PULMONARY DIFFUSING CAPACITY FOR CARBON MONOXIDE IN SHEEP

by

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I. INTRODUCTION

The physiology of the respiratory system in domestic animals has not been studied to the extent that its importance deserves. In many aspects not even normal values have been established.

There is a definite need for a series of studies in order to establish normal values and techniques to permit the clinical appreciation of the respiratory function in domestic animals. Most of the efforts in this area have been conducted on anesthetized animals. This is unfortunate because anesthetized animals are not normal.

The estimation of pulmonary diffusing capacity is a good indicator of the efficiency of pulmonary function. It gives an estimation of the area available for diffusion and the condition of the air-blood barrier. In pulmonary adenomatosis, emphysema, and pneumonia the estimation of pulmonary diffusing capacity could be of importance in the diagnosis and prognosis.

The primary objectives of this investigation were to estimate pulmonary diffusing capacity in normal sheep and to study the effectiveness of this measurement method in detecting artificially-produced alveolar lesions.

II. REVIEW OF LITERATURE

A. Symbols

All of the symbols used in the present work are those based on a report in Federation Proceedings (83). These symbols were selected by a group of American pulmonary physiologists in 1950.

A dash (-) above any symbol indicates a mean value.

A dot (•) above any symbol indicates a time derivative.

1. Symbols for gases

a) Primary symbols

V = gas volume

$\dot{V}$ = gas volume/unit time

P = gas pressure

$\bar{P}$ = mean gas pressure

F = fractional concentrations in dry gas phase

f = respiratory frequency (breath/unit time)

D = diffusing capacity

R = respiratory exchange ratio

BP = barometric pressure

b) Secondary symbols

$_I$ = inspired gas

$_E$ = expired gas

$_A$ = alveolar gas

$_T$ = tidal gas

$_D$ = dead space gas

$_B$ = barometric

STPD = 0°C, 760 mm Hg, dry

BTPS = body temperature and pressure saturated with water vapor

ATPS = ambient temperature and pressure saturated with water vapor

2. Symbols for blood

a) Primary symbols

Q = volume of blood

$\dot{Q}$ = volume flow of blood/unit time

C = concentration of gas in blood phase

S = % saturation of Hb with O_2 or CO

b) Secondary symbols

a = arterial blood

v = venous blood

c = capillary blood

3. Symbols for lung volumes

VC = vital capacity

IC = inspiratory capacity

IRV = inspiratory reserve volume

ERV = expiratory reserve volume

FRC = functional residual capacity

RV = residual volume

TLC = total lung capacity

B. Definition of Pulmonary Diffusing Capacity

The molecules of O_2 and CO_2, which are transferred because of their differences in partial pressure between blood and alveolar gas, cross a number of membranes and liquid interphases which present a certain resistance to their passage. This resistance limits the rate of diffusion from one side of the alveolar wall to the other.

The simple relationship between the quantity of a gas diffusing from one medium into the other per unit of time ($\dot{V}X$) and the difference between partial pressure of the gas in the alveolar gas (P_AX) and the mean pressure of the gas in the capillary ($P\bar{c}X$) is given by: $VX = DX(P_AX-P\bar{c}X)$. DX is the diffusing capacity of the lung for X. When VX is in ml/min STPD, and the pressures are in mm Hg, DX becomes the volume of gas which diffuses between blood and gas per minute and per mm Hg pressure difference.

$$DX\ (\text{ml/min mm Hg, STPD}) = \frac{VX\ (\text{ml/min, STPD})}{P_AX-P\bar{c}X\ (\text{mm Hg})}$$

Dejours (56), Comroe (57), Forster (12).

C. Techniques for Measuring Pulmonary Diffusing Capacity with Carbon Monoxide

The techniques most frequently used for measuring pulmonary diffusing capacity are those that use carbon monoxide. Bohr was the first to use CO (carbon monoxide) for the measurement of diffusing capacity of the lung. The idea was based on his realization that affinity of hemoglobin for CO was too great and that if one were careful to use small concentrations of CO in the inspired air, the HbCO concentration in the blood could be ignored.

Bohr assumed that the rate of combination of CO with intracellular Hb was instantaneous so that the CO that diffused across into the plasma was immediately taken up by Hb (hemoglobin).

There are three principal techniques for the determination of D_LCO (pulmonary diffusing capacity for CO). These are (1) steady state technique, (2) breath holding technique, and (3) rebreathing technique.

1. Steady state technique

There are two ways of measuring D_LCO with the steady state technique as follows:

a) Measuring the rate at which CO appears in the blood. Forbes et al. (29) and Roughton (76) have used this technique. This method demands measurements of the rate of change of average blood COHb concentrations and total blood volume.

b) Observing the rate at which CO disappears from the respired gas. This technique has been widely used and is described elsewhere (27, 35, 6).

In the steady state technique the critical point is the estimation of the alveolar pressure of CO (P_ACO). Forbes et al. (29) used an alveolar sampling technique to obtain P_ACO. Bates et al. (6) have used the end tidal sample technique with good results.

P_ACO can also be calculated from the Bohr relation:

$$P_ACO = \frac{(V_T \times P_ECO) - (V_D \times P_ICO)}{(V_T - V_D)}$$

For this last equation the dead space volume is required. Bates et al. (6) have shown that P_ACO calculated in this way becomes very sensitive to small

differences in the estimated volume of the dead space whenever tidal volume is small.

The estimation of P_ACO from alveolar air or from end tidal air presents the problem of the variation of P_ACO with time due to uneven distribution of inspired air (36) and to uneven distribution of ventilation with respect to blood flow.

Finlley et al. (27) devised a method for obtaining P_ACO from physiological dead space. This necessitates the obtainance of an arterial blood sample and the measurement of $PaCO_2$. The formula for calculating P_ACO has many different forms, the most simple being:

$$P_ACO = P_ICO - \frac{PaCO_2}{P_ECO_2} (P_ICO - P_ECO).$$

The formula used by Finlley et al. is:

$$P_ACO = (BP-47) \frac{F_ECO - r\ F_ICO}{1 - r}$$

where,

$$r = \frac{PaCO_2 - P_ECO_2}{PaCO_2} = \frac{V_D}{V_T}$$

The value of P_ACO so obtained is very sensitive to small differences in the data when either the ratio $PaCO_2/P_ECO_2$ is large or P_ICO-P_ECO is large.

Another factor to be determined in calculating D_LCO is the amount of CO that is diffusing into the blood. The equation used by Finlley et al. (27) was as follows:

$$\dot{V}CO = \dot{V}_E \left(F_ICO \frac{F_EN_2}{F_IN_2} - F_ECO\right).$$

Forster et al. (33) have determined that the rate of CO uptake in milliliters per minute depends upon the inspired PCO, total minute ventilation, alveolar ventilation, and total D_LCO; this also assumes that these variables are uniformly distributed throughout all the alveoli in the lung.

The percentage of the inspired CO that is taken up by the blood over a period of several minutes gives an index of diffusing capacity which is called fractional CO uptake (27).

$$\text{Fractional CO uptake} = \frac{\text{CO uptake (ml/min)}}{\text{CO inspired (ml/min)}} \times 100$$

Bates (5) measured fractional CO uptake. They found it to be 53% in young people and 47% in people with average age of 59.

Finlley (27) found fractional CO uptake to range between 42% to 61%.

Comroe (17) mentions that a fractional CO uptake of less than 30% is indicative of impairment of diffusion.

Forster et al. (33) have shown that the fractional CO uptake is independent of the inspired CO tension and is therefore a function of total ventilation, alveolar ventilation, and D_LCO. The fact that fractional CO uptake depends on alveolar and total ventilation makes it a poor estimation of D_LCO.

An important question is how long should the animal breathe the mixture of gases containing CO to reach the so-called steady state? Forster et al. (33), considering respiration a continuous process and assuming a homogeneous

lung breathing from a mixture of gases containing CO, stated that theoretically the P_ACO exponentially approaches a steady state. The exponential constant is equal to alveolar ventilation (D_LCO 713/V_A). Bates et al. (6) found that stable end expiratory CO tension was reached after 7 breaths in normal men. Finlley et al. (27) found the steady state present after breathing the mixture for 2 minutes. Shepard et al. (82) reported that the steady state was not always reached after 2 minutes of breathing a gas mixture.

2. The breath holding technique

Krogh was the first to develop the breath holding technique for studying D_LCO. He derived an equation describing P_ACO as a function of time, considering the lung as a single well mixed bag, from which it was possible to calculate D_LCO.

$$P_ACO = Pi_ACO \times e^{\frac{-D_LCO\ (BP-47)t}{V_A}}$$

Pi_ACO = initial alveolar PCO.

If the log of P_ACO is plotted against time, a straight line should be obtained.

Forster et al. (34) suggested modifying the breath holding technique of Krogh by using a gas mixture containing about 10% helium in addition to a low concentration of CO, and collecting one expired sample at the end of the breath holding period. The initial PCO was calculated from the dilution of the helium and the initial concentration of CO. The same authors found that plotting the log of P_ACO against time gave a line that was concave upward. The most probable explanation for this is the existence of different

values of D_LCO/V_A. Marks et al. (55) have suggested that when the breath holding technique is used and samples of gas are taken from the parts of the lung with good D_L (D_LCO/V_A greater than average), it will overestimate the true D_LCO. However, if the areas with good D_LCO are at the same time the areas that receive most of the inspired gas, this will bring the fraction D_LCO/V_A to average values.

Ogilvie et al. (61) described the technical details of the modified breath holding technique. They also investigated the possibility that alveolar samples from different parts of the lungs give different values of P_ACO/Pi_ACO by collecting samples after 1 liter and after 2.5 liters. On the average D_LCO calculated from the later sample was 10% greater than that calculated from the earlier sample. According to Fowler (36) the earlier sample should contain a greater proportion of gas from the better ventilated alveoli.

3. Rebreathing technique

Kruhoffer (51) has developed an original method for measuring D_LCO in which the subject rebreathes from a bag of approximately 6 liters at a rate of about 25 liters per minute for roughly 30 seconds. The rate of CO disappearance is determined by taking a gas sample from the system after approximately 12, 20, and 30 seconds. Knowing the total volume of the system (bag + lungs) one can calculate rebreathing D_LCO from the equation developed by Krogh. Addition of He (20%) to the initial mixture permits the evaluation of the original volume of the lung.

In defining pulmonary diffusing capacity the formula used for any gas (X) was as follows:

$$D_LX(\text{ml/min mm Hg, STPD}) = \frac{VX\ (\text{ml/min, STPD})}{P_AX - P\bar{c}X\ (\text{mm Hg})}$$

where $P\bar{c}X$ stands for mean pressure of X in the pulmonary capillary blood. According to Bohr's idea the affinity of the Hb for CO was so high that pressure of CO in blood was for all practical purposes equal to zero. This is not absolutely true as has been demonstrated by many workers. Forster et al. (33) recognized that if there is COHb in the mixed venous blood there will be a definitive equilibrated capillary PCO equal to P_{CO_2} x [COHb]/210 [HbO_2] which should be subtracted to give the correct value for the diffusion gradient. Forster (32) has shown that if one assumes normal values of physiological dead space, D_LCO, total CO combining power of the blood, and blood COHb saturation, equilibrated capillary PCO will increase approximately 1/200 of alveolar PCO per minute when breathing a mixture containing 21% O_2 at rest. Linderholm (54) has made a detailed study of the same problem. Many different techniques have been proposed to measure this equilibrated PCO. Carlsten et al. (15) have calculated the equilibrated PCO from blood [COHb]. Linderholm (54) calculated the equilibrated PCO by rebreathing in a closed system. Foster et al. (35) calculated the equilibrated PCO by a technique that requires rebreathing in a closed system or breath holding for 2 minutes.

Roughton and Forster (78) demonstrated that approximately one-half of the total resistance to diffusion of CO from alveolar gas to capillary blood while breathing air exists between the plasma and the interior of the red blood cell. They derived the following equation:

$$1/D_L = 1/DM + 1/\theta Vc$$

$1/D_L$ = total resistance to diffusion, $1/DM$ = resistance of the membrane, $1/\theta Vc$ = resistance of the blood, θ = rate of gas uptake by ml of normal whole blood per minute for a partial pressure of 1 mm Hg of CO, and Vc = average amount of blood in ml in the pulmonary capillary bed. The equation is solved most conveniently by plotting $1/D_L$ against $1/\theta$ giving a straight line whose intercept equals $1/DM$ and whose slope equals $1/Vc$.

θ has been measured for red cell suspensions in vitro at 37°C (77, 79, 40, 59).

Roughton et al. (76) found a value of Vc = 70 ml in man. Roughton and Forster (78) found a value of Vc equal to 73 ml. Gibson et al. (39) have measured the blood content of the lung parenchyma in normal dogs immediately after death and found an average of 0.125 ml of blood per gram of tissue.

D. Technique for Measuring Pulmonary Diffusing Capacity with Oxygen

To measure the diffusing capacity of the lung with O_2 the following measurements must be made: (1) ml of O_2 transferred from alveolar to blood/minute, (2) the mean alveolar oxygen pressure, and (3) the mean pulmonary capillary pressure.

The first is measured easily, since it is the O_2 consumption of the animal per minute.

The estimation of P_AO_2 presents no major problem since P_AO_2 can be obtained from an alveolar sample or calculated from the alveolar air equations. In general, the estimation of P_AO_2 presents the same problems as the estimation of P_ACO discussed earlier.

The critical point in this technique is the estimation of the $P\bar{c}O_2$. This is not the simple average of venous and arterial blood pressures of O_2 since the rate of uptake of O_2 into the blood is proportional to the difference between alveolar gas and capillary blood PO_2 at each point.

Fortunately, using Bohr's integration procedure, one can compute the mean capillary PO_2 if one knows four things (17, 32, 30, 22, 75):

(1) The pressure of O_2 in the blood just at the beginning of the pulmonary capillaries (mixed venous blood).

(2) The pressure of O_2 in the alveoli, which determines the pressure gradient across the alveolar capillary membranes.

(3) The pressure of O_2 in the blood just at the end of the pulmonary capillaries.

(4) The physiological oxygen-hemoglobin dissociation curve.

It is possible to measure the first, estimate the second, and consult tables or graph for the fourth.

The estimation of the end capillaries PO_2 has been accomplished by Lilienthal et al. (53) and Riley and Cournand (71).

E. Pulmonary Diffusing Capacity in Man

Values for pulmonary diffusing capacity in man are abundant. The value depends on the method used, sex, age, body size, body weight, and many other factors (63).

The relation of pulmonary diffusing capacity to body size has been studied by Forster et al. (33). They found that D_LCO increases with body size.

The effect of size on the value of pulmonary diffusing capacity has been studied by Bates et al. (6), Krogh (50), Kruhoffer (51), and Ogilvie (61). They reported that females had a lower D_L. This could be due to their smaller body size.

The effect of ages on D_L has been extensively studied. Bates (5) found that fractional CO uptake was 53% in young males with an average age of 28, and 47% in subjects of average age 59. Cohn et al. (16) reported that maximal diffusing capacity is greatly decreased by age. Burrows et al. (13) have established that D_L by the breath holding technique or D_L by the steady state technique decreases with age. The causes for this are not clear; it could result from a decrease in the actual capacity of the capillary bed from alterations in the factors that control the number of active capillaries, or abnormalities of distribution of blood and gas in the lung (30). McGrath and Thomson (56) have also found that D_LCO is affected by age.

The variations of D_L with exercise has also been extensively studied since Krogh (50) first showed that D_L increased with exercise; this increase that has been confirmed by all investigators has been found to be greater in athletes by Bannister et al. (3).

The exact mechanism of the increase in D_L with exercise is not known but it seems reasonable to assume that the surface area of the capillary bed increases either by dilatation of patent vessels or the opening of previously closed vessels as a result of neuroendocrine factors (32).

Roughton (76) found that the volume of blood in the pulmonary capillary (Vc) increases with exercise. Riley et al. (73) have measured D_LO_2

at increasing levels of exercise in three normal subjects and concluded it rises at first and eventually reaches a plateau.

MacNamara et al. (57) have also shown that D_L rose steadily with increasing ventilation. They suggested that the area of the blood gas interphase is the important factor underlying the increase in D_L that occurs during exercise and during voluntary hyperventilation.

Giammona and Daly (38), working with children between 4-13 years old, found also an increase in D_L with exercise.

Ross et al. (74), using the breath holding and steady state techniques, confirmed that D_L increased with exercise; cardiac output did not bring about any change in D_L. They also tested the effect of hyperventilation and found that it increased D_L. Engorging the lung with blood brought about only minor changes in D_L.

Bishop et al. (9) found that with exercise Vc is about twice as great as the increase in DM. This indicates that the volume and surface area of the pulmonary capillaries increases with exercise.

The variation of D_L with changes in alveolar value was first reported by Krogh; he found an increase in D_L with increased lung volumes.

MacNamara et al. (57) and Ross et al. (74) have shown that steady state D_LCO apparently does not increase with increasing mean alveolar volume in a given individual.

The effect of alveolar oxygen tension (P_AO_2) upon D_LCO has also been studied. Forster et al. (33), Roughton (76), and Roughton and Forster (78) have shown that increasing alveolar O_2 tension lowers D_LCO and vice versa.

Cender and Forster (14) found an increase of 10-15% in resting D_LCO at an alveolar PO_2 of 60 mm Hg as compared with that of breathing air.

Forster et al. (35) measured D_LCO at alveolar O_2 tensions from 40 mm Hg to 600 mm Hg. They found that D_LCO decreases with increasing alveolar O_2 tensions, varying as much as 5-fold over the entire range.

Forbes et al. (29) measured the CO uptake at a simulated altitude of 16,000 feet (P_AO_2 = 40 mm Hg) and found no relative increase over that at sea level. Kreuzer and Van Lookeren (49), working at high altitude, found no increase in D_LCO or D_LO_2 with respect to sea level values. Lilienthal and Pine (52) have studied the effect of O_2 or CO uptake at sea level and at high altitude.

It has been demonstrated by many investigators that the alveolar tension of CO_2 affects the value of D_LCO. Forster et al. (34) found that D_LCO increases if 6% CO_2 is given with the inspired air; they used the breath holding technique.

Hyde et al. (45) found that an increase in P_ACO_2 causes an increase in D_LCO in the isolated perfused cat lung.

Rankin et al. (69) determined D_LCO with 10% CO_2 added to the inspired air. D_LCO increased by 5.3%; if permitted to breathe 7.3-7.8% CO_2 for 10 minutes, D_LCO increased 24.5%. The authors thought that CO_2 produced an increase in Vc during 10% CO_2 inhalation.

Body position is another factor that affects D_L. Bates and Pearce (7) measured D_LCO by the breath holding technique in normal subjects lying and sitting. The values for subjects lying down were about 20% greater than those sitting. They suggested that the cause of the greater value in subjects lying down is either an actual increase in the capillary bed or a change in the distribution of diffusing surface in relation to alveolar ventilation.

Ogilvie et al. (61) investigated the same problem and got values of D_LCO that were 14% greater for supine as compared to sitting, and in addition a decrease of 13% for standing as compared with sitting measurements.

Holmgran and Svanborg (43) studied the effects of shifting positions of the body on both hemodynamics and respiratory variables.

Frayser et al. (37) and Otis (62) have studied the effect of increased temperature on pulmonary diffusing capacity.

The effects of uneven distribution on D_L is widely documented and rather confusing.

Piiper et al. (65) and Riley and Cournand (71) have studied the effects of nonuniformity of alveolar ventilation in relation to capillary diffusing surface and found that it will lead to a decrease in steady state D_LCO.

Rahn (68) and Riley et al. (72) have studied the effect of pulmonary blood flow in relation to alveolar ventilation and they found that this affects the steady state alveolar CO_2 tension and the physiological dead space.

Read et al. (70), in a theoretical analysis of the magnitude of error which nonuniformity within the lungs may introduce into a steady state physiological dead space method of estimating D_LCO, concluded that if nonuniformity of ventilation is introduced into an initially uniform lung model, no error in D_L will occur; however, when redistribution of blood takes place, the error in D_L will be an overestimation which may in some cases reach infinity or become negative.

F. Pulmonary Diffusing Capacity in the Sheep

No reference has been found in the literature searched regarding normal values of pulmonary diffusing capacity in the sheep.

G. Pulmonary Diffusing Capacity in Domestic Animals Other Than Sheep

Studies of D_L in domestic animals have been confined to dogs and cats. These studies have usually been done with animals under anesthesia. Niden et al. (60) described a method for measuring D_LCO in dogs called "equilibration technique" with continuous analysis of the expired gas. The value found in dogs weighing approximately 20 kg was 21.3 ml/min mm Hg.

Kentera et al. (48), studying pulmonary hypertension in relation with D_LCO, found that there was no difference in D_LCO in the two groups of dogs they studied (high and low pulmonary arterial pressure). D_LCO in dogs with pulmonary pressure greater than 20 mm Hg was 1.0 ± .23 ml/min mm Hg kg and D_LCO equals 1.19 ± .15 ml/min mm Hg kg in the animals with pulmonary pressure lower than 20 mm Hg.

Jovasset-Strieder et al. (46) used 8 dogs and the single breath technique they found that in normal dogs D_LCO was equal to 27 ml/min mm Hg. DM (measured by the Roughton and Forster technique) was equal to 100 ml/min mm Hg and Vc obtained by the same technique was equal to 67 ml.

Young et al. (85) have used 14 dogs and the Ogilvie technique for measuring D_LCO. Their results agree with those obtained by other workers.

Burrows and Niden (13) found that artificially-produced hemorrhagic shock decreased D_LCO in dogs producing at the same time a nonuniform D_L/V_A ratio.

Glauser (41) has measured D_LCO for individual lungs (left and right) by means of bronchospirometric techniques in 8 male dogs. The technique for the estimation of D_LCO was that described by Ogilvie. He also measured lung volume by neon dilution techniques. The results reported for an average weight and average surface area for lung volume are as follows:

left lung = 1,080 ml STPD

right lung = 1,226 ml STPD

total (both lungs) = 2,280 ml STPD

The difference in volume between the left and the right lung was not found to be significant.

The results reported for an average weight and average surface area for D_LCO are as follows:

left lung = 11 ml/min mm Hg (43.4%)

right lung = 14 ml/min mm Hg (56.6%)

total (right and left) = 25 ml/min mm Hg (100%)

The difference between right and left lung D_LCO was found to be significant.

The total D_LCO expressed by kg was found equal to 1.14 ml/min mm Hg kg.

Duke and Stedeford (26) have worked with cats under anesthesia and found that the mean D_LO_2 was .91 ml/min mm Hg kg. They also found that cooling the animal reduced D_LO_2. In this work mean pulmonary capillary PO_2 was estimated equal to $PaO_2 - 1/3(PaO_2 - PvO_2)$.

H. Respiratory Data of Domestic Animals

The bibliography concerning respiratory physiology in domestic animals is very limited and in many aspects normal values have not been established.

Purchase (66) has studied peak inspiratory and peak expiratory flow rates, minute volume and tidal volume in 12 anesthetized horses and five conscious cows using a close fitting mask.

Patterson et al. (64), working with oxen, determined some respiratory variables; using an open circuit method they found a value of 2.21 l/min or .27 l/min/m^2 for O_2 consumption. Minute ventilation averaged 82 l., tidal volume 3 l. Dead space (calculated by Bohr equations) was 1.44 l. Alveolar PO_2 by end tidal sampling was found to be 119 mm Hg and PCO_2 equal to 26 mm Hg. PaO_2 by equilibration technique was 78 mm Hg and $PaCO_2$ equals 33 mm Hg.

Wittke (87), using face masks, Douglas bags, and a gas meter, has determined the respiratory volumes of 5 mature cattle of different breeds and weights. For cattle of medium size at rest, the mean value was 86 ± 10 l/min for minute volume, 3.5 ± .2 l. for tidal volume. Respiratory rate was 25 ± 3 per minute.

Bianca et al. (8) reported total plasma CO_2, pH of venous blood, PCO_2 of venous blood, respiratory rate, minute volume, and tidal volume values obtained from 11 Ayrshire calves 7-11 months old.

Fisher (28) studied pulmonary ventilation of cows by means of a face mask. The minute volume found in the normal animals was 25 l/min. Respiratory rate was 34 per minute, and tidal volume was 740 ml.

Amoroso et al. (1) have studied the pattern of air flow and tidal volume. They found a decrease in the respiratory rate as weight increases; this, however, was not true for cows.

The information with respect to respiratory physiology in sheep is almost all related to studies of temperature regulation and metabolism in these animals. These studies have been carried out using tracheal intubation in some cases; Webster and Cresswell (86) found the use of tracheotubes to be convenient and recommended them in calorimetric determinations.

Blaxter and Joyce (11) have used tracheal intubation in animals with permanent fistulas measuring and analyzing the expired air in sheep. Using the Douglas bag technique they found an O_2 consumption of 16 l/hr, CO_2 production of 13 l/hr, and CH_4 production (eliminated through the lung) 0.14 l/hr. The CO_2 and CH_4 production determined by the Douglas bag technique was found to be less than that determined in a respiratory chamber. They also reported a considerable increase in O_2 consumption and CO_2 production between the 5th and 10th minute after feeding.

Blaxter (10) has studied respiratory metabolism in the female sheep fed iodinated casein using spirometer techniques with direct analysis of expired air. He reported a reduction of the tidal volume with increasing respiratory rate. The value for tidal volume was found to be equal to: $401.3-(1.887 \times f)$.

In another experiment Joyce and Blaxter (47) studied respiration in sheep in cold environments; they found the oxygen consumption to be 13-18 l/hr under normal conditions. In cold environments this value increased to 20 l/hr; this increase in oxygen consumption was found to be linearly

correlated with an increase in pulmonary ventilation, mostly due to an increase in tidal volume.

Halmagyi et al. (42) reported a series of determinations on 3 to 5-year-old wethers that were obtained with the animals under anesthesia. Arterial blood oxygen capacity was 14.33 volumes. SaO_2 equals 87.6. Oxygen consumption was 154 ml/min/m^2 BSA at STPD. CO_2 production was 116 ml/min/m^2 BSA at STPD. $\dot{V}_E$ = 6.89 l/min/m^2 BSA at BTPS, $\dot{V}_T$ = 249 ml/m^2 BSA at BTPS, $\dot{V}_A$ = 240 l/min/m^2 BSA at BTPS, $PaCO_2$ = 41.8 mm Hg.

Dawson and Evans (21), in a study of the effect of hemoglobin types on the cardiorespiratory system of sheep, reported that sheep with HbA had higher O_2 content in both arterial and venous blood than those of the sheep with HbB. The CO_2 content of the blood of animals with HbA had a tendency to be lower than that of sheep with HbB both in arterial and venous blood. Under light anesthesia the respiratory minute volume of sheep with HbA were significantly higher than those of sheep with HbB; there was no difference in respiratory rate.

The following findings were reported by the authors:

		HbA	HbB
(1)	Oxygen content (vol.%)		
	art.	15.8	12.9
	ven.	10.4	7.6
	art. ven. difference	5.4	5.3
(2)	Oxygen saturation (%)		
	art.	91.6	83.0
	ven.	59.8	48.8
	art. ven. difference	31.9	34.2

		HbA	HbB
(3)	CO_2 content (vol.%)		
	art.	47.0	51.8
	ven.	52.9	56.0
	art. ven. difference	5.9	4.2
(4)	Resp. rate (breath/min)	17.9	19.3
(5)	Tidal vol. (ml)	114.5	84.0
(6)	Min. vol. (l.)	2.04	1.62

Cross et al. (19) reported a study of 16 adult sheep under anesthesia and with tracheal cannulas. The following are figures for five adult sheep.

	1	2	3	4	5
Age (years)	7	6	6	1.7	5
Weight (kg)	31	55	80	57	33
Surface area (m^2)	.98	1.17	1.49	1.07	.75
SaO_2 (%)	80	95	84	83	90
SvO_2 (%)	50	55	46	32	49
End exp. PO_2 (mm Hg)	99	120	111	118	114
End exp. PCO_2 (mm Hg)	32	30	33	33	37
Min. volume (l.)	9.7	9.9	8.4	8.8	45
Resp. rate	34	30	28	26	16
O_2 consump. (ml/min)	209	257	234	252	149

Purves (67), studying the effect of breathing 100% O_2 in the newborn lamb, gives the following average values for 50 lambs that were under anesthesia obtained using a body plethysmograph:

Ventilation = 0.18 to 0.37 l/kg/min BTPS

He found ventilation significantly related to body weight.

$$y\ (l/min) = 0.123 + 0.261\ kg$$

No relation was found between ventilation and age. PaO_2 reported by this author was 57-84 mm Hg for anesthetized and 85-97 mm Hg for unanesthetized lambs.

Huisman et al. (44) reported that the different types of Hb in sheep had different affinities for oxygen and that they possessed different dissociation curves.

Barron (4) found that the O_2 capacity of the blood of lambs changes, dropping towards the adult level within the first three weeks after delivery. The dissociation curve shifts rapidly to the right during the first 15 days after birth and then gradually to reach the adult field between 34 to 48 days after birth.

III. MATERIALS AND METHODS

In the present study six large, western, crossbred sheep (wethers), were used. They were fed hay and water ad lib. and remained at all times under constant conditions of temperature (22°-23°C) and humidity.

The animals were surgically prepared by establishing a tracheal fistula at the level of the midcervical region and by cannulation of the femoral artery with medical grade vinyl tubing. The vinyl tubing had a 0.067" I.D. and a 0.107" O.D. (24).[1] To maintain the tracheal fistula a plastic Dyson-type tracheotube was used.

The technique used to measure pulmonary diffusing capacity was the steady state technique described by Finlley et al. (27).

The animal was put in a crate at least 15 minutes prior to the start of the experiment and the trachea was intubated using an endotracheal tube (size 42 French)[2] with an inflatable cuff. A local anesthetic[3] was applied to the outside portion of the endotracheal tube prior to insertion.

The endotracheal tube was connected to a two-way valve.[4] The inspiration side of the valve was connected to a gas tank containing a mixture of air and 0.0975% CO.[5] The expiration side of the valve was connected to a

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[1]Becton, Dickinson and Co.

[2]Magill's endotracheal tubes.

[3]Cyclaine hydrochloride. 5%, Jelly. Merck Sharp and Dohme.

[4]Swivel "Y" inhaler valve. Ohio Chemical & Surgical Equipment Co.

[5]Matheson Company, Inc.

200 l. Douglas bag.[1] A Collins giant three-way valve,[1] located between the animal and the Douglas bag, permitted the expired air to be collected in the Douglas bag or exhausted to the air. The dead air space of the valve and endotracheal tube was found to be approximately 35 ml.

The animal was allowed to inhale from the tank and to exhale into the Douglas bag for two minutes, at which time the Collins giant three-way valve was opened to the air and the Douglas bag was evacuated by means of a vacuum pump (this permitted flushing the system). The Collins giant three-way valve was then reopened to the Douglas bag and expired gas was collected for approximately two minutes, during which time a blood sample was being taken anaerobically from the arterial catheter into a 30 ml heparinized plastic syringe. The respiratory rate was also recorded with a pneumograph connected to a pressure transducer and was recorded with a multichannel recorder.[2]

The pH of the blood was measured with a pH meter.[3]

The analyses of expired gas (O_2, N_2, CO_2, CO) and blood gas (O_2, CO_2) were made with a gas chromatograph apparatus.[4]

Carbon dioxide partial pressure in the arterial blood was calculated by means of the Henderson-Hasselbalch equation (18, 20). For this purpose

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[1]Warren E. Collins, Inc.

[2]Sanborn Company.

[3]Beckman Model 1019 research pH meter with Model 28505 thermomatic constant temperature block.

[4]Loenco Model AD 2000 Respiration and Blood Gas Analyzer with Model AD 200 Loenco-Hackney vortex blood gas extractor.

arterial blood was centrifuged under mineral oil and then pH and CO_2 content of the plasma were determined. A correction for temperature was made due to the fact that the calculation of $PaCO_2$ was made for a 37°C temperature and the animal temperature was approximately 38°C (12).

The amount of gas expired was measured with a wet test meter.[1] The volume obtained was corrected for STPD and BTPS conditions.

One of the animals was exposed to NO_2 in order to produce alveolar lesions which would interfere with the pulmonary diffusing capacity. The animal was exposed first to approximately two liters of the gas for 10-minute periods in four consecutive days. Following this, the animal was exposed to approximately four liters of the gas for 20-minute periods in four consecutive days. After each of these two periods of exposure, pulmonary diffusing capacity was estimated.

Due to the fact that considerable quantities of eructated gas enter the respiratory tract (23, 25), it was necessary to intubate the trachea. It was then discovered that tracheal intubation caused certain effects on the animal and thus observations pertaining to this were necessary. For this purpose, the animals were put in a crate and left there undisturbed for a reasonable period of time. Pre-intubation blood samples were collected and after this the Dyson-type tracheotube was removed and the trachea was intubated using an endotracheal tube with an inflatable cuff, as previously described. Postintubation samples were taken at different time intervals. The endotracheal tube was then removed and the Dyson-type tracheotube was replaced. At this time blood samples were again taken.

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[1]Precision wet test gas meter. Sargent S-39467.

All blood samples were anaerobically obtained and kept in a 10 ml heparinized plastic syringe.

In each of the blood samples the following determinations were made:

(1) Blood pH

(2) Packed cell volume using a microhematocrit method.[1]

(3) Hemoglobin using a hemoglobinometer.[2]

(4) Oxygen and carbon dioxide content with a gas chromatograph apparatus.

The above experiment was repeated ten times. In four experiments blood pressure and respiratory rate were recorded.

Since there was a tendency for the packed cell volume to decrease in the above experiments, a splenectomy was performed to determine the part the spleen might have played in this phenomenon.

Finally, to determine the effect of the increased pressure upon the trachea as caused by the inflated cuff, an animal was fitted with a special endotracheal tube possessing two cuffs, one at each end. This was applied through the tracheostoma. This allowed for maintenance of pressure while also permitting the respiratory and eructated gases to follow the normal pathway.

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[1]International microhematocrit centrifuge Model MB.

[2]Lumetron Model 15.

IV. RESULTS

The values for diffusing capacity (D_LCO) ranged from 11.71 to 22.79 ml CO/min mm Hg m^2 B.S.A. Fractional CO uptake ranged from 21.94% to 56.00%.

The results of the measurement of D_LCO and complementary data are presented in Table 1.

The exposure of animal No. 1 to NO_2 for four days produced a drop of D_LCO from 22.29 to 16.73 ml/min mm Hg m^2; the exposure to NO_2 of the same animal for four more days produced an additional drop in D_LCO from 16.73 to 7.15 ml/min mm Hg m^2. The first four days of treatment also dropped the SaO_2 from 85.1 to 80.0%; four days of additional treatment failed to produce further decrease in SaO_2.

The results of the effect of NO_2 treatment on D_LCO, pH and SaO_2 are summarized in Table 2.

The results of the tracheal intubation in five animals on Hb, PCV, pH, CO_2, O_2 and arterial blood pressure are presented in Table 3. In the cases in which more than one determination was made, the average and the standard error of the mean are presented.

In all the animals a drop in the values for Hb, PCV, and O_2 was produced by the intubation. Although the most significant drop occurred between 80-135 minutes after intubation, it was possible to detect changes as soon as 15 minutes after the introduction of the cannula in some of the animals. In all the animals, with the exception of No. 1, the values for Hb, PCV, and O_2 show a tendency to increase after the removal of the endotracheal tube.

TABLE 1. D_LCO and complementary data for four sheep

	Sheep			
	1[a]	2	3	4
Weight (kg)	48	94	46	65.5
pH (art. blood)	7.582	7.546	--	7.546
Sa_{O_2} (%)	85.1	97.1	87.3	84.5
Ca_{O_2} (vol.%)	10.69	15.74	13.21	13.36
P_AO_2 (mm Hg)	120	116	108	127
$\dot{V}$ (ml)	7,642	12,755	19,570	17,702
V_T (ml)	437	478	200	369
V_D (ml)	127	146	120	150
D_LCO (ml/min. mm. Hg)	24.29	36.25	12.30	23.46
D_LCO (ml/min. mm. Hg kg.)	.506	.382	.267	.358
D_LCO (ml/min. mm. Hg m^2)	22.29	22.79	11.71	18.05
Fractional CO uptake (%)	56.00	50.66	21.94	39.58

[a]Average of two determinations.

TABLE 2. Effect of NO_2 treatment on D_LCO, pH and SaO_2 on sheep No. 1

	Treatment		
	Normal[a]	4 days of NO_2 2 l. for 10 min.	4 days of NO_2 4 l. for 20 min.
pH (art. blood)	7.582	7.575	7.536
SaO_2 (%)	85.1	80.0	80.0
D_LCO (ml/min. mm. Hg)	24.29	18.24	7.80
D_LCO (ml/min. mm Hg kg.)	.506	.380	.162
D_LCO (ml/min. mm. Hg m^2)	22.29	16.73	7.15

[a]Average of two determinations.

TABLE 3. Effects of tracheal intubation on sheep

	Hb (gm/100 ml)	PCV (%)	pH (art. blood)	CO_2 (vol.%)	O_2 (vol.%)	Blood pressure (mm Hg)
Sheep No. 1						
P[a]	9.10	25.0	7.495	53.84	11.18	---
CI[b]	8.95	24.5	7.575	47.05	11.72	---
CO[c]	8.65	24.4	7.536	50.67	10.63	---
Sheep No. 2 (Av. 3 ± $S\bar{x}$)						
P[a]	13.51 ± .16	40.69 ± .70	7.555 ± .01	48.95 ± .62	17.23 ± .23	103 ± 3.33
CI[b]	12.71 ± .16	36.80 ± 1.03	7.605 ± .02	50.63 ± 1.87	16.47 ± .27	103 ± 5.68
CO[c]	12.86 ± .17	38.06 ± .11	7.586 ± .01	50.95 ± 1.96	16.64 ± .30	100 ± 10.00
Sheep No. 3 (Av. 3 ± $S\bar{x}$)						
P[a]	8.05 ± .45	23.34 ± 1.04	7.493 ± .01	66.48 ± 2.59	10.76	---
CI[b]	7.75 ± .44	22.39 ± 1.09	7.579 ± .01	63.41 ± 1.76	10.73	---
CO[c]	8.20 ± .60	23.64 ± 1.30	7.509 ± .01	60.84 ± 1.47	11.30	---
Sheep No. 4						
P[a]	12.30	34.5	7.605	48.27	15.01	115
CI[b]	11.35	30.8	7.646	49.24	13.50	105
CO[c]	11.80	31.4	7.632	49.51	13.70	105

[a]P = presample

[b]CI = 80-135 min. after tracheal intubation.

[c]CO = 30-90 min. after removal of endotracheal tube.

TABLE 3 (Continued)

	Hb (gm/100 ml)	PCV (%)	pH (art. blood)	CO_2 (vol.%)	O_2 (vol.%)	Blood pressure (mm Hg)
Sheep No. 5 (Av. 2 ± $S\bar{x}$)						
P[a]	8.46 ± .56	24.96 ± 1.37	7.511 ± .02	58.12	---	---
CI[b]	7.54 ± .05	22.66 ± .49	7.578 ± .01	52.96	---	---
CO[c]	8.35 ± .39	25.03 ± 1.39	7.572 ± .01	54.17	---	---

Sheep No. 2 was splenectomized and the effect of the tracheal intubation on Hb, PCV and pH was determined. The results are shown in Table 4.

Finally, the results of the tracheal intubation of sheep No. 1 with the double catheter are shown in Table 5.

TABLE 4. Effect of endotracheal intubation on sheep No. 2 after splenectomy

	Hb	PCV	pH
p[a]	11.95	34.0	7.550
CI[b]	11.95	34.6	7.619
CO[c]	11.95	34.8	7.624

[a]P = presample

[b]CI = 80-135 min. after tracheal intubation.

[c]CO = 30-90 min. after removal of the endotracheal tube.

TABLE 5. Effect of endotracheal intubation with a double cuffed endotracheal tube on sheep No. 1

	Hb	PCV	pH
P[a]	7.2	21.2	7.574
DCI[b]	6.8	20.3	7.573
CI[c]	6.8	20.3	7.562
CO[d]	7.0	21.0	7.587

[a]P = presample

[b]DCI = 90 min. after introduction of the double cuffed endotracheal tube.

[c]CI = 60 min. after removal of the double cuffed endotracheal tube; an introduction of the endotracheal tube.

[d]CO = 50 min. after removal of the endotracheal tube.

V. DISCUSSION

In the D_LCO experiments the time allowed to reach the so-called steady state and flushing of the system was selected on the basis of previous reports on humans (33, 6, 27). This time was considered adequate to allow the achievement of the steady state and to avoid the buildup of COHb in the blood. The importance of the equilibrated COHb tension as a factor that should be subtracted from the calculated P_ACO has been previously recognized (32, 33, 58, 80). In sheep No. 1, the equilibrated COHb tension was found small enough to be negligible. This could be explained on the basis of the low concentration of CO used (0.0975%) and the short time of exposure.

Since the CO concentration was low, the response and accuracy of the gas chromatograph apparatus were tested. This response was found to be linear within 2% error.

From Table 1 it is evident that in two animals (sheep 1 and 2) the obtained values of D_LCO are very close to each other when they are expressed per m^2 of B.S.A. These two sheep presented a regular respiration during the determinations. In sheep No. 3, D_LCO is low when compared to Nos. 1 and 2, but this animal had a very high respiratory frequency (100 resp./min.). Sheep No. 4 had a respiratory frequency of 44 and a D_LCO value closer, but still lower than the values from sheep Nos. 1 and 2. This seems to indicate that when respiratory frequency increases, and dead space ventilation increases, there is a tendency for the calculated value of D_LCO to decrease. This could be explained by the fact that the steady state technique of Finlley et al. (27) is very sensitive to small

differences in the true values and in those obtained experimentally when tidal volume is small (27). There is also evidence that an increase in tidal volume increases the estimated value of D_LCO by the steady state method (2).

If the values of D_LCO in sheep Nos. 1 and 2 are considered to be representative of the true value of D_LCO in this species, the obtained value of D_LCO in sheep No. 3 should be disregarded as unreliable. The technique used is estimated to have a 25% error (27) and the difference between the average of D_LCO of sheep Nos. 1, 2 and 4, and that of sheep No. 3 is of the order of 50%.

All of the above seems to coincide with the fact that in the other two sheep the high respiratory frequency made it impossible to get an estimation of D_LCO. The calculated P_ACO made the values of D_LCO small enough to be rejected without hesitation.

The steady state technique of Finlley is likely to give good results in animals with regular quiet respiration. Unfortunately, sheep are very irregular in their respiratory pattern. This fact limits the application of this technique to sheep.

In the D_LCO determination it was necessary to intubate the trachea to avoid contamination of the expired gases by the eructated gases that have a very high CO_2 content (23, 25). The introduction of the endotracheal tube produced some physiological changes, mainly an increase in the respiratory rate and a drop in the Hb and PCV values. The decrease in Hb affects the value of D_LCO since it affects the θ factor in the equation

$$\frac{1}{D_L} = \frac{1}{DM} + \frac{1}{\theta V_c} \quad (78).$$

θ, in this case, is the capacity of the blood for CO which is highly dependent on the Hb content.

If the values of D_LCO obtained are compared with those reported for humans (2, 32, 33, 81), they are found to be higher. However, they are closer to the values reported for dogs (41, 60). The values of fractional CO uptake are within the values considered normal for humans (5, 17, 27). The fractional CO uptakes in sheep Nos. 3 and 4 are low; this may be due to the possibility of part of the CO inspired being limited to the dead space where no significant CO absorption is known to occur.

The results for SaO_2 in all four animals and the values for $\dot{V}$ and V_T found in sheep Nos. 1 and 2 agree with the results of other workers (42, 21, 19).

No report has been found with respect to P_AO_2 and V_D in sheep, but the results obtained in this work seem to be within reasonable limits.

As previously stated, sheep No. 1 was exposed to NO_2, an irritant gas known to produce pulmonary lesions consisting mainly of edema and epithelial cell proliferation. NO_2 has been used before to produce pulmonary lesions in studies of pulmonary adenomatosis in cattle (81). In sheep No. 1 there is a decrease in the calculated D_LCO, especially after the second treatment (four liters of NO_2 for 20 min. each day for 4 days). This drop indicates the presence of an alveolo-capillary block, actually an increase in the total air-blood barrier. The failure of SaO_2 to drop is not a contradiction of the above, since it is known that in the presence of an alveolar capillary block the SaO_2 tends to remain normal and it falls only in extreme cases or when the animal is exercised (31).

Although tracheal intubation has been used for physiological studies (11, 23, 25), there is little information on the effect of the introduction of the cannula on the physiology of the animal. In this experiment it has been found that the introduction of the endotracheal tube produced a drop in the Hb and PCV values and O_2 content of the blood; this seems to be related to a decrease of red blood cell numbers in the circulation; the red blood cells may be trapped or stored in the spleen as indicated in the failure to decrease in animal No. 2 after splenectomy. The possibility of red blood cell destruction is discarded because no signs of hemolysis have been seen after the tracheal intubation. After the tracheotube is removed, there is a tendency for the PCV, Hb and O_2 content values of the blood to increase. There is also the possibility of fluid displacement into the circulatory system; however, this has not been studied.

The fact that the drop of PCV and Hb occurred in the sheep which was fitted with the double cuffed endotracheal tube indicates that at least in this sheep the blocking of the eructated gases that normally go to the lungs was not the cause for the PCV and Hb decrease.

The pH increased during intubation and decreased after the catheter was removed. For unexplained reasons the CO_2 content of blood did not correspond to the changes in pH in sheep Nos. 2, 3 and 4; in animals 1 and 5 there was good agreement between the pH values and the CO_2 content of blood.

In each case the value selected was the most representative in the time intervals chosen. No test of significance was conducted because these values were not randomly selected.

VI. SUMMARY

Six surgically prepared sheep were used in a series of experiments designed to measure pulmonary diffusing capacity for CO using the steady state technique.

The steady state technique of Finlley et al. was found to be adequate in sheep with normal respiratory frequency. Sheep with high respiratory frequency gave low values of D_LCO.

One sheep was exposed to NO_2, and a reduction in the estimated value of D_LCO was found after the treatment.

The effect of tracheal intubation on Hb content, PCV values, O_2 content, CO_2 content, and pH of arterial blood was studied.

A small, but consistent, drop of the PCV value and Hb content was found after tracheal intubation.

Following the removal of the endotracheal tube there was a tendency for the Hb and PCV values to return to pre-intubation levels.

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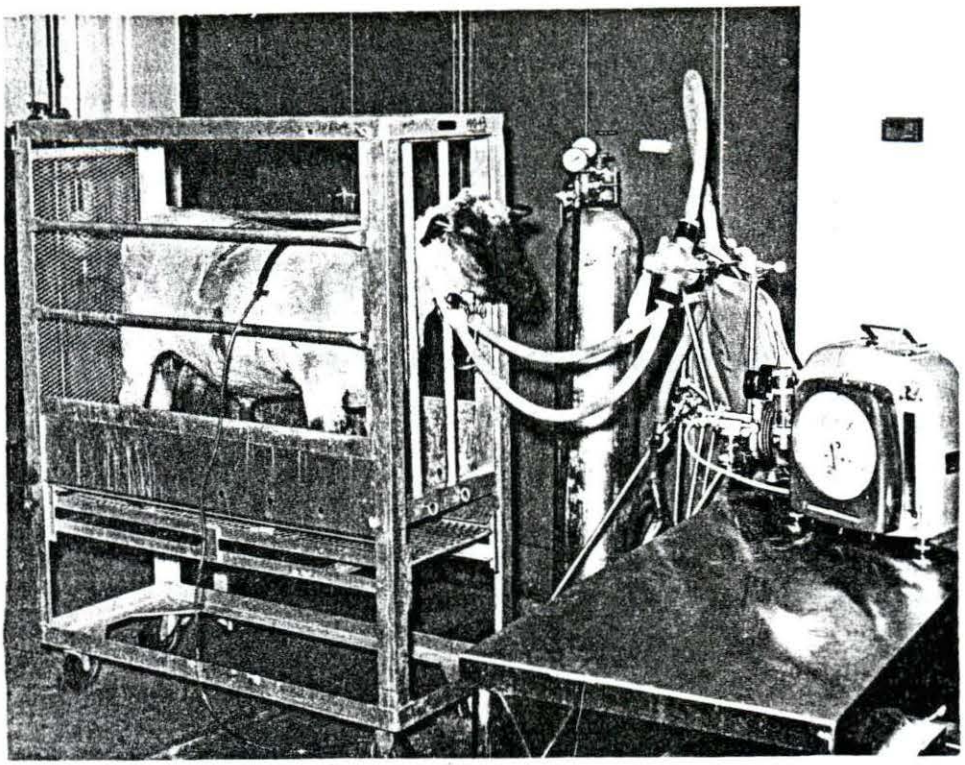
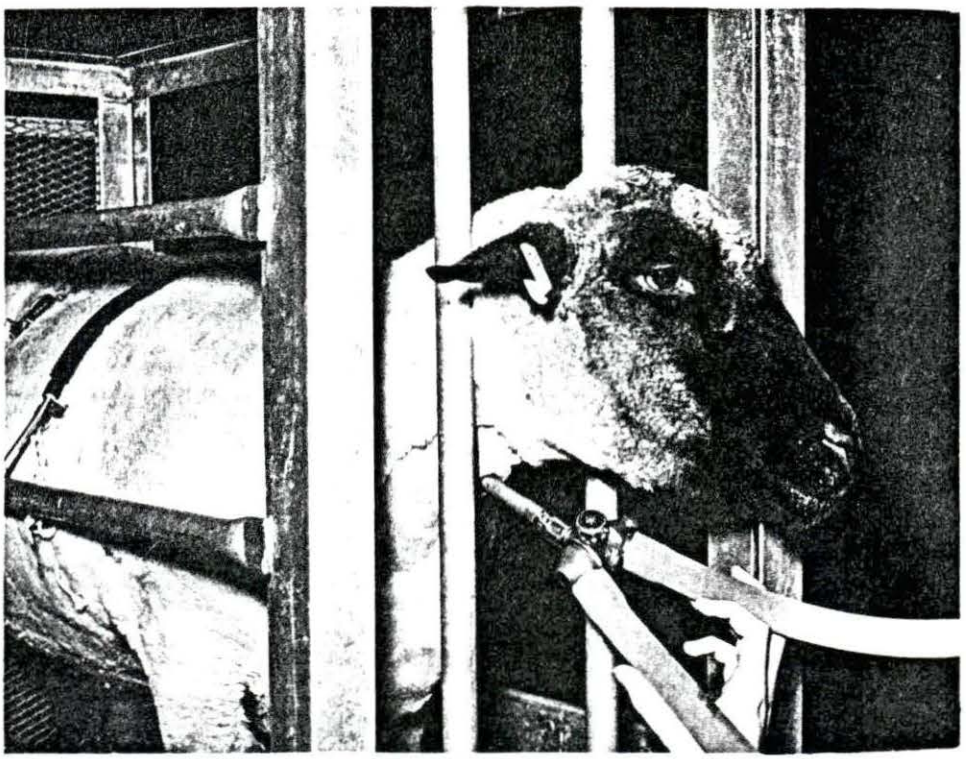
IX. APPENDIX

Figure 1. Animal and equipment used for collection and measurement of expired gases in D_LCO determinations

Figure 2. Close-up of the two-way valve and endotracheal tube used in D_LCO determinations.





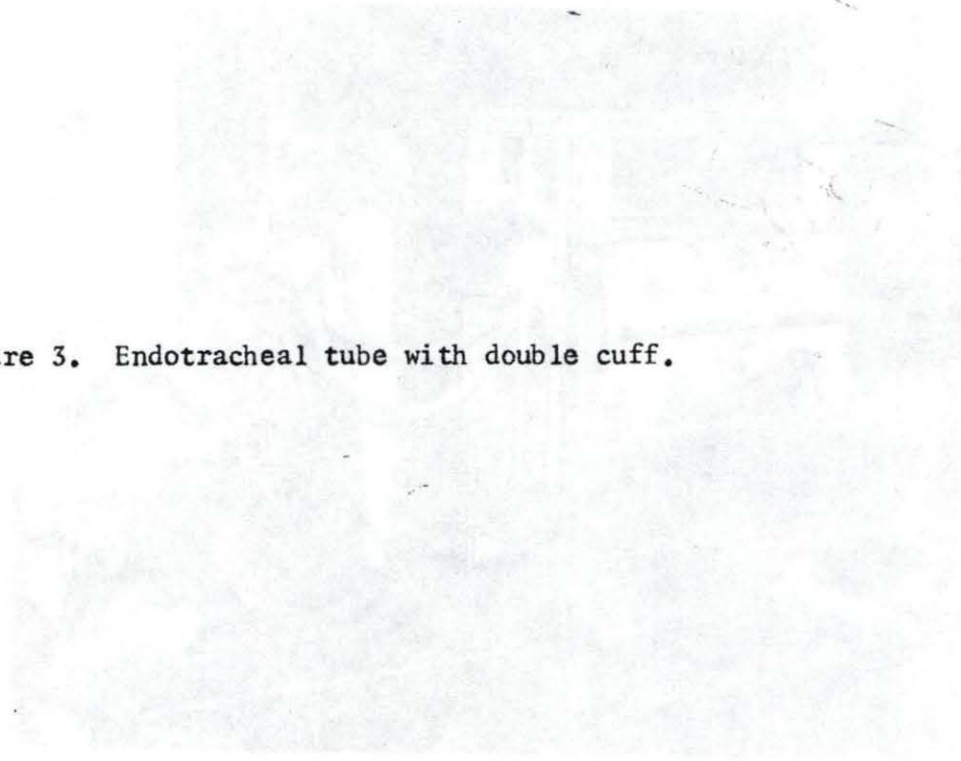


Figure 3. Endotracheal tube with double cuff.

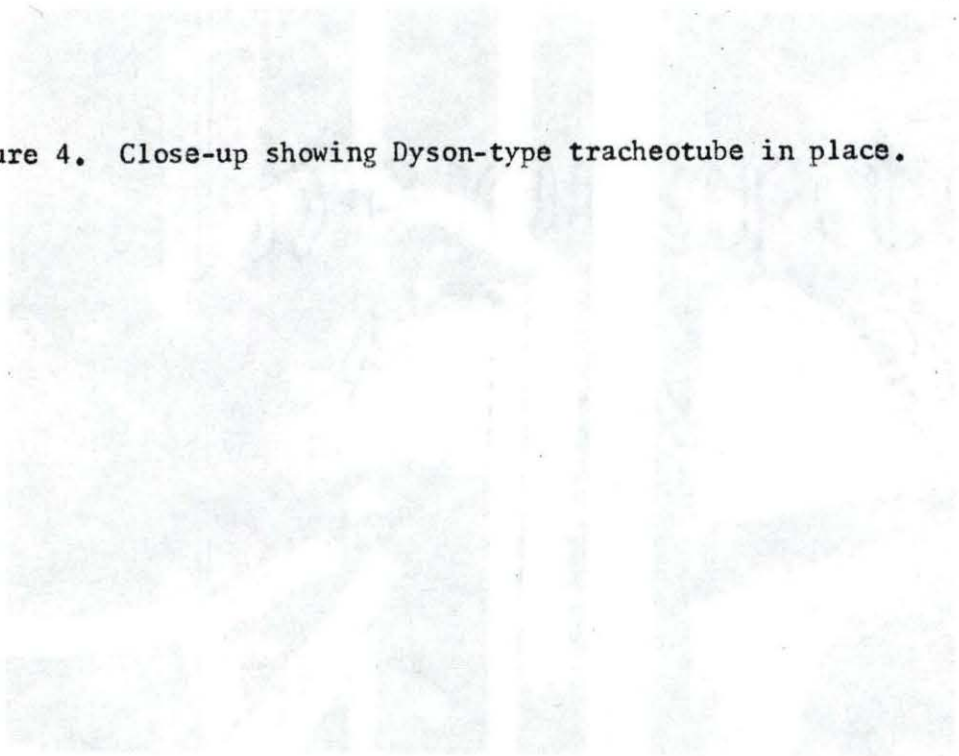


Figure 4. Close-up showing Dyson-type tracheotube in place.

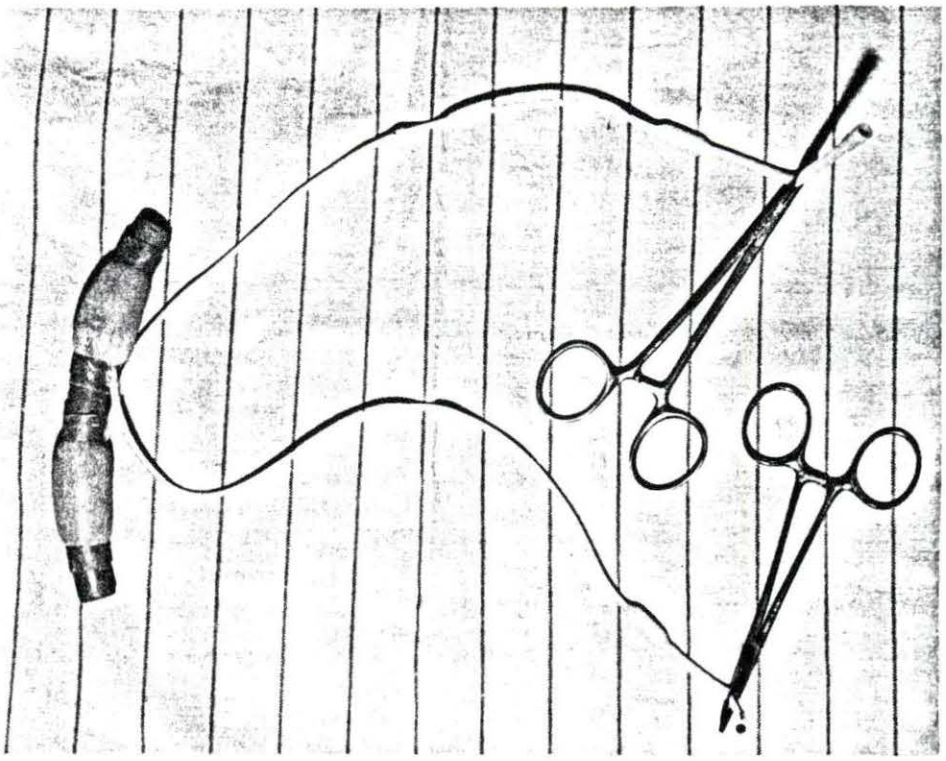


Figure 5. Equipment used for the determination of expired gases and blood gases.

Figure 6. Equipment used for measuring pH, hemoglobin, and PCV in blood samples.