Anesthesiology
56:97–106, 1982

Changes in Lung Membrane Diffusing Capacity for Oxygen Produced by Halothane

John K. Stene, M.D., Ph.D., Raymond B. Laravuso, M.D.,† Barry Burns, Ph.D.‡*

The effect of halothane on membrane diffusing capacity for O_2 (DMO_2) was measured in isolated left lower lobes of dog lungs using the sodium dithionite method. At 25° C, halothane reduced DMO_2 according to the regression equation: per cent control DMO_2 = −4.85(per cent halothane) + 97.5 (r = −0.55, P = 0.0007). Although DMO_2 was reduced from control by halothane administration, lung volume (V_L) increased at higher halothane concentrations and tended to restore DMO_2 by increasing surface area. There was a better correlation between the DMO_2/V_L ratios and per cent halothane: per cent (DMO_2/V_L) = −5.76 (per cent halothane) + 95.6 (r = −0.65, P = 0.00003). Effects of halothane on DMO_2 and V_L were reversible and were not influenced by gas mixing efficiency since argon dilution half-times over two decades were unchanged by halothane. It is unlikely that altered vascular recruitment affected the measured DMO_2 since resistance to blood flow was unchanged. We conclude that halothane decreases DMO_2 by either decreasing the physical diffusion coefficient (D′) for O_2 or decreasing the effective O_2 solubility (α), or both, in the alveolar-capillary membrane. (Key words: Anesthetics, volatile: halothane. Lung: alveolar-capillary membrane; blood flow. Measurement techniques: diffusing capacity; sodium dithionite. Oxygen: diffusion.)

THE RESISTANCE to gas diffusion through the anatomic alveolar-capillary membrane (DM) is the ultimate limitation for pulmonary gas exchange. Although the absolute value of DM for any gas is not known with certainty, an understanding of anesthetic effects on DM is necessary to appreciate fully the effects of anesthesia on gas exchange. The consistently observed widening of the alveolar-arterial O_2 difference in anesthetized patients and animals[1,2] could be due, in part, to anesthetic-induced changes in DMO_2. Using conventional gas exchange methodology, it is extremely difficult to separate changes in DM from distributional inequalities of ventilation, blood flow, or other factors such as hemoglobin amounts and reaction rates. To date, we are unaware of any studies of the effects of anesthesia on DMO_2.

* Assistant Professor, University of Maryland.

† Assistant Professor, Department of Anesthesiology, University of Wisconsin, School of Medicine Madison, Wisconsin 53792.

‡ Senior Research Scientist, University of Maryland.

Received from Critical Care Research, University of Maryland Institute for Emergency Medical Services, Baltimore, Maryland. Accepted for publication July 27, 1981. Supported in part by USPHS Grant HL10342 and the American Heart Association. Preliminary findings presented at the Molecular Mechanisms of Anesthesia Symposium, Seattle, Washington, 1979.

Address reprint requests to Dr. Burns: Critical Care Research, MIEMSS, 22 South Greene Street, Baltimore, Maryland 21201.

Theory

The Fick equation is commonly used to describe transfer of matter by diffusional processes and we use this theoretic formulation as the basis for interpretation of our membrane diffusing capacity results:

$$J = -AD' \frac{\Delta C}{\Delta x} \quad (1)$$

where:

J = flux (ml/s)
A = surface area for diffusion (cm^2)
D′ = kinematic diffusion coefficient (cm^2/s)
ΔC = concentration gradient
Δx = thickness of homogeneous barrier (cm)

In order to apply equation 1 to diffusion of gas in tissue, the term ΔC must be replaced by $\alpha\Delta P$, which gives the gaseous concentration difference. The Bunsen solubility coefficient of the gas in the membrane (α) converts the partial pressure (P) to volumes of dissolved gas:

$$J = -AD' \frac{\alpha\Delta P}{\Delta x} \quad (2)$$

By dividing both sides of equation 2 by ΔP (in mmHg) and changing seconds to minutes, the units of Fick flux are converted to those of a membrane diffusing capacity $ml \cdot min^{-1} \cdot mmHg^{-1}$:

$$DM = J/\Delta P = -\frac{A}{\Delta x}(\alpha D') \quad (3)$$

It is clear from inspection of equation 3 that membrane diffusing capacity depends only on the two anatomic properties ($A/\Delta x$) and the two physical properties of the membrane ($\alpha D'$). In the absence of lung volume changes, exposure of the lung to anesthetics would not be likely to alter the membrane surface area (A) or the average membrane thickness (Δx), but may change the physical diffusion coefficient (D′) or effective tissue solubility (α) for the diffusing species. In our experiments, any observed changes in DM would therefore be directly proportional to changes in D′ or α in equation 3 if surface area and thickness did not vary, as would be the case with a constant lung volume.

A New Method for Measuring DM

In order to test the hypothesis that anesthetic exposure alters DM by affecting α or D′ for a gas such as O_2, it

0003-3022/82/0200/0097 $01.00

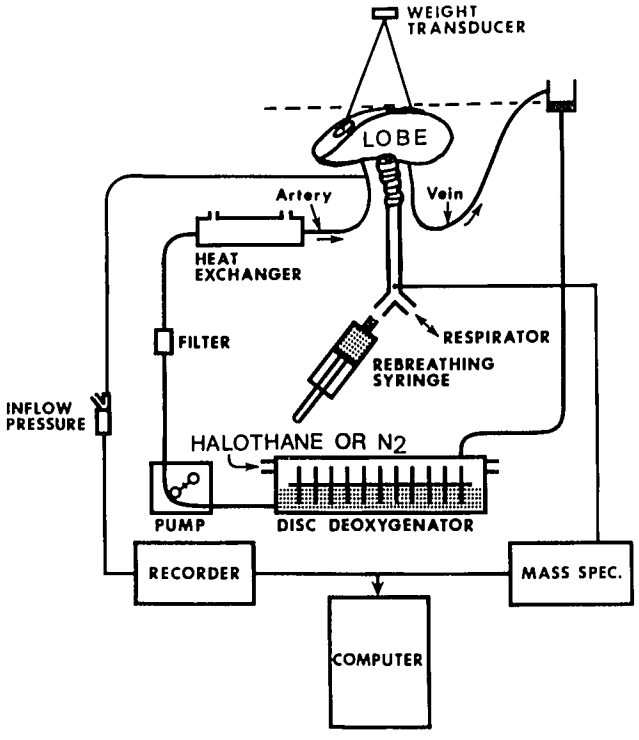

FIG. 1 Excised lobe preparation. Perfusion circuit consists of pump, pressure transducer, filter, flow probe (not shown), heat exchanger, isolated lobe, outflow column for setting "downstream" pressure (zone III) and deoxygenator (disk type). Partial pressures of gases were sampled by capillary leak of mass spectrometer at a point between bronchus and rebreathing syringe. Data were recorded on a Gould-Brush 8-channel recorder and digitized on a PDP 11 (Digital Equipment Corporation) computer for subsequent display and analysis.

is first necessary to have some direct measure of membrane resistance. Recently Burns and Shepard[3] have described a direct method for measuring DMO_2 in excised lungs perfused with the chemical sodium dithionite (DTT). In this preparation, capillary P_{O_2} is everywhere zero, independent of capillary volume or perfusion rate, and DM can be measured by a simple rebreathing technique. We have applied this method in the present study to determine the effects on DMO_2 of exposure to clinical concentrations of halothane (see appendix).

Materials and Methods

Mongrel dogs of either sex (15–25 kg) were anesthetized with ketamine (10 mg/kg), scopolamine (1–2 mg), and diphenhydramine (50 mg). The femoral artery was cannulated, the dog was heparinized (10,000 units) and one liter of blood rapidly collected in a chilled flask. The left lower lobe of the lung was immediately resected through a left lateral thoracotomy (left fifth intercostal space), suspended horizontally and ventilated by its bronchus. The lobe was supported by two adhesive ECG pads (Beckman) attached to the pleural surface with Eastman 910 bonding agent and hung from a strain gauge (Grass) so that the venous outflow was at the level of the top of the lobe. Changes in weight were continuously recorded. The pulmonary artery and vein were connected to the closed perfusion circuit illustrated in figure 1. The perfusion circuit consisted of a gas exchanger, roller pump (Sarns), heat exchanger, blood filter (Bently), pulmonary artery pressure (P_{PA}) transducer, and an electromagnetic blood flowmeter (Carolina Medical).

The autologous blood was cooled to 25° C and equilibrated with 95 per cent N_2 and 5 per cent CO_2 by recirculating through the gas exchanger prior to perfusing the lobe. The lobe was ventilated with this same gas mixture using a Harvard animal respirator set at 35 breaths/min and 200 ml tidal volume. End-expiratory pressure was 6 cmH_2O and all rebreathing measurements were begun at end-expiration. Immediately before lung perfusion, 10 g DTT dissolved in 100 ml distilled water and titrated to *p*H 7.4 with NaOH was added to the original one liter of perfusate. The addition of DTT increased osmolality from 350 to 400 mOs/kg and reduced the hematocrit by approximately 15–20 per cent (combined dilution and hypertonicity).

Pulmonary Vascular Reactivity

A temperature of 25° C and ventilation with 95 per cent N_2/5 per cent CO_2 was used to prolong lung viability[4] and prevent depletion of DTT between measurements of DMO_2. The hypoxic pulmonary artery vasoconstrictive response has been reported absent at temperatures of 25° C or less,[5] and we did not observe increased P_{PA} under the conditions of anoxia and hypothermia in our experiments. A passive, relaxed pulmonary vascular bed is advantageous because it results in a more even capillary recruitment and maintains low values of P_{PA}, helping to prevent edema formation.

DMO_2 Measurement

The lobes were manually rebreathed (100 to 200 ml tidal volume) with 2 per cent O_2, 5 per cent CO_2, balance N_2 for 5–10 s for the DMO_2 measurements (fig. 2). Between rebreathing measurements, the lung was ventilated by the respirator with 95 per cent N_2, 5 per cent CO_2. All measurements were made at blood flow rates of 115 to 300 ml/min which gave a P_{PA} of 13 to 20 mmHg. This flow rate and perfusion pressure were shown in previous experiments[6] to provide maximal gas exchange and a fully recruited vascular bed. We have verified this for the present experiments by showing that when the vascular bed is fully recruited with DTT, DMO_2 is relatively constant with further increases in flow (table 1).

A capillary leak mass spectrometer probe placed be-

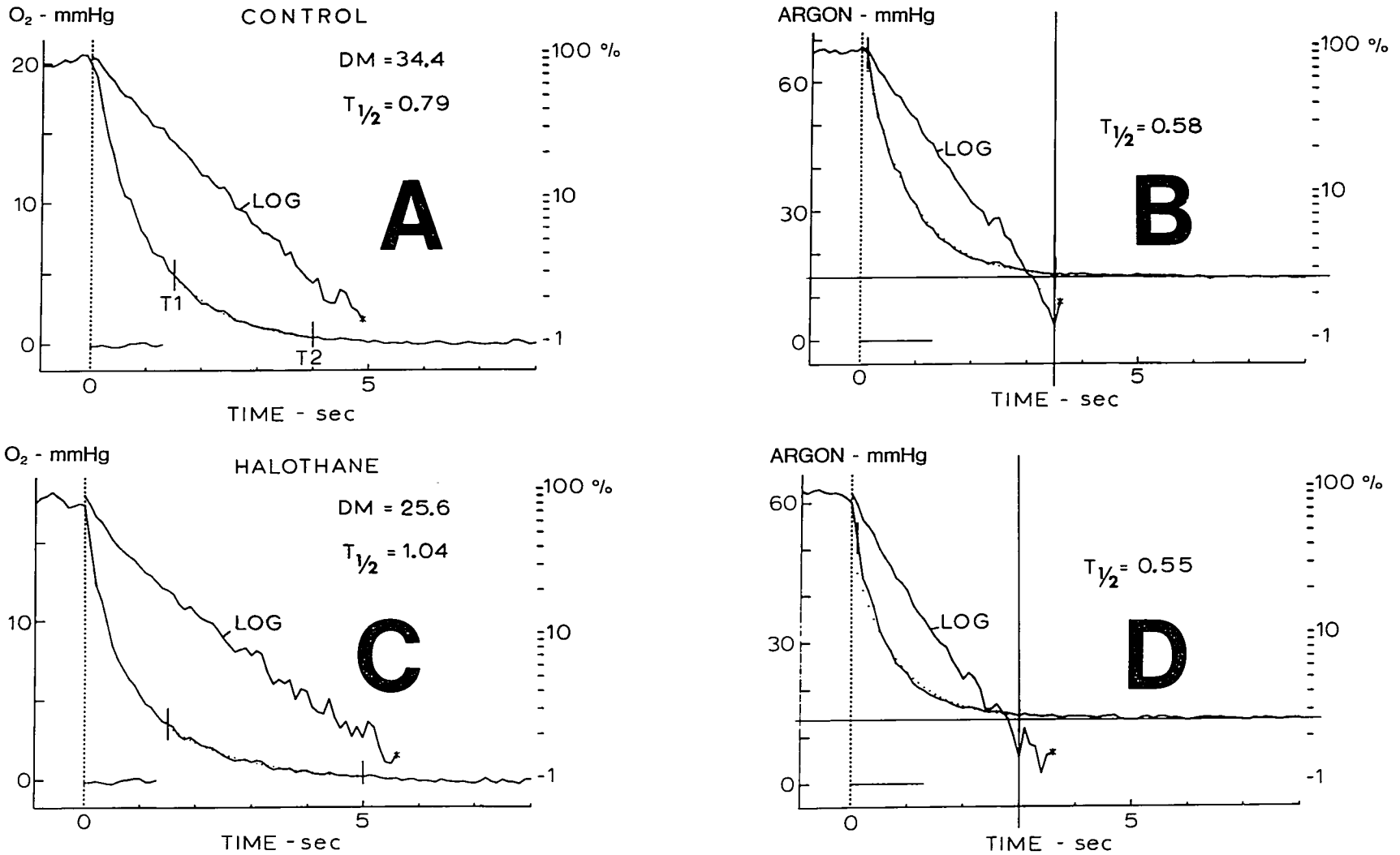

FIG. 2 Appearance and analysis of raw data. Washins photographed from screen of Tektronix 4012 terminal used for interactive determination of O_2 and Ar $t_{1/2}$. All runs scaled to 8 s duration, linear scale at left, log scale at right. Time zero marks beginning of rebreathing (±0.2 s). *A*. Control O_2 alveolar disappearance curve (Dog 2, run 1) and semilog plot generated by computer (LOG) over two decades. Log plot was fit by a straight line (not shown) over the interval (T_1–T_2) indicated in linear washin. Slope of straight line from log plot was used for $t_{1/2}$ (0.79 s) determination and calculation of DMO_2 (34.4 $ml \cdot min^{-1} \cdot mmHg^{-1}$ STPD). Half-time determination was begun at 1.5 s into washin, a point at which mixing was more than 80 per cent complete (see *B*). *B*. Simultaneous argon (Ar) washin during measurement (*A*). Ar dilution was used to calculate lung volume using the known syringe volume prior to rebreathing. The semilog plot of Ar washin (0.1–3.5 s) was also used to evaluate mixing efficiency ($t_{1/2}$ = 0.58 s). The appearance of a straight log plot over two decades indicates minimal inhomogeneities in the distribution of $\dot{V}/V$ ratios. Ventilatory rate of approximately 120/min was required to make mixing faster than the 0_2 disappearance rate in (*A*). Horizontal and vertical cursors delineate asymptote and T_1, T_2 for linear regression on log plot for $t_{1/2}$ determination. *C*. Same lobe as in (*A*), but O_2 disappearance in presence of 1.7 per cent halothane (Dog 2, run 4). DM is slightly different (25.6) than table 2 value due to small variations in positioning hairline cursors (not shown) for asymptote selection and regression interval during replotting and reanalysis. Lobe was ventilated with O_2 briefly prior to this measurement (table 2) but DM was not much different than previous measure before O_2 exposure indicating absence of significant extravascular DTT. Overall $t_{1/2}$ (1.04 s) was noticeably slower on halothane and DM was reduced. *D*. Simultaneous Ar washin for (*C*). Note Ar mixing $t_{1/2}$ (0.55 s) is virtually unchanged and log plot still approximates a single exponential (dotted line superimposed on lower Ar washin from T = 0.2 s to vertical cursor).

TABLE 1. Flow Effects on DMO_2

Dog No.	DMO^*_2	V_L (ml)	O_2 $t_{1/2}$ (s)	Ar $t_{1/2}$ (s)	$\dot{Q}$ (ml/min)	P_{FA} (mmHg)	Wt (g)
1	38.7	591	0.91	0.50	40	8.2	62
	52.6	584	0.67	0.56	115	12.4	67.3
	58.2	585	0.60	0.51	300	19	73.7
2	19.9	357	1.18	0.52	40	11	50.5
	32.9	383	0.76	0.60	115	15	52.5
	32.4	372	0.75	0.49	230	18.5	57
3	33	329	0.66	0.29	40	10	40
	41.8	319	0.52	0.28	113	19	42.5
	43.9	330	0.51	0.30	190	22	44
4	30.5	353	0.73	0.44	36	8.7	42
	44.7	355	0.53	0.38	120	13	48
	42.5	327	0.53	0.40	230	17	50

* DMO_2 = $ml \cdot min^{-1} \cdot mmHg^{-1}$ (STPD).

tween the rebreathing syringe and the bronchus (fig. 1) measured the disappearance of O_2 and the dilution of argon (Ar). Ar dilution was used to calculate the lung volume. The O_2 disappearance curve and the argon dilution curve were converted by the laboratory minicomputer into two decade semilog plots from which disappearance half-times were measured (fig. 2). The DMO_2 was calculated from a modification of the Krogh[7] equation:

$$DMO_2 = K \cdot V_{LTPS} \quad (4)$$

where: DMO_2 is in $ml \cdot min^{-1} \cdot mmHg^{-1}$ (STPD); K is a numerical constant consisting of the proportional rate constant ($-\ln 0.5/t_{1/2}$), the conversion of seconds to minutes, atmospheres to mmHg, and lung temperature and pressure (LTPS) to standard conditions (STPD). V_{LTPS} is the volume of lung, tubing (14 ml), and syringe (100–200 ml) in ml; $t_{1/2}$ is half-time for O_2 disappearance in seconds. At one atmosphere (760 mmHg) and a lung temperature of 25° C, K = $[(-\ln 0.5)\ (60)\ (273.1)/(760)\ (273.1 + 25)\ (t_{1/2})]$ or $.05/t_{1/2}$. Thus, under our experimental conditions, equation 4 can be rewritten:

$$DMO_2 = 0.05\ V_{LTPS}/t_{1/2} \quad (5)$$

Oxygen is consumed by the DTT during the rebreathing measurements and there is a small decrease (<2 per cent) in the total system volume (V_{LTPS}) as this occurs. The volume change was so small that it did not significantly alter the calculated DMO_2 and was ignored. In addition, we always used lung volume determined by Ar concentration at the end of rebreathing in the calculations, further minimizing any systematic effects of the 2 per cent volume change on the results.

HALOTHANE ADMINISTRATION

Following control measurements of DM, halothane was administered to the lung from a Fluotec MK-II (cyprane) vaporizor installed in line with the 95 per cent N_2 and 5 per cent CO_2 gas source. The halothane was administered simultaneously through a T-connection to both the gas exchanger (blood phase) and the respirator ventilating the bronchus (gas phase). The alveolar halothane concentration was measured by the mass spectrometer during rebreathing (alveolar plateau). Although the vaporizor was opened maximally (4 per cent) the lung halothane concentration was always less than this maximal value after the 6 to 8 min exposure duration. Although the exposure duration was intentionally short to minimize effects of any potential lung deterioration, and to avoid large changes in weight during exposure or recovery, maximal alveolar halothane levels were generally two to five times the minimal alveolar concentration (MAC) for clinical anesthesia.

DMO_2 measurements were made during the washin of halothane at approximately 3-min intervals. The halothane was discontinued after 6 to 9 min, and DMO_2 measurements were repeated approximately every 3 min in most experiments, until the halothane concentration was reduced to near control. The mass spectrometer and vaporizor halothane calibrations were checked against a volumetric standard and an infrared analyzer for accuracy. When the data were analyzed, the halothane effect was independent of whether the halothane level was increasing or decreasing; we have combined all washin and washout values for statistical analysis. In the calculations of results, DMO_2 as per cent of control DMO_2 prior to halothane administration was plotted against associated halothane concentration and fitted with a straight line by least squares linear regression (fig. 3*A, B*). The slopes of the lines from the least squares regression analysis were evaluated by Student's *t* test for significance of correlation.[8] We used a computer program to calculate the direct probability integral from our *t* statistic and the appropriate degrees of freedom.

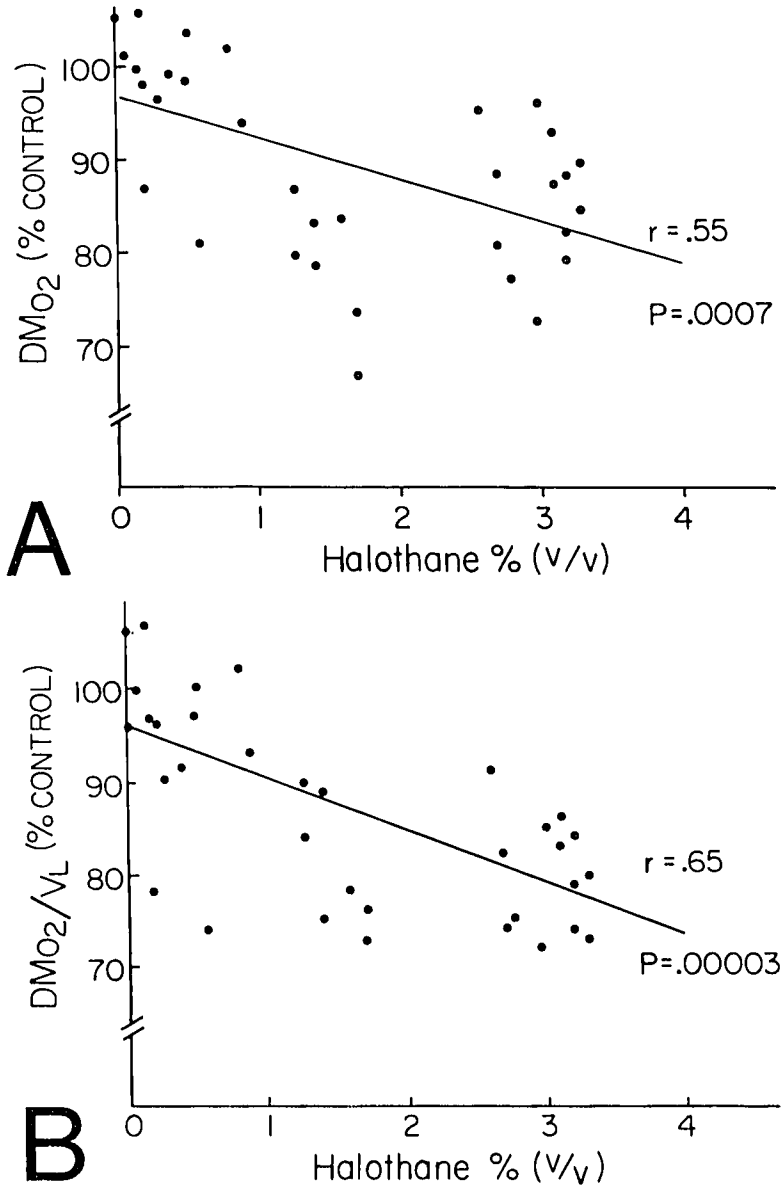

FIG. 3 Linear regression analysis of data from table 2. *A*. Plot of per cent control DM versus per cent halothane concentration. Correlation ($r = -0.55$) is significant but there is considerable scatter. *B*. Same data as in (*A*), except DMO_2 normalized for individual V_L values since lung volume tended to increase at higher halothane concentrations. Note improvement in correlation ($r = -.65$) and significance level. Overall relationship (slope) still approximately the same with 20–25 per cent decrease in DMO_2/V_L at 3.3 per cent halothane.

Validation of Method

Blood Flow. Table 1 illustrates the effects of increasing blood flow rates on the DMO_2. Argon mixing $t_{1/2}$ was unaffected by blood flow as would be expected, but DMO_2 increased with increasing flow until all the capillaries were recruited with fluid containing DTT (P_{PA} = 13–20 mmHg). Further increases in flow beyond maximal recruitment did not significantly influence DMO_2. As long as the capillaries contained sufficient DTT to keep P_{O_2} at zero from beginning to end-capillary, the effect of blood flow variation was negligible. We made our control and halothane DMO_2 measurements at a $\dot{Q}$ and P_{PA} associated with complete recruitment based on results shown in table 1.

Respiratory Rate. We have examined the effects of rebreathing rate on the measured DMO_2 and argon mixing half-time in table 2. DMO_2 nearly doubles as rebreathing rate is increased from 40–120/min, with relatively little further change up to the highest rate

TABLE 2. Rebreathing Rate Effects on DMO_2

Rebreathing Rate	DMO_2	V_L (ml)	O_2 $t_{1/2}$ (s)	Ar $t_{1/2}$ (s)
45	18.8	313	1.14	*
60	38.1	323	0.58	(0.45/1.0)†
120	40.4	330	0.55	0.36
180	43.1	335	0.52	0.28

DMO_2 = $ml \cdot min^{-1} \cdot mmHg^{-1}$ (STPD); $\dot{Q}$ = 190 ml/min; P_{PA} = 18–20 mmHg.

* Waveform could not be used with semilog regression technique.

† Multiple exponential resolved into two components (fast and slow half-times) over two decades on log plot.

tested (180/min). Argon mixing half-time showed a similar plateau with increasing ventilatory rate. Convective mixing is very important and can be rate limiting when using the DTT method due to the large magnitude of DMO_2. In order to minimize effects due to mixing rate limitation or ventilation/volume ($\dot{V}/V$) inequalities, we have used a rebreathing rate greater than 120/min in the present study.

Results

Table 3 gives the experimental data for nine lung lobes in which the effects of halothane on DMO_2 were determined at 25° C. The control value for each animal represents the DMO_2 immediately prior to halothane administration. Halothane caused a reversible decrease in DMO_2. The argon mixing half-times in table 3 were

TABLE 3. Data Summary

Dog No. (wt-kg)	Per cent Halothane	DMO_2*	Per cent Control	V_L (ml)	DMO_2/VL (× 100)	O_2 $t_{1/2}$ (s)	Ar $t_{1/2}$ (s)	$\dot{Q}$ (ml/min)	P_{EA} (mmHg)	Wt (g)
1	0.00	58.2	100.0	585	9.95	0.60	0.46	300	19.0	73.7
(20.9)	0.89	54.7	93.9	590	9.27	0.65	0.53	300	20.0	75.5
	1.26	50.4	86.6	565	8.92	0.68	0.55	300	20.0	78.0
	1.26	46.5†	79.9	559	8.32	0.73	0.56	300	19.5	78.5
	0.37	57.8†	99.3	640	9.03	0.65	0.55	300	19.5	80.0
	0.15	58.1	99.8	603	9.64	0.62	0.54	300	19.2	80.0
	0.07	58.9†	101.2	589	10.0	0.60	0.54	300	19.0	80.0
2	0.00	34.4	100.0	427	8.06	0.79	0.58	230	18.2	58.5
(19.1)	1.40	28.7	83.4	401	7.16	0.90	0.59	230	18.5	60.2
	1.70	23.0	66.8	392	5.87	1.10	0.53	230	18.6	60.5
	1.70	25.4†	73.8	415	6.12	1.04	0.55	230	18.0	62.0
	0.50	33.9	98.5	419	8.09	0.79	0.50	230	18.3	66.0
	0.13	38.4	111.6	420	9.14	0.70	0.49	230	18.0	71.0
	0.00	37.1†	107.8	435	8.53	0.74	0.44	230	17.2	80.0
3	0.00	57.9	100.0	526	11.0	0.55	0.40	260	15.0	63.4
(18.2)	2.96	42.2†	72.9	531	7.95	0.77	0.44	260	14.0	72.5
	0.00	61.0†	105.4	576	10.6	0.57	0.46	260	11.5	87.0
4	0.00	42.5	100.0	327	13.0	0.53	0.39	230	17.0	50.0
(22.7)	1.40	33.4†	78.6	344	9.71	0.69	0.42	230	16.9	53.5
	1.60	35.6†	83.8	350	10.2	0.65	0.40	230	16.5	53.5
	0.56	34.5†	81.2	361	9.56	0.69	0.44	230	16.5	54.0
	0.17	36.9	86.8	365	10.1	0.65	0.40	230	16.0	55.0
5	0.00	64.0	100.0	515	12.4	0.53	0.32	115	15.5	63.0
(19.1)	2.80	49.3	77.0	527	9.36	0.70	0.35	115	14.5	59.0
	3.20	56.5	88.3	543	10.4	0.63	0.34	115	15.0	63.0
	3.20	52.7	82.3	536	9.83	0.67	0.34	115	14.5	63.0
6	0.0	89.2	100.0	750	11.9	0.54	0.44	175	15.5	101
(21.8)	3.1	83.0	93.0	810	10.2	0.61	0.52	175	16.0	101
	3.3	80.1	89.8	840	9.54	0.65	0.51	175	16.0	104
	0.3	85.8	96.2	798	10.8	0.59	0.51	175	16.3	106.5
7	0.0	56.2	100.0	441	12.7	0.54	0.23	115	12.5	51.0
(20.2)	3.0	53.8	95.7	498	10.8	0.62	0.28	115	11.5	55.5
	3.3	47.5	84.5	512	9.28	0.71	0.28	115	11.5	58.6
	0.5	58.6	104.0	475	12.3	0.55	0.26	115	12.5	58.6
8	0.0	75.1	100.0	478	15.7	0.43	0.32	285	15.8	71.0
(19.1)	2.6	71.4	95.1	498	14.3	0.46	0.31	285	15.5	73.5
	3.1	65.7	87.5	502	13.1	0.51	0.36	285	14.8	74.0
	0.8	76.6	102.0	479	16.0	0.42	0.36	285	15.0	76.0
9	0.0	43.9	100.0	330	13.3	0.51	0.30	190	22.0	44.0
(19)	3.2	34.2	79.4	349	9.8	0.68	0.37	190	21.5	45.0
	2.7	38.2	88.1	348	11.0	0.61	0.35	190	21.0	46.5
	2.7	34.9†	80.9	353	9.9	0.67	0.27	190	20.3	46.1
	0.2	43.1	98.0	335	12.8	0.52	0.28	190	20.0	47.0

* DMO_2 = $ml \cdot min^{-1} \cdot mmHg$ (STPD).

† Five breaths of room air prior to DM measurement.

simultaneously measured during rebreathing for DMO_2 but were not correlated with halothane concentration ($r = 0.20$, $P = 0.22$). This indicates that halothane administration did not result in gross ventilation/volume ($\dot{V}/V$) inequalities which might have influenced the measured DMO_2.

Figure 2 shows the effect of halothane on both a typical O_2-disappearance curve and a simultaneous argon washin. We have combined all the halothane washin and washout points for linear regression analysis in figure 3*A* in which DMO_2 is plotted as a percentage of the control value *vs.* halothane concentration. Although we had attempted to hold lung volume (and surface area) constant, there were slight increases in V_L at the higher halothane concentrations (dogs 6–8). Increased V_L may have interfered with the halothane effect since DM is proportional to V_L. To correct for any effects of increasing V_L we also normalized DMO_2 by V_L. These data are reported as percentage of control DM/V_L ratios (fig. 3*B*), with normalization for lung volume increasing both the correlation coefficient of regression and the significance level of correlation.

Pulmonary artery pressure at constant flow was unchanged by halothane indicating that the available or perfused fraction of the microcirculation was unchanged during the experiments (table 3). There was a slight, permanent increase in lobe weight during halothane exposure in all lobes. We attribute this to increased vascular permeability caused by halothane itself. This increased vascular permeability was rarely observed in the control period or in other experiments in which halothane was not administered. The halothane-related extravascular water accumulation was probably perivascular or peribronchial since DM was not much altered before and after brief O_2 ventilation (to deplete extravascular DTT) following the weight gain (table 3), and returned to control after halothane elimination (see discussion). Fluid collection in the alveolar gas exchanging membranes would have altered DM by virtue of the increased thickness or diffusion distance.

Discussion

According to equation 3, the reversible effects of halothane could be explained by either alterations in the anatomic factors (A, Δx) or changes in the physical properties (α, D′) of the blood–gas barrier.

Anatomic Factors

Halothane could decrease DMO_2 by decreasing surface area for gas exchange. This would require either de-recruitment of capillaries (and increased P_{PA} due to increased resistance) or a decreased lung volume—neither of which was observed. In fact, the gas volume of some lobes actually increased at higher halothane concentrations but the decreased DMO_2 persisted despite the increase in surface area. Normalizing the DMO_2 for lung volume changes gives a more accurate representation of the halothane effect (fig. 3*B*).

Thickness (Δx) of the blood–gas barrier might be increased by halothane leading to decreased DMO_2. Halothane is known to cause a volume increase in cell membranes,[9] but the change is small (0.1–0.3 per cent) and may not necessarily result in thickness changes since the surface area could vary as well. This small percentage increase in membrane volume would be unlikely to explain the large decrease in DMO_2 that we have observed.

Physical Properties

If the decrease in DMO_2 with halothane is not due to anatomic factors, then it must be due to changes in the physical properties of the barrier (either α or D′). At present, we are unable to distinguish between the separate effects related to solubility or diffusivity changes. The propensity for anesthetics to order cell water and enhance clathrate formation[10] may decrease the effective diffusivity for O_2 through the aqueous compartments of cells comprising the blood–gas barrier. It is well known that the gaseous diffusion coefficients through ice are at least five orders of magnitude less than those through water.[11]

Evidence for a high diffusion resistance of membrane lipids is lacking. In earlier studies, we reported that the Q_{10} for DMO_2 (ratio DMO_2 at 35° C/DMO_2 at 25° C) is 1.2–1.3 over the temperature range 5–35° C.[12] This Q_{10} is consistent with an aqueous O_2 diffusion pathway.[13] Cell membrane lipids also are believed to undergo a change-in-state at low temperature with significantly increased diffusion resistance.[14] Since we have not observed a critical temperature for DMO_2 we infer either that the lipid diffusion pathway is not a primary limitation in DMO_2 or that lung lipids do not have a sharp temperature transition. In support of this, recent studies of O_2 diffusion through red blood cell membranes before and after removal of membrane lipids have failed to show any effects of these lipids on O_2 diffusion resistance.[15] Thus, any effects of halothane on the lipid components of the lung cell membranes are unlikely to affect DMO_2. We must conclude, as in the preliminary studies,[16] that halothane does not decrease DMO_2 by decreasing A or by increasing Δx, and that the state of membrane lipids is probably not important as a significant factor in overall diffusion resistance.

Limitations of the Method

We are assuming that DTT remains in the capillaries so that the effective O_2 diffusion distance is from alveolar

surface to capillary lumen. This assumption is supported by recent studies using labeled $(SO_4)^=$, a molecule similar in size and charge to dithionite $(S_2O_4)^=$. It has been shown that the lung is very impermeable to $(SO_4)^=$ which has the same volume of distribution as blue dextran, (an intravascular marker) over at least 10–15 min.[17,18]

If DTT did penetrate the capillary endothelium instantaneously—and there is no evidence for this—then the measurements that we have reported might reflect the diffusing capacity of the alveolar epithelium, or only half the barrier. Even under these extreme and hypothetical conditions, our interpretation of the halothane effects would still be valid. Electron micrographs of the lungs taken post-DTT perfusion in preliminary studies show normal ultrastructure and no evidence of disruption of tight junctions or edema.[11]

In addition, if significant amounts of DTT were present in the interstitium during our measurements, the diffusion distance would change during the rebreathing measurement as O_2 depleted the extravascular DTT stores. This would result in a variable diffusion distance and would produce a multiple-exponential O_2 disappearance log plot. This has not occurred in our study since we have consistently observed a single exponential (fig. 2*A*) O_2 disappearance curve over one decade.

We believe that lung viability is preserved despite the lack of O_2 in the alveoli or capillaries. According to the transplant literature, lung viability is best preserved in an atmosphere of 95 per cent N_2/5 per cent CO_2 at reduced temperature.[4] Anoxia alone does not affect capillary permeability[19,20] or the sensitivity to metabolic poisons[21] in excised dog lobes.

Absolute Magnitude of DMO_2

The control values of DMO_2 in this study are approximately 20 per cent greater than that first reported using this method.[3] There are several reasons for this change. In the earlier experiments, the rebreathing dead space was larger and the relative efficiency of gas mixing reduced. In recent studies,[22] we have increased rebreathing rate and tidal volume, reduced system dead space, improved mass spectrometer response time, used higher DTT levels, and conducted the experiments at blood flow rates giving more uniform capillary recruitment (P_{PA} = 13–20 mmHg) with DTT-containing plasma.[6]

We also have attempted to validate DM values obtained during rebreathing by subsequently measuring the steady state values while ventilating the lung with 2 per cent O_2, 5 per cent CO_2, balance N_2. In general, steady state results have equaled 70 to 95 per cent of the rebreathing values at blood flow rates consistent with maximal recruitment, but at necessarily reduced ventilatory rates (35/min steady state *vs.* 120/min rebreathing). Since there is a dependence of DTT DMO_2 on ventilatory rate, the steady state method may be inaccurate because a lower respiratory rate must be used for an accurate end-tidal measurement. However, it is important to note the approximate agreement since extravascular DTT would not contribute significantly to the steady state results unless DTT equilibrium were virtually instantaneous for intravascular and extravascular compartments.

The only independent measures of DMO_2 that we are aware of would be those based on lung morphometry, using measured thickness, surface area, α and D'. The predicted DMO_2 using these methods still exceeds our values using DTT by 20 to 30 per cent, but morphometry is remarkably close to the DTT value especially when compared with the very large discrepancies (25-fold) between morphometric and conventional physiological estimates of DM.[23]

Facilitated Diffusion

The presence of a pulmonary O_2/CO carrier was originally proposed by Burns and Gurtner[24] and later shown to be influenced by methoxyflurane exposure in sheep as measured using DLco.[25] The presumed carrier was tentatively identified as cytochrome P-450. At the time of these early studies on pulmonary facilitated diffusion, there was considerable uncertainty concerning the true magnitude of anatomic membrane DM in the lung and conventional reasoning was that fully half the resistance to CO uptake in the DLco was due to membrane diffusion limitation. It should be stressed that "membrane" for DLco refers to the anatomic barrier and the plasma and red cell diffusion resistances in series since there is no way to separate these individual components. Given this potentially large apparent membrane resistance, we believed there was an obvious role for a pulmonary carrier for CO (and O_2). The early results demonstrating saturation kinetics[26] and drug inhibition could equally well have been explained by variations in theta (θ, the diffusing capacity of 1 ml blood[27]) and this possibility was never ruled out.

The facilitated diffusion theory is effectively disproven by much of our recent work.[10,14,28] Since the true membrane DM is nearly 25 times larger than previously believed, the actual anatomic membrane diffusion limitation in the conventional DLco is not 50 per cent as we believed, but is less than 4 per cent of the total diffusion resistance. This means that earlier interpretations supporting a carrier were incorrect in placing the rate-limiting process in the alveolar-capillary membrane and the effects of halothane *in vivo* cannot be due to membrane carrier inhibition.

There is more direct evidence against a cytochrome

P-450 type carrier, however. Burns and Shepard have recently shown that the DTT DMO_2 was unchanged when the primary inert gas (88 per cent N_2) was replaced by CO in excised lobes.[22] Since both O_2 and CO would bind to cytochrome P-450 with a 1:1 relative affinity, the presence of 88 per cent CO should have competetively reduced any facilitated O_2 flux (with only 2 per cent O_2 in the rebreathing mixture). Although the DTT diffusing capacity was developed as a method to prove the existence of a pulmonary O_2 carrier, it has ultimately disproven the membrane carrier hypothesis.

Clinical Significance

The role of diffusion tends to be ignored because it is difficult to assess with conventional gas exchange methods, and venous admixture or shunt seem to account for the increased (A–a) DO_2.[29,30] A decrease in DMco with halothane anesthesia was recently reported by Zebrowski and Smith[31] using the method of Roughton and Forster[27] to estimate the membrane component of DLco. Although they measured a 25 per cent reduction in membrane diffusing capacity with halothane concentrations in the range of our study, these changes were not great enough to influence arterial oxygenation. The hypothetical membrane component of the DLco referred to in the Roughton-Forster method represents the diffusibility of CO in the alveolar-capillary membrane, across the plasma barrier surrounding red cells and in the red cell hemoglobin solution within the pulmonary capillary—and not across the alveolar membrane[32] alone as is often inferred.

The results of Zebrowski and Smith are not directly comparable with our own since additional diffusion resistances are involved in the DLco; however, it is provocative that the membrane component of the Roughton-Forster DLco was reduced by about the same fractional amount as the DMO_2 with halothane in the present study. It is reported that the diffusivity and solubility of O_2 in plasma and red cell hemoglobin solutions are approximately the same as in lung tissue.[11] At a molecular level, halothane may affect the true membrane, plasma, and red cell diffusion pathways in the same way and account for these similar findings using the DLco and DTT DMO_2. Changes in DLco also could be due to effects of halothane on theta (θco), but this has not been studied to our knowledge.

Bergman has measured the DLco before and after halothane anesthesia in patients and reported no significant change.[30] These results could not be readily compared with those of Zebrowski and Smith because Bergman's measurements were done at a single alveolar P_{O_2}. When we reexamined Bergman's results, there appeared to be a real increase in DLco after halothane, but this was not detected with the independent groups t test which was used (personal communication). If the one subject in Bergman's study which had a missing measurement (no. 2) is omitted, a paired comparisons t test can be used and gives a significant increase ($P = 0.01$) in DLco with anesthesia. This increase in DLco is not surprising, considering the pulmonary vasodilitation encountered with halothane and the pronounced sensitivity of DLco to changes in pulmonary capillary volume—a mechanism that also was suggested by Bergman.[30]

Our results are the first directly measured O_2 membrane diffusing capacity changes during anesthetic administration, and although we have observed a 20 to 25 per cent reduction in the true membrane diffusing capacity, these membrane diffusion changes alone cannot account for the widened (A–a) DO_2 during anesthesia. We believe that a similar impairment by halothane on O_2 (and CO) diffusion through the plasma and red cell hemoglobin may occur, but this could not account for the clinically observed widening of the (A–a) DO_2 either. Our data indicate that O_2 gas exchange deficits during anesthesia in humans are most likely due to ventilation/perfusion mismatch in the lung.

The authors thank Drs. R. H. Shepard, G. Nagel and M. C. Rogers for their assistance.

References

1. Rehder K, Sessler D, Marsh HM: General anesthesia and the lung. Am Rev Respir Dis 112:541–563, 1975
2. Fukuma S, Wildeboer-Venema FN, Horie S, et al: Pulmonary diffusing capacity in the dog as influenced by anesthesia and ventilatory regime. Respir Physiol 8:311–331, 1970
3. Burns B, Shepard RH: DL_{O2} in excised lungs perfused with blood containing sodium dithionite ($Na_2S_2O_4$). J Appl Physiol 46:100–110, 1979
4. Toledo-Pereyra LH, Northrup WF, Humphrey EW, Najarian JS: Maintenance of lung viability for transplantation after long periods of hypothermic perfusion. J Surg Res 18:99–106, 1975
5. Nilsen KH, Hauge A: Effects of temperature changes on the pressor response to acute alveolar hypoxia in isolated rat lungs. Acta Physiol Scand 73:111–120, 1968
6. Burns B, Shepard RH: Relationship between pulmonary arterial blood flow and rebreathing DLO_2 using the dithionite method in excised lungs. Am Rev Respir Dis 117:319, 1978
7. Krogh M: The diffusion of gases through the lungs of man. J Physiol (Lond) 49:271–300, 1915
8. Snedecor GW, Cochran WG: *Statistical Methods*. Ames, Iowa, The Iowa University Press, 1967, p 184
9. Miller KW, Paton WDM, Smith RA, et al: The pressure reversal of general anesthesia and the critical volume hypothesis. Molec Pharmacol 9:131–143, 1973
10. Pauling L: A molecular theory of general anesthesia. Science 134:15–21, 1961
11. Fenichel IR, Horowitz SB: The transport of nonelectrolytes in muscle as a diffusional process in cytoplasm. Acta Physiol Scand 60 (suppl 221):1–63, 1963
12. Burns B, Shepard RH: Q_{10} for the dithionite DO_2 and simultaneous DL_{CO} in excised lungs. Fed Proc 38:965, 1979
13. Grote J: Die Sauerstoffdiffusionskonstanten im Lungengewebe und Wasser und ihre Temperaturabhangigkeit. Pfluegers Arch 295:245–254, 1967
14. White SH: Phase transitions in planar bilayer membranes. Biophys J 15:95–117, 1975

15. Rotman H, Ikeda I, Chiu CS, et al: Resistance of red blood cell membrane to oxygen uptake. J Appl Physiol 49:306–310, 1980
16. Burns B, Stene JK, Laravuso RB, et al: Molecular factors influencing pulmonary alveolar-capillary membrane oxygen diffusion resistance during anesthesia, *Molecular Mechanisms of Anesthesia*, Progress in Anesthesia. Volume 2. Edited by Fink RB. New York, Raven Press, 1980, pp 389–396
17. Charles JM, Menzel DB: Heavy metal enhancement of airway sulfate absorption in the perfused rat lung. Res Commun Chem Pathol Pharmacol 15:627–639, 1976
18. Charles JM, Anderson WG, Menzel DB: Sulfate absorption from the airways of the isolated perfused rat lung. Toxicol Appl Pharmacol 41:91–99, 1977
19. Fisher AB, Hyde RW, Reif JS: Insensitivity of the alveolar septum to local hypoxia. Am J Physiol 223:770–776, 1972
20. Nicoloff DM, Ballin HM, Visscher MB: Hypoxia and edema of the perfused isolated canine lung. Pro Soc Exp Biol Med 131:22–26, 1969
21. Goodale RL, Goetzman B, Visscher MB: Hypoxia and iodoacetic acid and alveolocapillary barrier permeability to albumin. Am J Physiol 219:1226–1230, 1970
22. Burns B, Shepard RH: Membrane diffusion: Comparison between dithionite DO_2 and DLco, Progress in Respiration Research, Volume 16. Edited by Hertzog G, Piiper J, Scheid P. Basil, S. Karger AG, 1981, pp 130–141
23. Weible ER: The structural basis of alveolo-capillary gas exchange. Physiol Rev 53:419–495, 1973
24. Burns B, Gurtner GH: A specific carrier for oxygen and carbon monoxide in the lung and placenta. Drug Metab Dispos 1:347–379, 1973
25. Burns B, Cha YN, Purcell J: A specific carrier for O_2 and CO in the lung: Effects of volatile anesthetics on gas transfer and drug metabolism. Chest 69:316–321, 1976
26. Mendoza CH, Peavy B, Burns B, et al: Saturation kinetics for steady-state pulmonary CO transfer. J Appl Physiol 43:800–884, 1977
27. Roughton FJW, Forster RE: Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J Appl Physiol 11:290–302, 1957
28. Burns B, Shepard RH: Relationship between the DO_2 and the DLCO: Does membrane diffusion resistance limit the conventional DLCO or DLO_2? Am Rev Respir Dis 119:293, 1979
29. Weenig CS, Pietak S, Hickey RF, et al: Relationship of preoperative closing volume to functional residual capacity and alveolar-arterial oxygen difference during anesthesia with controlled ventilation. ANESTHESIOLOGY 41:3–7, 1974
30. Bergman NA: Pulmonary diffusing capacity and gas exchange during halothane anesthesia. ANESTHESIOLOGY 32:317–324, 1970
31. Zebrowski ME, Smith TC: Pulmonary capillary hemodynamics and diffusion limitations. ANESTHESIOLOGY 53:S410, 1980
32. Mochizuki M: Graphical Analysis of Oxygenation and Co-combination Rates of the Red Cells in the Lung. Tokyo, Hirokawa Publishing Co., 1975, p 66
33. Lambeth DO, Palmer G: The kinetics and mechanism of reduction of electron transfer proteins and other compounds of biological interest by dithionite. J Biol Chem 248:6095–6103, 1973

APPENDIX

Dithionite Reaction Scheme

There are two basic mechanisms[33] for reduction of oxidants by DTT ($Na_2S_2O_4$). One mechanism involves $S_2O_4^{=}$ (dimer) and the other SO_2^- (monomer). With the dimer as the reducing species, the reaction can be formally written as:

$$S_2O_4^{=} + (O_2) = S_2O_4^- + (O_2)^- \quad (1)$$

$$S_2O_4^- + (O_2) = 2(SO_2) + (O_2)^- \quad (2)$$

The reaction is a competetive second-order process and the product $S_2O_4^-$ of the first reaction is a highly reactive species that should rapidly reduce another (O_2) in the second reaction or reform $S_2O_4^{=}$.

With the monomer as the reducing species, the following reaction is seen in which the monomer is in equilibrium with the dimer:

$$S_2O_4^{=} \leftrightarrow 2(SO_2^-) \quad (3)$$

The monomer is very reactive and has a relatively low equilibrium concentration. At high $S_2O_4^{=}/(O_2)$ ratios, (O_2) will be reduced with first order kinetics:

$$SO_2^- + (O_2) = SO_2 + (O_2)^- \quad (4)$$

The gas SO_2 is derived from reactions involving both the dimer and monomer, and rapidly forms sulfurous acid and sulfates in aqueous media. We have sought to minimize the formation of acid reaction products in the pulmonary capillaries by performing the rebreathing measurements of DMO_2 at a relatively low initial alveolar P_{O_2} levels (10–15 mmHg $P_{A_{O_2}}$). Generation of heat and acid reaction products from DTT measurements at high $P_{A_{O_2}}$ levels (>60 mmHg) tends to cause the lungs to deteriorate with rapid edema and evolution of CO_2 into alveolar air as the bicarbonate buffer system responds to the very low capillary plasma *p*H. In the absence of large amounts of O_2, the excised, DTT-perfused lungs are remarkably stable at 25° C for long periods (>1 hour).