a double-indicator-dilution technique by injecting cold hypertonic saline into the right atrium. Q_{P+LH} was calculated as the product of thermodilution cardiac output and the difference in mean transit time between temperature and conductivity changes sensed in the pulmonary artery and aortic arch respectively.

We showed, based on data from CHAPTER 4, that Q_c could be satisfactorily derived from just two DL_{CO} determinations. Q_c and Q_{P+LH} decreased to a similar degree with increasing alveolar volume, 106 (95%CI: 53-158) and 111 (95%CI: 75-146) ml respectively for each litre BTPS increase in alveolar volume. A significant partial correlation coefficient (adjusted for differences between pigs) of the relationship between Q_c and Q_{P+LH} of 0.40 ($p<0.001$) was found. These data indicate that forced inflation of the lungs causes a considerable shift of blood from the capillary part of the pulmonary circulation into the systemic circulation. Such a shift would be disadvantageous to gas transfer during mechanical ventilation if lung inflation is deep.

The relation between capillary (Q_c) and total pulmonary blood volume (Q_P) may, however, be more complicated. If a left heart volume of about 20 ml was assumed, a mean Q_c/Q_P ratio of 0.75 would be found. As total pulmonary blood volume values were in proportion to those reported for other species (human), Q_c values were likely to be overestimated. This may have been due to CO back pressure, which was considerable. Even though our data were corrected for back pressure, large errors could have crept into the estimate of the CO diffusing gradient because of numerous approximations in the calculation. We reasoned that the relative high values of back pressure in pig blood could be ascribed to differences in theta (rate of uptake of CO by intracellular haemoglobin) and Haldane's affinity constant M (a measure of the competition of CO with O_2 for unoccupied haem binding sites), which are both lower in pigs than in humans.

CHAPTER 6 comprises some final considerations with regard to difficulties in the interpretation of DL_{CO} values and comparison between different studies. Attention was paid to differences in alveolar O_2 tension, haemoglobin concentration, CO back pressure (i.e. CO tension in blood which lowers the diffusion gradient for CO) and lung volume. Furthermore, uncertainties in the determination of DL_{CO} and the in vitro estimates of theta came up for discussion, both of which will affect the capillary blood volume estimates. Subsequently, the use of pigs as a model in pulmonary CO transfer studies was discussed. and finally, some suggestions for future research were considered.

SAMENVATTING

Tijdens een geforceerde inademing onder beademingscondities neemt het totale longbloedvolume af. Aangezien onze interesse uitgaat naar de gasoverdracht van de longblaasjes (alveoli) naar het bloed in de longhaarvaten (capillairen), wierp dit de vraag op of het capillaire deel van de longcirculatie evenzo zou afnemen. Dit zou nadelig zijn voor de gasoverdracht terwijl beademing juist wordt toegepast om de gasoverdracht te verbeteren.

In dit proefschrift zijn resultaten weergegeven van een aantal onderzoekingen naar de gasoverdracht in de longen en veranderingen in het (capillaire) longbloedvolume tijdens beademing. Aangezien er over dit onderwerp nog maar weinig bekend is, hebben we voor dit onderzoek gekozen voor varkens met gezonde longen als eerste aanzet om de gasoverdracht tijdens beademing in kaart te brengen. Een procedure is opgezet waarmee de gasoverdracht, ook wel diffusiecapaciteit genoemd, tijdens beademing kan worden bepaald door middel van inflatie (opblazen) van de longen met een gasmengsel dat een lage concentratie koolmonoxide (CO) bevat.

In ***HOOFDSTUK 1*** zijn een aantal begrippen aan de orde gekomen. Er is een historisch overzicht gegeven van de ontwikkeling van methoden om de diffusiecapaciteit (DL_{CO}) te bepalen. DL_{CO} wordt omschreven als het volume CO dat de longmembraan per tijdseenheid passeert als het partiële drukverschil tussen gas in de alveoli en dat in de capillairen één eenheid van druk bedraagt. De eenheid van DL_{CO} is $\mu mol.s^{-1}.kPa^{-1}$ (SI eenheden) of $ml.min^{-1}.mmHg^{-1}$ (traditionele eenheden). Vervolgens is uitgelegd hoe het capillaire longbloedvolume kan worden berekend uit bepalingen van de DL_{CO} bij verschillende zuurstof (O_2) concentraties in het inademingsgas. Hierbij wordt gebruikt gemaakt van de vergelijking zoals opgesteld door Roughton and Forster in 1957 (schematisch weergegeven in FIGUUR 1 van HOOFDSTUK 1). Zij toonden aan, dat het omgekeerde van DL_{CO}, d.w.z. de totale weerstand die CO bij de diffusie in de longen ondervindt, bepaald wordt door de som van een tweetal weerstanden: 1) de weerstand die het gas ondervindt bij passage van de longmembraan (die alveoli en capillairen van elkaar scheidt), hiertoe wordt gerekend alles wat zich tussen het gas in de alveoli en de membraan van de rode bloedcellen in de capillairen bevindt; 2) de weerstand die het gas ondervindt bij de diffusie in de rode bloedcel en chemische binding aan het hemoglobine. Voorts is een aantal veranderingen die de circulatie ondergaat tijdens beademing vergeleken met die tijdens spontaan ademen. Voor interpretatie van de resultaten in dit proefschrift is het belangrijk deze verschillen te kennen. Als laatste is in een kort overzicht de inhoud van de komende hoofdstukken

gegeven.

In ***HOOFDSTUK 2*** is het effect van een geforceerde inflatie van de longen op de diffusiecapaciteit van koolmonoxide bestudeerd en wel bij verschillende longvolumes. Hiervoor zijn metingen verricht aan varkens onder narcose en met spierverslapping. In dit hoofdstuk wordt de opzet van een inspiratoire pauze-procedure (IP-procedure) beschreven, die te vergelijken is met de "single-breath" (letterlijk vertaald: een enkele teug) procedure die wordt toegepast tijdens spontaan ademen. De IP-procedure begint met een inflatie van de longen, gevolgd door een pauze van bepaalde duur (de inspiratoire pauze) op het inflatievolume-niveau en eindigt met een expiratie (uitademing). Van belang is dat de gehele procedure, alsmede de beademing tussendoor, computergecontroleerd plaatsvond. Hierdoor is het mogelijk de procedure te standaardiseren en de longen met een constante snelheid op te blazen en leeg te laten lopen. Tijdens de IP-procedure werd een gasmengsel ingeblazen dat 0,3% CO, circa 5% helium, 20 of 30% O_2 en aanvullend stikstof bevatte. Aangezien het gas helium niet wordt opgenomen in bloed, zal het zich verdelen over het gasvolume dat in de longen aanwezig is voorafgaand aan de adempauze. Hierbij treedt een verdunning van het gas op. Uit de verdunningsfactor van helium kan het longvolume berekend worden. Voor de berekening van de diffusiecapaciteit van de longen voor CO moeten het longvolume, de inspiratoire pauzeduur en de afname in de CO concentratie tijdens de inspiratoire pauze bekend zijn. Voor berekening van de afname in de CO concentratie werd de CO concentratie van zowel het ingeademde als het uitgeademde gasmengsel gemeten met behulp van een massaspectrometer. Bij de berekening van DL_{CO} werden correcties doorgevoerd voor de tijd en het volume van de CO opname (zie FIGUUR 2 van HOOFDSTUK 2), de dode ruimte van de apparatuur en de luchtwegen, en de CO spanning in het capillaire bloed, ook wel "tegendruk" genoemd. De tegendruk van CO in bloed wordt bepaald door de blootstelling aan CO anders dan door de IP-procedure van dat moment zelf, zoals door roken, beroep, woonomgeving (industrieën) of voorafgaande IP-procedures.

In een groep van vijf varkens (gemiddeld lichaamsgewicht 12,7 kg), werd het longvolume vergroot door het opleggen van een positieve eind-expiratoire druk, in de wandelgangen PEEP genoemd, van 2 of 10 cm waterdruk (cmH_2O), bij gelijkblijvend inflatievolume. Op beide longvolume-niveaus werd de pauzetijd gevarieerd van 1 tot 8 seconden om na te gaan of de CO concentratie tijdens de inspiratoire pauze

exponentieel afneemt. Dit laatste is een voorwaarde voor toepassing van de conventionele formule om DL_{CO} te berekenen. In een andere groep van negen varkens (gemiddeld lichaamsgewicht 11,2 kg), werd het longvolume vergroot, bij een constante PEEP van 2 cmH_2O, door verschillende inflatievolumes op te leggen: 15, 20, 25 en 30 ml per kg.

Op beide PEEP-niveaus werd een exponentiële afname in de CO concentratie gevonden. DL_{CO} veranderde niet bij vergroting van het longvolume, ongeacht of dit door middel van PEEP of inflatievolume bewerkstelligd was. Aangezien het waarschijnlijk is dat de diffusiecapaciteit van de longmembraan toeneemt bij toename van het longvolume (gebaseerd op bevindingen tijdens spontaan ademen en morfologische onderzoeken), zou een afname in capillair longbloedvolume het constant blijven van DL_{CO} kunnen verklaren.

Bovenstaande hypothese kan worden getoetst door het capillaire longbloedvolume (Q_c) en de membraandiffusiecapaciteit (D_M) onder beademingsomstandigheden bij verschillende longvolumes te bepalen. Hiertoe dient de DL_{CO} bij verschillende alveolaire O_2 spanningen bepaald te worden om gebruik te kunnen maken van de Roughton-Forster vergelijking: $1/DL_{CO} = 1/D_M + 1/(\theta_{CO} \times Q_c)$. De waarde van θ_{CO} (uit te spreken als "theta voor CO"), d.w.z. de bindingssnelheidsconstante van CO aan hemoglobine in de rode bloedcellen, dient dan wel bekend te zijn. Aangezien geen gegevens met betrekking tot θ_{CO} voor varkensbloed beschikbaar waren in de literatuur, hebben wij deze bepaald in bloed van vijf verschillende zeugen, waarvan de resultaten beschreven zijn in ***HOOFDSTUK 3***.

θ_{CO}, uitgedrukt in het aantal ml's CO dat per ml bloed opgenomen wordt per minuut per mmHg CO drukverschil (traditionele eenheden), werd bepaald voor elk bloedmonster van de vijf varkens bij 37°C en een pH van 7,4, bij vier tot vijf verschillende O_2 spanningen. Voor de bepaling werd gebruik gemaakt van een zogenaamd "continuous-flow rapid-mixing" apparaat (dus een continue stroom met snelle menging). De bepalingsmethode berust op een snelle menging van een rode bloedcelsuspensie waarin geen carboxyhemoglobine (hemoglobine waaraan CO gebonden is) aanwezig is en een bufferoplossing waarin een bepaalde hoeveelheid CO is opgelost. Vervolgens wordt er op verschillende tijdstippen na de menging gemeten hoeveel carboxyhemoglobine gevormd is. Dit gebeurt met behulp van spectrofotometrische apparatuur.

Voor bloed van elk varken werd de relatie bepaald tussen het omgekeerde van θ_{CO} ($1/\theta_{CO}$) en de zuurstofspanning (P_{O2}), wat een rechtlijnig verband oplevert, resulterend in vijf rechte lijnen. Aangezien er geen verschil was tussen de hellingen en de hoogte van deze vijf lijnen, kon een gemeenschappelijke vergelijking voor het bloed van de vijf varkens opgesteld worden: $1/\theta_{CO} = 0{,}0084 \times P_{O2} + 0{,}63$. De helling van deze lijn (0,0084) was beduidend steiler dan de hellingen van de lijnen gerapporteerd voor mensen [Krawiec *c.s.*, 1983] en honden [Crapo *c.s.*, 1989] gemeten onder dezelfde omstandigheden op hetzelfde laboratorium. Indien voor de berekening van het capillaire bloedvolume in varkens gebruik zou worden gemaakt van de theta-waarden van mensen of honden, dan zou dit resulteren in een onderschatting van Q_c van ongeveer 50%.

In ***HOOFDSTUK 4*** is beschreven hoe de theta-vergelijking uit HOOFDSTUK 3 werd gebruikt bij de bepaling van het capillaire bloedvolume en de membraandiffusie-capaciteit in acht varkens (gemiddeld lichaamsgewicht 11,2 kg) onder narcose en met spierverslapping. DL_{CO} werd bepaald (zoals beschreven in HOOFDSTUK 2) met behulp van de IP-procedure bij vier verschillende longvolumes, en op elk longvolume-niveau bij drie verschillende alveolaire O_2 spanningen. Het longvolume werd vergroot door 15, 20, 25 of 30 ml per kg lichaamsgewicht in te blazen bij een PEEP van 2 cmH_2O. Aangezien de theta-vergelijking van HOOFDSTUK 3 gebaseerd is op normaal bloed dat maximaal 0,2 ml CO per ml bloed kan bevatten, ervan uitgaande dat de hemoglobine-concentratie 8,9 $mmol.L^{-1}$ (=14,4 $g.dl^{-1}$) bedraagt, werden de theta-waarden aangepast aan de hemoglobine-concentraties die gemeten werden bij de varkens.

DL_{CO} nam, bij een inspiratoire O_2 concentratie van 30%, gemiddeld met 11,8 $\mu mol.s^{-1}.kPa^{-1}$ af voor elke liter toename in alveolair volume. Het capillaire bloedvolume nam bij eenzelfde toename in alveolair volume met 241 ml af, en de membraandiffusiecapaciteit vertoonde een lichte stijging van 2,7 $\mu mol.s^{-1}.kPa^{-1}$. We hadden een sterkere toename in de diffusiecapaciteit van de membraan verwacht. Dat dit niet het geval was, is waarschijnlijk te wijten aan het (functioneel) verloren gaan van delen van de alveolaire-capillaire membraan voor gasoverdracht ten gevolge van de tegelijkertijd optredende afname in het capillaire bloedvolume. De afname in het capillaire bloedvolume kan verklaard worden door het samendrukken van de longcapillairen door de toegenomen druk in de longblaasjes tijdens de geforceerde inademing en door het uitrekken van longweefsel.

De waarden van het capillaire bloedvolume zoals vermeld in HOOFDSTUK 4 zijn relatief groot in vergelijking met waarden die verkregen werden tijdens beademing in andere zoogdieren zoals mens en hond. Een ander bijkomend probleem was, dat de afname van het *capillaire* bloedvolume bij een toename in het longvolume groter was dan de afname van het *totale* longbloedvolume, zoals door Versprille en medewerkers (1990) in soortgelijke varkens is gevonden. Uit de literatuur is bekend dat veranderingen in het bloedvolume van het capillaire deel (kleine vaten) en dat van de grotere vaten van de longcirculatie kunnen verschillen. Deze kennis is echter gebaseerd op waarnemingen in vitro. Daarom is door ons de relatie bestudeerd, in het intacte organisme, tussen veranderingen van het bloedvolume in het capillaire deel van de longcirculatie en die in de totale longcirculatie onder beademingsomstandigheden in tien varkens (gemiddeld lichaamsgewicht 10,5 kg). Dit onderzoek is beschreven in ***HOOFDSTUK 5.***

Capillair bloedvolume werd afgeleid van DL_{CO} bepalingen bij twee verschillende alveolaire O_2 spanningen en gebruikmakend van de Roughton-Forster formule (zie HOOFDSTUK 2 t/m 4). Totaal longbloedvolume inclusief het bloedvolume in de linker harthelft (Q_{P+LH}) werd bepaald met behulp van een dubbele-indicator-dilutie techniek. Hiervoor werd een kleine hoeveelheid koude hypertonische zoutoplossing (NaCl) geïnjecteerd in de rechterboezem van het hart. Door in de longslagader de veranderingen in temperatuur en in de lichaamsslagader de verandering in zoutconcentratie op geleide van de elektrische geleiding van het bloed te volgen, kon een schatting gemaakt worden van het volume dat tussen deze twee meetplaatsen ligt, dus Q_{P+LH}. Dit volume werd berekend als het verschil in gemiddelde looptijd van de koude en de zout "deeltjes" tussen aankomst in de longslagader en aankomst in de lichaamsslagader respectievelijk, vermenigvuldigd met het hartminuutvolume.

Het afleiden van het capillaire bloedvolume uit slechts twee bepalingen van DL_{CO} bleek afdoende (gebaseerd op data van het onderzoek in HOOFDSTUK 4). Q_c en Q_{P+LH} namen in dezelfde mate af met een toename in alveolair longvolume, respectievelijk 106 en 111 ml voor elke liter toename in alveolair volume. Uit dit resultaat werd geconcludeerd dat geforceerde inflatie van de longen leidt tot een aanzienlijke verplaatsing van bloed, van de capillairen naar de grote systeemcirculatie. Er lijkt zich dus geen bloed op te hopen in de grotere vaten van de longcirculatie. Zo'n verplaatsing

van bloed is nadelig voor de gasoverdracht als tijdens beademing grote volumes worden ingeblazen.

De relatie tussen het capillaire en het totale deel van de longcirculatie kan echter wel eens anders zijn. Als er van wordt uitgegaan dat het linkerhartvolume in varkens van 10,5 kg ongeveer 20 ml bedraagt, dan zou het capillaire bloedvolume ongeveer 75% van het totale longbloedvolume uitmaken wat onwaarschijnlijk hoog lijkt. Aangezien het totale longbloedvolume overeenkomt met waarden gevonden voor mensen, als gecorrigeerd wordt voor het verschil in lichaamsgewicht, lijkt het waarschijnlijk dat de capillaire bloedvolumes overschat zijn. De tegendruk van CO in het capillaire bloed van de varkens was aanzienlijk. Hoewel de diffusiegradiënt voor deze tegendruk gecorrigeerd was, kunnen toch aanzienlijke fouten in de bepaling van de gradiënt zijn geslopen door de vele benaderingen in onze berekeningen. De gemiddeld hogere waarden van de tegendruk van CO in varkensbloed in vergelijking met die in menselijk bloed kunnen verklaard worden door: 1) de lagere bindingssnelheidsconstante van CO aan hemoglobine van varkens, en 2) de lagere affiniteit van het varkenshemoglobine voor CO in competitie met O_2.

In ***HOOFDSTUK 6*** is een aantal aspecten van de onderzochte problemen aan een nadere beschouwing onderworpen. Het vergelijken van DL_{CO} waarden tussen verschillende onderzoeken blijkt niet op eenvoudige wijze tot conclusies te kunnen leiden aangezien de bepaling van DL_{CO} beïnvloed kan worden door talrijke factoren (zie TABEL 1 van HOOFDSTUK 6). In de tekst is nader ingegaan op verschillen in alveolaire O_2 spanning, hemoglobine-concentratie, tegendruk van CO in bloed, en longvolume. Ook zijn een aantal onzekerheden in de afleiding van het capillaire bloedvolume aan de orde gekomen, voortvloeiend uit de DL_{CO} bepaling en de in vitro bepaling van theta voor CO. Verder zijn achtereenvolgens besproken: de geschiktheid van het varken als model in onderzoeken naar de gasoverdracht in de longen, en enkele suggesties voor verder onderzoek.

Wat de luisteraar onthoudt

100%	informatie: alles wat er te zeggen valt;
90%	informatie: wat ik belangrijk vind;
80%	informatie: wat ik zelf daarvan weet;
70%	informatie: wat ik paraat in mijn geheugen heb;
60%	informatie: wat ik daarvan helder kan uitdrukken;
50%	informatie: wat daarvan overkomt op een ander;
40%	wat de ander daarvan begrijpt;
30%	wat de ander daarvan gelooft;
20%	wat de ander daarvan belangrijk vindt;
10%	wat de ander daarvan onthoudt.

(Uit "Smetten op de witte jas"
- onder redactie van JJE van Everdingen, 1993)

DANKWOORD

"Souvent trop rechercher fait trop trouver aussi."
- Wie te veel onderzoekt vindt vaak meer dan hem lief is. -
JEAN BERTAUT

Op sommige momenten bekroop mij dit gevoel tijdens mijn verkenningstocht naar de overdracht van koolmonoxide in de longen. Het onderzoek hiernaar leek dan net als het gas zelf: reukloos maar verraderlijk. Zulke momenten, twijfels, maar gelukkig ook "eureka" gevoelens horen er nou eenmaal bij, maar het was fijn om te weten, dat er dan altijd personen waren waarop ik kon steunen of die meedeelden in mijn vreugde. Graag wil ik die mensen persoonlijk bedanken en allen die mij met raad en daad hebben bijgestaan en hebben bijgedragen aan de totstandkoming van dit proefschrift. Dat ik (mogelijk) hierbij personen ten onrechte vergeet te noemen, is helaas onvermijdelijk.

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(ERASMUS in een brief aan zijn vriend Thomas More)

CURRICULUM VITAE

Francis te Nijenhuis is op 11 februari 1966 te Oldenzaal geboren. In 1984 behaalde zij het V.W.O. diploma aan het Twents Carmellyceum te Oldenzaal. Daarna begon zij de studie Geneeskunde aan de Rijksuniversiteit Groningen (R.U.G.). In het studiejaar 1987/88 nam zij deel aan een onderzoek naar het "Metabolisme van het hart" bij de vakgroep Kindergeneeskunde en Heelkunde aan de R.U.G. onder leiding van Prof.Dr. J.R.G. Kuipers. In 1988/89 was zij werkzaam als student-assistent bij het tweedejaars practicum Fysiologie. Daarnaast deed zij onderzoek naar de anaërobe drempel bij sporters met behulp van fietsergometrie bij de vakgroep Bewegingswetenschappen en Fysiologie aan de R.U.G. onder leiding van Prof.Dr. P. Rispens. Het doctoraalexamen behaalde zij in januari 1989.

De hierop volgende (basis) co-assistentschappen volgde zij in het Medisch Spectrum Twente te Enschede. Voor het drie maanden durende keuzeco-schap koos zij voor de afdeling Interne Geneeskunde in het St. Elisabeth Ziekenhuis te Tilburg. Het wetenschappelijk keuze-project vervulde zij aan de afdeling Kindergeneeskunde van het Catharina Ziekenhuis te Eindhoven. Hier onderzocht zij het effect van een onderhoudsbehandeling met inhalatie-corticosteroïden op de lengtegroei in prepuberale kinderen met astma onder leiding van Dr. J.J.J. Waelkens.

Na het behalen van het artsexamen in december 1991 trad zij als Assistent in Opleiding (AIO) in dienst van de Erasmus Universiteit Rotterdam. Tot 1 september 1996 werkte zij aan haar promotie-onderzoek aan het Pathofysiologisch Laboratorium van het Instituut Longziekten onder leiding van Prof.Dr. A. Versprille. Een onderdeel hiervan, het in vitro onderzoek naar de bindingssnelheidsconstante van koolmonoxide aan intracellulair hemoglobine, heeft zij, onder leiding van Prof.Dr. R.E. Forster, uitgevoerd aan het "Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, USA" (augustus-september 1993 en januari-maart 1995). Vanaf 1 oktober 1996 zal zij als Assistent Geneeskundige niet in Opleiding (AGNIO) verbonden zijn aan het Sophia Kinderziekenhuis Rotterdam.