THE MEASUREMENT OF PULMONARY DIFFUSING CAPACITY FOR CARBON MONOXIDE BY A REBREATHING METHOD*†

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The pulmonary diffusing capacity for oxygen ($D_{L_{O_2}}$) is of great physiological and clinical significance (1). Its measurement, however, is relatively complex (2). Pulmonary diffusing capacity for carbon monoxide ($D_{L_{CO}}$) which, it is usually assumed,[1] can be converted to $D_{L_{O_2}}$ from the known solubilities and molecular weights of the two gases, is, in general, more readily measured. Further, since the rate of reaction of carbon monoxide with hemoglobin has been measured (4) *in vitro* while that of oxygen has not, determination of $D_{L_{CO}}$ at two or more alveolar oxygen tensions makes possible the computation of pulmonary capillary blood volume and pulmonary membrane diffusing capacity (5–7).

The methods used for measuring $D_{L_{CO}}$ are the single breath technique of Krogh (8) as modified by Ogilvie, Forster, Blakemore and Morton (9), the steady state technique using an independent measurement of the physiological dead space (10), the steady state technique using an alveolar sample (11) and a rebreathing technique using $C^{14}O$ (12). The present paper presents a modification of the rebreathing method utilizing stable CO and continuous analysis.

METHODS

A. Experimental procedure. The apparatus shown in Figure 1 was used in these studies. The subject's vital capacity and one second vital capacity are first determined. The analyzer circuit of the apparatus is then flushed with tank oxygen. A sealed bag containing a volume of 0.3 per cent CO and 10 per cent He in air (or in oxygen)[2] equal to the subject's one second vital capacity is attached to the three-way tap, the clamps on the bag are removed and the bag and analyzer circuit mixed by the pump.[3] A bag-in-box device attached to a spirometer has been found convenient for filling the bag. The subject then expires fully through the open arm of the three-way tap, following which the tap is turned to connect him with the bag. The subject rebreathes into the bag for 30 to 45 seconds at a rate of 30 cycles per minute in time to a metronome, endeavoring to empty the bag on each inspiration. At the end of this period the tap is closed, the pump is stopped and the bag clamped and removed. Before and after the measurement of diffusing capacity the equilibrium pCO of the subject's blood is determined by a rebreathing technique (13). The bag is then analyzed for oxygen, carbon dioxide and helium by passing its contents successively through a paramagnetic oxygen analyzer,[4] an infrared CO_2 analyzer[5] and a katharometer.[6] Carbon dioxide is adsorbed before the sample is passed through the katharometer.

B. Calculations. The volume of the lung-bag system is calculated from the equation:

$$V_s = \frac{F_b V_b}{F_s},$$

where V_s = volume of the lung-bag system in milliliters STPD, V_b = volume of rebreathing bag in milliliters STPD, F_b = concentration of helium in the bag at start of rebreathing and F_s = concentration of helium in bag at end of rebreathing. V_b is obtained from the record of the spirometer attached to the bag-in-box from which the bag is filled. F_b and F_s are determined by katharometer analysis of the gas mixture used and of the bag contents at the end of rebreathing. Since CO_2 is absorbed before the gas is passed through the katharometer, this last reading is multiplied by $1 - \frac{\text{per cent } CO_2}{100}$ to obtain the final helium concentration.

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[1] This assumption has recently been challenged by Bates (3).

[2] Matheson Co., E. Rutherford, N. J.

[3] Dyna-Pump, Fisher Scientific Co., Pittsburgh, Pa.

[4] Model E2, Beckman Instrument Co., Pasadena, Calif.

[5] Model 16, Liston Becker Division, Beckman Instruments, Fullerton, Calif.

[6] Cambridge Instrument Co., New York, N. Y.

The subject's residual volume may readily be calculated by subtracting the volume of the bag and the dead space of the apparatus (150 ml.) from the total volume thus obtained.

The CO concentration during rebreathing is recorded by a direct writing oscillograph moving at 1 cm. per second. This record is measured at any desired interval, usually five seconds. From this measured deflection is subtracted the deflection produced by passing 5 per cent CO_2 through the CO analyzer. In two subjects the error introduced by the use of a standard correction for CO_2 was investigated by placing an infrared CO_2 analyzer in the circuit and recording simultaneously CO and CO_2 concentration in the bag. Each point of the CO record could then be corrected for the deflection due to the CO_2 present at that point by using a calibration curve for the sensitivity of the CO analyzer to CO_2. The points thus obtained were indistinguishable from those obtained by using a standard correction.

After the measured points are corrected for CO_2, the CO concentrations are read from a calibration curve and these values are corrected for the back pressure of CO in the pulmonary capillaries. The back pressure is calculated by interpolating between the initial and final equilibrium CO values obtained as described above and multiplying this interpolated value by the fraction pO_2 (bag)/pO_2 (equilibrium sample). This correction is only approximate since it assumes that all hemoglobin (Hb) is in the form O_2Hb.

After this final correction, the values for CO concentration are plotted against time on semilogarithmic paper. A line is drawn through the points beginning with the final concentration. From this line, values for CO concentration at any convenient time interval are obtained and substituted into Krogh's equation (8):

$$D = \frac{Vs}{Pb(t_2 - t_1)} \ln \frac{Fs_{t_1}}{Fs_{t_2}},$$

where Fs_{t_1} = concentration of CO (dry) in the lung bag system at time t_1, Fs_{t_2} = concentration of CO (dry) in the lung bag system at time t_2, D = D_{LCO} = apparent diffusing capacity of the lungs for CO in milliliters per millimeter Hg × minute, Pb = pressure of the dry gases in the lung-bag system, $(t_2 - t_1)$ = time of rebreathing in minutes and ln = natural logarithm.

C. Other methods. Vital capacity and one second vital capacity were measured by a rapidly moving spirometer kymograph.[7] Residual volume was measured as described above or by a closed circuit helium method (14). Maximum breathing capacity was measured with the use of a high velocity valve[8] and a 120 L. Tissot type spirometer. The single breath diffusing capacity was measured as described by Ogilvie and co-workers (9). The steady state diffusing capacity was measured by the method of Filley, MacIntosh and Wright (10); P_{ACO_2} was obtained from direct measurement of CO_2 content and pH by the use of nomogram (15). Arterial oxygen saturation was measured as described by Peters and Van Slyke (16).

RESULTS

The absorption of CO was measured continuously during a 30 to 45 second period of rebreathing 578 times in 128 subjects. In 571 studies (98.9 per cent) the disappearance of CO was apparently exponential for a period of 15 seconds or more. The exponential fall of CO began within the first five seconds of rebreathing in 236 studies (40.8 per cent), between six and 10 seconds in 229 studies (39.6 per cent), between 11 and 15 seconds in 80 instances (13.8 per cent) and between 16 and 20 seconds in 23 studies (4.0 per cent), 19 of which were of 45 seconds' duration and four of 35 seconds' duration. In four studies (0.7 per cent), all of 45 seconds, the

[7] H. S. Osborne, Havertown, Pa.

[8] Hans Rudolph, Kansas City, Mo.

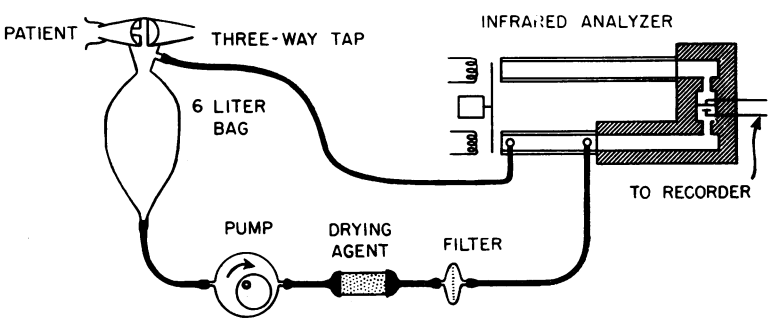

FIG. 1. DIAGRAM OF APPARATUS FOR MEASURING PULMONARY DIFFUSING CAPACITY BY REBREATHING

The pump circulates the contents of the bag through the drying agent (anhydrous $CaSO_4$), filter (Kel-F®, Liston-Becker Division, Beckman Instruments Inc.) and infrared CO analyzer (Model 15A, Beckman Instruments, Inc.) at the rate of 3 L. per minute. Volume of analyzer circuit is 150 ml. Inner diameter of openings to analyzer is one-eighth inch. Pressure at mouth during operation of pump is 0.3 cm. H_2O.

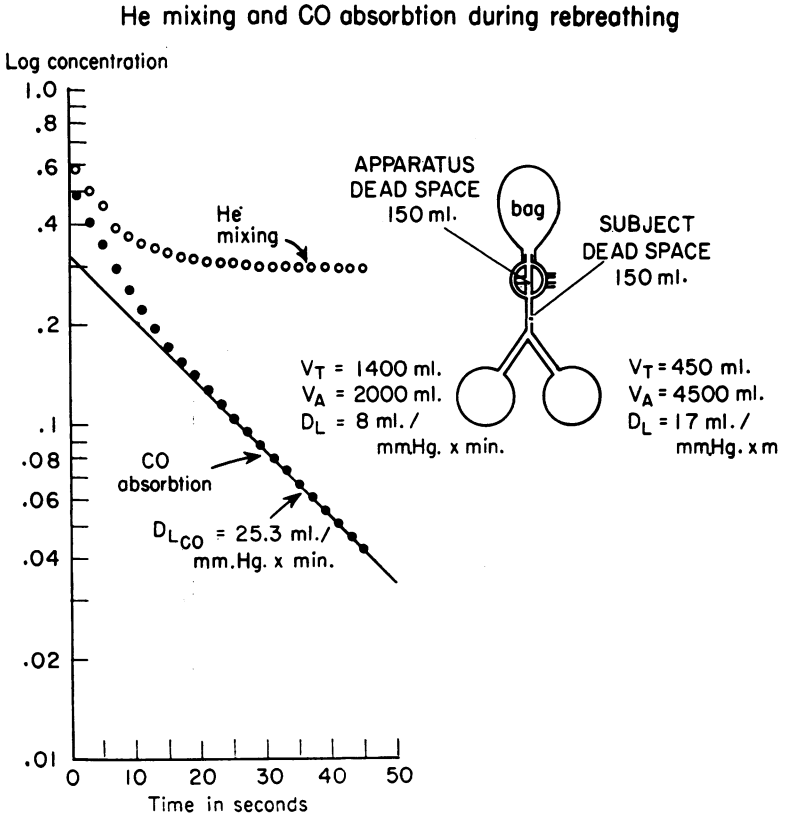

FIG. 2. HELIUM MIXING AND CARBON MONOXIDE ABSORPTION DURING REBREATHING

The concentrations of helium and CO are computed breath by breath for a 45 second period of rebreathing at 30 cycles per minute for the lung model shown at right. For explanation of abbreviations and comparison of different methods for measuring diffusing capacity in this lung model, see Table IV.

fall of CO was exponential after 21 to 25 seconds of rebreathing. When this apparently exponential decrease did not begin with the first measured point, the plot of log CO concentration prior to this time showed a gradually decreasing slope similar to that illustrated in Figure 2. This is probably due to concurrent mixing of the bag and the alveoli and diffusion from the alveoli into the blood. Those subjects in whom 10 seconds or more of rebreathing elapsed before CO concentration began to fall in an exponential manner usually had emphysema.

The continuous sampling method used in these studies was compared with discrete sampling at three points in five studies in four normal subjects (Table I). For each subject, three bags were filled to the same volume. The subject rebreathed into these bags for accurately timed periods of approximately 10, 20 and 30 seconds. The final CO concentration in each bag was measured, plotted against time and D_{LCO} calculated from the line connecting these points. As shown in Table I, this method of sampling did not give results significantly different from con-

TABLE I

*Effect of sampling method on D_{LCO}**

Subject	D_{LCO} Continuous sample	D_{LCO} Discrete sample
W. S.	28.8	28.6
E. C.	35.4	34.7
	16.1	15.4†
J. M.	28.2	26.9
R. G.	37.1	37.5
Average‡	29.13	28.63

* D_{LCO} = apparent diffusing capacity of lung for CO in milliliters per millimeter Hg × minute.
† pO_2 600 mm. Hg; other studies pO_2 100 mm. Hg.
‡ Average of five studies.

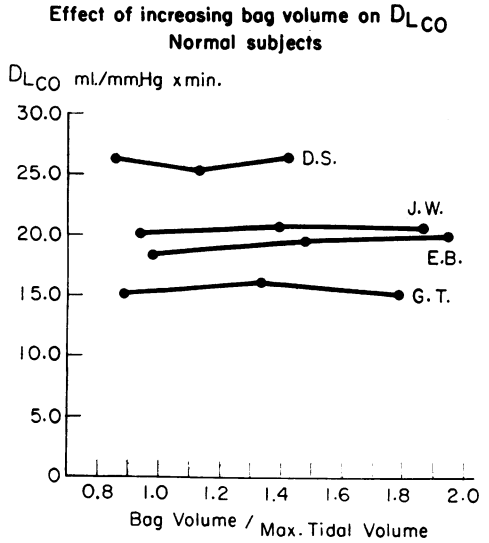

FIG. 3. EFFECT OF INCREASING BAG VOLUME ON DIFFUSING CAPACITY IN NORMAL SUBJECTS

tinuous analysis of the bag during a 30 second period.

The effect of increasing the apparatus dead space was studied in four normal subjects and five patients with emphysema by putting a greater volume in the rebreathing bag than the subject could inspire. For normal subjects the maximum tidal volume was assumed to be the one second vital capacity. Bag volumes greater than the one second vital capacity, then, represented an increase in apparatus dead space. As shown in Figure 3, such an increase did not change D_{LCO}.

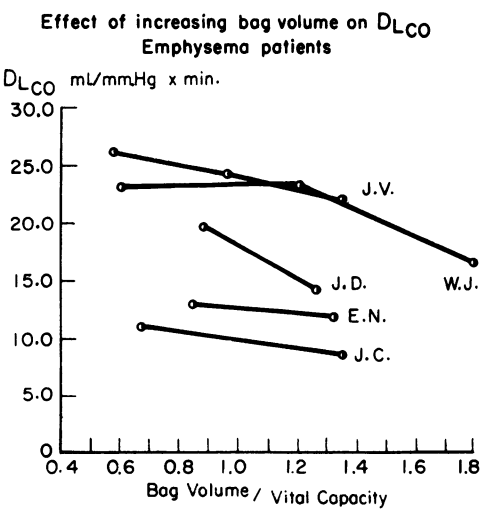

FIG. 4. EFFECT OF INCREASING BAG VOLUME ON DIFFUSING CAPACITY IN PATIENTS WITH EMPHYSEMA

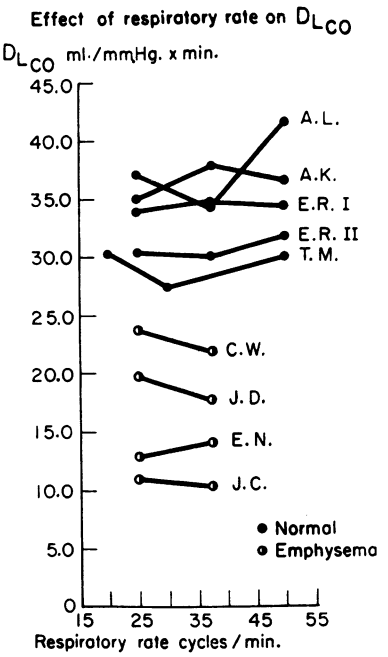

FIG. 5. EFFECT OF RATE OF REBREATHING ON DIFFUSING CAPACITY

This experiment was modified for the patients with emphysema. Previous experience with these patients had shown that when a volume of gas slightly less than the vital capacity was placed in the bag, the patient, after several

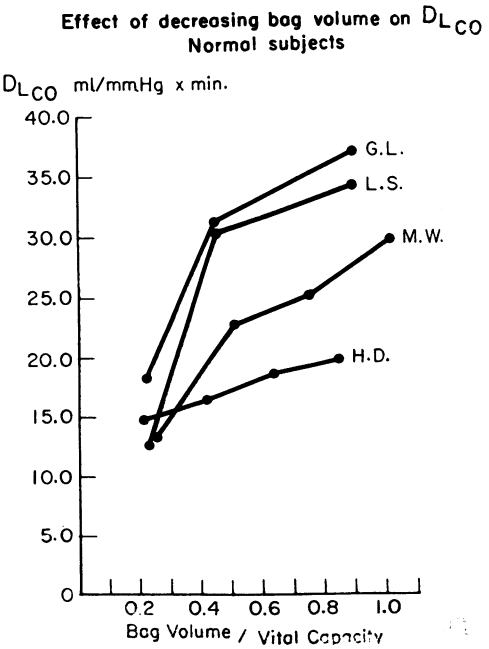

FIG. 6. EFFECT OF DECREASING BAG VOLUME ON DIFFUSING CAPACITY IN NORMAL SUBJECTS

breaths, emptied the bag completely on inspiration and yet had a tidal volume approximating his one second vital capacity. When the bag volume was appreciably less than the vital capacity, the tidal volume became very small because of air trapping during rapid expiration. Therefore, to increase the apparatus dead space, volumes of gas greater than the total vital capacity were placed in the bag. As shown in Figure 4, this led to lower values for calculated D_{LCO}.

The effect of changing rebreathing rate at the same bag volume was studied five times in four

TABLE II

Diffusing capacity of lungs in normal subjects (males)

Subject	Age	Ht.	Wt.	BSA*	VC	D_{LCO} RB	D_{LCO} SB	D_{LCO} SS
	yrs.	*in.*	*lbs.*	$M.^2$	*ml.*	*ml./mm. Hg × min.*		
H. B.	19	69	152	1.84	4,020	26.7	32.9	21.2
L. McL.	26	67	159	1.84	4,280	26.8	27.1	
M. N.	15	68	121	1.65	3,692	23.2	28.3	
W. S.	16	68	129	1.69	3,695	28.8	30.2	
J. H.	31	67	135	1.72	3,460	15.6	18.7	
J. P.	20	71	139	1.80	4,210	28.6	24.5	
C. S.	20	68	126	1.69	3,830	22.0	31.3	16.5
E. C.	31	73	178	2.06	5,025	35.4	34.8	
W. Z.	20	67	128	1.67	3,665	22.8	24.3	
J. S.	32	66	142	1.75	4,035	18.2	19.9	
R. G.	39	69	172	1.94	2,998	18.9	22.9	
C. B.	16	64	178	1.86	3,280	21.7	29.9	
H. L.	29	66	136	1.70	2,918	20.5	21.1	
T. E.	24	70	166	1.93	4,180	24.6	28.0	15.1
J. M.	25	74	186	2.11	4,800	37.5	35.0	
T. M.	25	68	168	1.91	5,419	27.5	35.5	
W. H.	47	71	151	1.88	3,760	21.9	21.8	18.5
W. S.	15	67	137	1.73	4,460	19.6	24.6	16.4
C. C.	18	70	145	1.83	4,700	33.2	32.0	
J. R.	29	68	185	1.98	4,270	23.1	28.6	
J. L.	31	73	209	2.20	4,300	26.3	32.8	
D. S.	20	71	162	1.93	4,000	26.8		
B. P.	15	70	150	1.85	4,720	30.9	25.3	
L. J.	30	73	185	2.09	4,130	34.4		
G. L.	18	65	150	1.76	4,112	37.0		
M. W.	21	70	148	1.84	3,620	29.7		
H. D.	32	72	159	1.93	4,346	19.8		
T. J.	23	67	130	1.68	3,580	25.0	26.7	
E. T.	19	70	136	1.78	3,195	18.2	17.8	
W. L.	33	74	187	2.11	4,890	40.4	41.2	
E. R.	24	72	188	2.07	5,640	32.1		
J. McG.	31	72	155	1.91	5,500	28.2		
A..Z.	23	73	205	2.18	5,150	30.5		
R. G.	25	73	180	2.06	4,935	37.1		
A. K.	23	69	158	1.91	5,000	35.1		
A. L.	24	71	160	1.92	5,104	37.1		
Average		69.62	158.20	1.88	4,157	27.3		
		Diffusing capacity of lungs in normal subjects (females)						
R. T.	32	60	111	1.45	3,000	18.5	24.0	
G. T.	30	66	134	1.69	3,100	15.3		
J. W.	38	66	200	2.01	2,600	20.2		
E. B.	26	67	116	1.60	3,500	18.4		
L. B.	30	65	125	1.62	3,050	18.7		
S. H.	20	62	103	1.45	2,830	19.4		
F. A.	23	65	127	1.62	3,725	25.8		
U. B.	28	64	160	1.78	3,003	26.5		
W. G.	35	64	135	1.65	2,585	18.3		
M. R.	22	67	137	1.73	2,794	21.3		
J. C.	32	64	134	1.65	2,330	14.4		
Average		64.55	134.73	1.66	2,956	19.7		

* Body surface area.

TABLE III

Diffusing capacity of the lungs in disease

Patient	Diagnosis	Age	Sex	Ht.	Wt.	BSA	VC	RV*	1″VC	MBC	S_{aO_2}	P_{aCO_2}	D_{LCO} RB	D_{LCO} SB	D_{LCO} SS
		yrs.		*in.*	*lbs.*	*M.²*	*ml.*	*ml.*	%	*L./min.*	%	*mm. Hg*	*ml./mm. Hg × min.*		
H. K.	Emphysema	52	M	64	159	1.78	1,134	3,355	41		79	49	22.6		5.5
R. O.	Emphysema	73	M	66	131	1.68	2,630	3,325	53	53	70	42	24.1		
D. W.	Emphysema	44	M		126		1,940	2,686	60				16.7	17.5	
P. B.	Emphysema	46	M	71	170	1.97	1,980	5,075	27	33.6	95	41	29.5	36.9	
E. R.	Emphysema	52	M	67	167	1.87	3,000	2,377	33		73	69	21.8	28.8	10.0
J. U.	Emphysema	68	M	68	160	1.85	2,640	2,977	42	52	96	34	26.1	19.0	
S. P.	Emphysema	64	M	68	140	1.77	2,740	3,980	42		96	30	19.7	14.0	
W. J.	Emphysema	57	M	72	103	1.61	1,685	5,420	38				23.3	16.9	
R. J.	Emphysema	53	M				1,762	2,244	50	37	94	36	20.7	24.6	
J. Y.	Emphysema	56	M	72	111	1.66	1,820	4,800	32		90	52	19.1		
J. D.	Emphysema	65	M	66	97	1.47	2,686	3,430	30	26	91	50	18.7		
N. F.	Emphysema	59	M	74	183	2.09	2,166	4,250	38				13.4		
C. W.	Emphysema	50	M	69	125	1.71	3,219	5,150	28	47			23.7		
E. N.	Emphysema	78	M	71	123	1.72	2,131	3,258	27		95	39	14.0		
G. V.	Emphysema	63	M	66	140	1.73	2,238	3,177	29	25			13.0	9.5	
C. S.	Emphysema	39	M	74	118	1 74	3,512	3,580	26	52			19.4		
J. C.	Emphysema	74	M	64	104	1.48	1,775	3,276	43	29			11.0		
W. M.	Emphysema, bullous	70	M	65	155	1.77	1,977	3,480	43		95	38	21.6	25.8	22.3
W. F.	Emphysema, bullous	57	M	67	110	1.58	1,985	2,021	41		85	44	9.0	8.2	
D. B.	Sarcoid	23	M	74	144	1.89	1,745	584	75	80.6	88	33	8.9	13.7	
M. B.	Sarcoid	24	M	67	126	1.66	1,250	682	100	123	98	37	9.4	12.8	13.0
W. S.	Sarcoid	32	M	69	196	2.05	2,550	1,156	78		98	36	18.5	28.0	12.6
J. S.	Sarcoid	36	M	68	140	1.76	3,478	1,151	88		98	36	24.4	21.7	27.6
W. Se	Fibrosis	64	M	66	177	1.89	1,506	1,024	73	81	92	34	6.0		
E. J.	Radiation fibrosis	57	F	63	119	1.56	2,450	1,482	52	67			17.2		
A. P.	Calcification	72	M	69	137	1.76	3,435	2,850	60	63.4			7.7		
R. J.	Anemia	45	M	70	147	1.84	3,655	1,210	81				11.9	13.8	
W. Sh	Anemia	38	F	62	156	1.74	2,474	790	76				10.9	14.7	
S. S.	Polycythemia	50	M	70	163	1.92	4,080	1,747	80		98	35	24.0	37.3	13.4
G. G.	Tuberculosis	51	M	68	152	1.82	3,790	2,015	71	134	98	30	21.6	24.0	
L. D.	Obesity	42	F	68	329	2.52	1,640	988	76				20.1		
H. B.	Barrel chest	66	M	74	190	2.00	5,905	2,250	76	133			26.3		
F. H.	Hypertension	65	M	72	194	2.10	3,380	1,976	77	71	96	33	20.0	22.6	
J. Sh	Mitral stenosis	28	F	65	143	1.72	1,284	1,427					12.6	13.1	

* Abbreviations are as follows: RV, residual volume; 1″ VC, one second vital capacity as per cent of total; MBC, maximum breathing capacity; S_{aO_2}, arterial oxygen saturation; P_{aCO_2}, arterial CO_2 tension. Other abbreviations are as in previous tables. VC and MBC are measured at BTPS; other volumes are measured at STPD.

normal subjects and in four patients with emphysema (Figure 5). Variance analysis of the data for normal subjects showed no significant difference among the rates studied. Three of the four patients showed a lower value for D_{LCO} at the higher rate.

The effect of decreasing bag volume below the vital capacity was studied in four normal subjects (Figure 6). There is a marked decrease in calculated D_{LCO} when the bag volume was about 25 per cent of the vital capacity, *i.e.*, when the total volume of the lung-bag system approximated the subject's functional residual capacity.

Our data on the rebreathing D_{LCO} in 47 subjects free from heart, lung or hematologic disease are shown in Table II. Data for the single breath D_{LCO} are available in 26 of these subjects and for the steady state D_{LCO} in five. In all cases, the rebreathing D_{LCO} was measured in the seated position, the gas breathed was approximately 0.3 per cent CO in air and the bag volume was equal to the one second vital capacity. Measurements of single breath and steady state D_{LCO} were made on the same day in the seated position. The lung volume for the single breath measurement was full inspiration and for the steady state determination, functional residual capacity. The rebreathing D_{LCO} was significantly related to the height ($r = 0.624$), weight ($r = 0.506$), body surface area ($r = 0.603$) and vital capacity ($r = 0.732$). The regression equations for these relations are: $D_{LCO} = 1.265$ height (in.) $- 60.98 \pm 5.28$; $D_{LCO} = 0.1361$ weight (lbs.) $+ 4.79 \pm 5.74$; $D_{LCO} = 22.77$ body surface area

($M.^2$) − 16.11 ± 5.39 and D_{LCO} = 5.92 vital capacity (L.) + 2.22 ± 4.61.

The coefficient of variation in 35 duplicate determinations of D_{LCO} in normal subjects was 6.1 per cent.

Since these subjects were young adults, no conclusions as to the relation of D_{LCO} to age can be drawn from our data.

Data on 34 patients with pulmonary, cardiac or hematologic disease are shown in Table III. Except for the emphysematous patients, measurements of D_{LCO} were carried out as in normal subjects. In the emphysematous patients, bag volume was approximately equal to total vital capacity, rather than one second vital capacity. Additional clinical data on these patients are found in Appendix I.

In 13 duplicate determinations of D_{LCO} in patients without airway obstruction, the coefficient of variation was 5.3 per cent. In 11 duplicate determinations in patients with emphysema, the coefficient of variation was 9.2 per cent.

Figure 7 shows a comparison of the rebreathing and single breath measurements of D_{LCO} in 26 normal subjects, 10 patients without airway obstruction and 10 patients with emphysema. Also shown are comparisons of rebreathing and single breath D_{LCO} at an alveolar pO_2 of about 600 mm. Hg in four normal subjects and five patients without airway obstruction. The regression line is for the data on normal subjects and patients without airway obstruction. As discussed below, different theoretical considerations apply to the measurement of D_{LCO} by rebreathing in emphysematous patients.

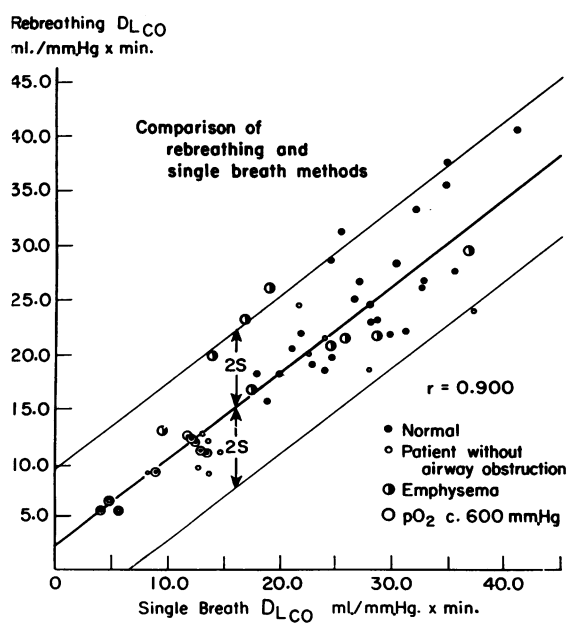

FIG. 7. COMPARISON OF MEASUREMENTS OF DIFFUSING CAPACITY BY REBREATHING AND SINGLE BREATH METHODS

The equation of the regression line is D_{LCO} (rebreathing) = 0.793 D_{LCO} (single breath) + 2.36 ± 3.68. "S" is the standard error of the estimate of this equation.

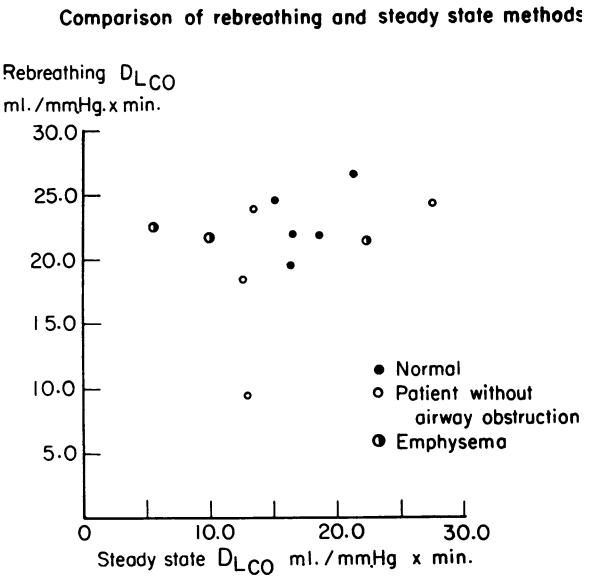

FIG. 8. COMPARISON OF MEASUREMENTS OF DIFFUSING CAPACITY BY REBREATHING AND STEADY STATE METHODS

Figure 8 shows a similar comparison of the steady state and rebreathing measurements of D_{LCO} for five normal subjects, four patients without airway obstruction and three patients with emphysema. No relation between the two measurements is evident from the rather few data available.

In Figure 9 the residual volume calculated from rebreathing data as explained above is compared with the residual volume determined in duplicate by the closed circuit helium method (14) in 28 normal subjects, 11 patients without airway obstruction and 13 patients with emphysema. The regression line shown is for the normal subjects and for the patients without airway obstruction.

DISCUSSION

Use of continuous sampling

Since we used stable rather than radioactive CO, direct comparison of our continuous sampling

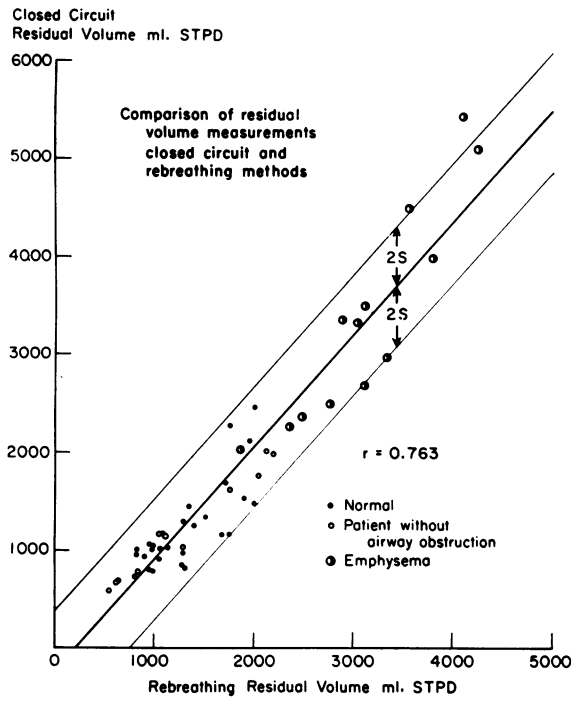

FIG. 9. COMPARISON OF RESIDUAL VOLUME MEASUREMENTS BY REBREATHING AND CLOSED CIRCUIT METHODS

The equation for the regression line is: residual volume (rebreathing) = 0.866 residual volume (closed circuit) + 228 ± 305. "S" is the standard error of the estimate of this equation.

technique with Kruhøffer's (12) technique of three discrete samples from the same rebreathing bag was not possible. However, our finding (Table I) that analysis of final CO concentration in each of three bags of the same volume gives the same rate of CO absorption as continuous sampling indicates the time lag introduced by passing gas through the analyzer circuit does not introduce any significant error into the data reported here.

Theory of carbon monoxide absorption during rebreathing

We have used Krogh's equation (8) to calculate D_{LCO} from the rate of fall of CO concentration during rebreathing. This is strictly valid only if the rate of rebreathing is infinitely fast. In this situation, the calculated D_{LCO} is independent of variations in the ratio of diffusing capacity to alveolar volume (D_L/V_A) or of diffusing capacity to alveolar ventilation ($D_L/\dot{V}_A$) among the various alveoli, since lungs, dead space and bag constitute a single well mixed system. Calculated D_{LCO} is also independent of increases or decreases in the volume of lungs, dead space or bag. The rate of rebreathing used in these studies (30 cycles per minute) is not "infinitely fast." However, it is sufficiently rapid to make the calculated D_{LCO} in normal subjects independent of increases in dead space (Figure 3). We, therefore, feel that the use of Krogh's equation (8) in such subjects is justified.

In subjects with emphysema, calculated D_{LCO} is not independent of increases in dead space (Figure 4). Rebreathing must be considered as occurring breath by breath at a finite rate. The rate of fall of CO is obtained by dividing the concentration of one breath by that of the preceding breath. The first two of a series of equations for the concentration of CO in the rebreathing bag at the end of the successive breaths are given in Appendix II. These equations are of increasing complexity and it has not been possible to obtain an equation that can be solved for D_L by dividing any such equation by that of the preceding breath.

Krogh's equation (8) can be applied to a rebreathing system that is ventilated breath by breath only if the following assumptions are made: *1*) that ratio of diffusing capacity to alveolar volume (D_L/V_A) is uniform for all alveoli. This same assumption is made in the single breath technique (17). *2*) The dead space of apparatus and subject is negligibly small. *3*) The time spent at end expiration is negligible in relation to the time spent in full inspiration.

The equation for the concentration of CO at the end of any breath then reduces to the product of a diffusion term and a mixing term. For the first two breaths these equations are:

diffusion term mixing term

$$F_{s_1} = F_I e^{-\frac{D_i P_b t}{V_{A_i}}} \times \sum_1^N \phi_i \frac{V_{T_i}}{V_{A_i}}$$

and

$$F_{s_2} = F_I \left(e^{-\frac{D_i P_b t}{V_{A_i}}}\right)^2 \times \sum_1^N \phi_i \frac{V_{T_i}}{V_{A_i}} \left(\frac{(V_A - V_T)_i}{V_{A_i}} + \sum_1^N \phi_i \frac{V_{T_i}}{V_{A_i}} \right),$$

TABLE IV

Calculated D_L in lung model*

Region I				Region II				Entire lung D_L			
V_{T_1}	V_{D_1}	V_{A_1}	D_{L_1}	V_{T_2}	V_{D_2}	V_{A_2}	D_{L_2}	RB	SB	SS	I + II
570	46	2,000	8	1,280	104	4,500	17	24.9	25.6	24.8	25
1,400	114	2,000	8	450	36	4,500	17	25.3	26.4	14.7	25
1,400	114	2,000	3	450	36	4,500	22	22.4	10.7	7.1	25
1,400	114	2,000	17	450	36	4,500	8	22.1	55.1	24.3	25

* Abbreviations are as follows: D_L, diffusing capacity of lung or region; V_T, tidal volume of region; V_A, volume of region at full inspiration; V_D, dead space of region. Other abbreviations are as used in previous tables. V_T, V_A and V_D are milliliters STPD; D_L is milliliters per millimeter Hg × min.

where F_I = concentration of CO in inspired gas, F_{s_1} = concentration of CO in bag at end of Breath 1, F_{s_2} = concentration of CO in bag at end of Breath 2, D_i = diffusing capacity of ith alveolus, V_{A_i} = volume of ith alveolus at full inspiration, V_{T_i} = tidal volume of ith alveolus and ϕ_i = fraction of total V_T contributed by ith alveolus. If complete mixing occurs on the first (or any subsequent) breath the mixing terms become equal. Consequently, the rate of fall of CO concentration obtained by dividing the equation for Breath 1 by that of Breath 2 is:

$$F_{s_2} = F_{s_1} e^{-\frac{D_i P_b t}{V_{A_i}}}$$

The second and third assumptions made above, however, are certainly incorrect. Further, we have continuously analyzed the helium concentration of the rebreathing circuit and found that slow mixing continues throughout a 45 second period in emphysematous patients (18).

Thus the use of Krogh's equation (8) to calculate D_{LCO} in a rebreathing system which is ventilated breath by breath at a finite rate may lead to errors in the value obtained. To evaluate the magnitude of these errors we have approached the problem empirically, using a mathematical model of the lung consisting of two regions (Figure 2). D_L/V_A and $D_L/\dot{V}_A$ are uniform within the region and may or may not be uniform between the regions (Table IV). With some labor, the concentration of an insoluble gas (helium) and a diffusible gas (CO) may be calculated at the end of each of the 23 breaths during a 45 second rebreathing period at 30 cycles per minute by expanding the equations in Appendix II. When the concentrations of CO are plotted on semilogarithmic paper, a final phase in which the disappearance of CO is apparently exponential is invariably obtained. A similar exponential phase was also obtained in a few calculations on a model lung consisting of four regions. When ventilation of the regions is uneven (Figure 2) slow mixing of helium continues during the apparently exponential disappearance of CO. The D_L calculated from this final phase of the CO curve approaches the true D_L of the model most closely when both D_L/V_A and $D_L/\dot{V}_A$ are uniform (Example 1, Table IV), but the variations from the true D_L are relatively small when D_L/D_A and $D_L/\dot{V}_A$ are not uniform as compared with the variations produced by non-uniform D_L/V_A in the single breath D_L and by non-uniform D_L/V_A in the steady state D_L.[9] From the data shown in Table IV and other similar calculations we conclude that the value of D_{LCO} calculated by a rebreathing method when the lungs are ventilated breath by breath as in emphysema, although approximate rather than exact, is not grossly in error.

Any practical consequence of this theoretical advantage, then, must rest on the demonstration that either D_L/V_A or $D_L/\dot{V}_A$ is not uniform in the lungs of the subject under study. In normal subjects ventilation is uneven to some degree (19), but Marshall (20) has concluded from fractional analysis of expired air that $D_L/\dot{V}_A$ is uniform. The evidence bearing on the uniformity of D_L/V_A in normal subjects is conflicting. Forster, Fowler, Bates and Van Lingen (21) from data on breath-holding for different periods of

[9] The theoretical values for the steady state and single breath techniques in these model lungs are calculated from the equations given by Forster, Fowler and Bates (17). The total alveolar volume during breath-holding is 6,500 ml. and the duration of breath-holding is 10 seconds. The effective tidal volume during the steady state technique is one-third that during rebreathing and the rate is 10 cycles per minute.

time thought that it was not, while Marshall (20), after repeating the same experiment, concluded that D_L/V_A is uniform. In emphysematous patients Marshall (20) found that CO concentration varied during a single expiration and concluded that $D_L/\dot{V}_A$ is non-uniform. Surprisingly, he also found that the ratio of an insoluble gas (helium) to CO during an expiration was uniform, indicating the same ratio of D_L/V_A throughout the lung. Ogilvie, Forster, Blakemore and Morton (9) also found that the ratio of He to CO was the same in an "early" as in a "late" expiratory sample in patients with emphysema.

If D_L/V_A is uniform in the lungs of normal subjects, there should be a high correlation between the rebreathing and single breath methods in these subjects. Such a correlation is found in our data (Figure 7). Further, the points for patients with emphysema scatter about the same regression line, supporting Marshall's (20) conclusion that D_L/V_A is uniform in these patients. From the available evidence, therefore, the theoretical advantage that the rebreathing D_{LCO} is independent of non-uniform D_L/V_A (infinite rate) or relatively insensitive non-uniform D_L/V_A (finite rate) does not make the rebreathing D_{LCO} more valid than the single breath D_{LCO}.

If the same type of reasoning is applied to the relation between the steady state D_{LCO} and the rebreathing D_{LCO}, one would predict a high correlation between the two methods for normal subjects and a poor correlation for emphysematous patients. These predictions are not supported by our data (Figure 8). However, no conclusions seem warranted because *1*) the data are relatively few, *2*) the lung volumes during the measurements of rebreathing and steady state D_{LCO} differ. Marshall (20) has pointed out that steady state D_{LCO} increases with lung volume and we have found a similar relation of rebreathing D_{LCO} to lung volume (Figure 6). *3*) The steady state measurements were performed at rest and the potential errors of the steady state method are greatest at rest (10).

Practical advantages of rebreathing

The following practical advantages are unique to the rebreathing method:

1) The method incorporates a continuous internal check on CO analysis. The required measurements of CO are made within seconds of each other, while the rebreathing is going on. Further, the concentration of CO should fall exponentially during a valid study. Thus, any error due to malfunction of the analyzer or leak in the system is readily detected.

2) A measurement of residual volume is obtained simultaneously with a measurement of D_{LCO}. There is a satisfactory correlation between the residual volume so obtained and a measurement using a seven minute, closed circuit, helium technique (14) (Figure 9). A corollary advantage of the rebreathing method is that calculation of the total volume of the lung-bag system by helium dilution guards against errors which may occur in the single breath method through failure of the subject to expire to his residual volume at the start of the procedure.

3) A complete measurement of D_{LCO} is very rapid. CO analysis is completed during rebreathing. The record of the CO analyzer may be measured at a minimum of three points while the rebreathing bag is analyzed for helium and carbon dioxide. All measurements and calculations can be completed in 10 minutes or less.

The following advantages of the rebreathing method are shared by either the steady state or the single breath methods:

1) No blood samples are required. *2*) Rebreathing is readily carried out by patients and untrained subjects. *3*) The method can be used in subjects with a low vital capacity. *4*) Required analysis can be done by physical methods and without highly skilled personnel.

Disadvantages of the rebreathing method

The rebreathing method has the following disadvantages:

1) Rebreathing involves considerable exercise of the respiratory muscles. We have measured the oxygen consumption[10] during rebreathing in six normal subjects by sampling continuously the oxygen concentration in the lung-bag system with a mass spectrometer.[11] The difference in

[10] Strictly, we have measured the rate of oxygen uptake by the lungs. This may not be equal to oxygen uptake by the tissues during this brief, unsteady state.

[11] Model 21–611 Consolidated Electrodynamics Corp., Pasadena, Calif.

oxygen concentration between 10 and 30 seconds was multiplied by the total volume of the system and divided by the time interval to give the data shown in Table V. For comparison, the basal oxygen consumptions of these subjects, computed from body surface area using the standards of Aub and DuBois (22) are also shown. The oxygen consumption during rebreathing is about twice the basal level. The increase in D_{LCO} corresponding to this increment in oxygen consumption apparently varies somewhat with the method. From the data collected by Forster (1), doubling the basal oxygen consumption might increase the steady state D_{LCO} by as much as 20 per cent, but would change the single breath D_{LCO} by about 5 per cent.

The oxygen cost of hyperventilation, however, is greater in emphysematous patients than in normal subjects (23). The practical importance of the fact that rebreathing is moderately severe exercise in a patient with emphysema depends on the extent to which this patient can increase his D_{LCO} on exertion. Bates, Knott and Christie (24) have reported that the steady state D_{LCO} does not increase in emphysematous patients during treadmill exercise.

2) Alveolar pO_2 falls during rebreathing. Roughton, Forster and Cander (4) have shown that the reaction rate of CO with hemoglobin is inversely proportional to pO_2; hence, the lower the alveolar pO_2 the greater D_{LCO}. With the changes in alveolar pO_2 during rebreathing, however, this effect cannot be large. In a subject with a capillary blood volume of 80 ml. and a membrane diffusing capacity of 50 ml. per mm. Hg × min., D_{LCO} would be 27.2 ml. per mm. Hg × min. at an alveolar pO_2 of 105 mm. Hg and 29.6 ml. per mm. Hg × min. at an alveolar pO_2 of 65 mm. Hg.

3) Alveolar pCO_2 rises during rebreathing. Rankin, McNeill and Forster (25) found that hypercapnia increased the single breath D_{LCO}. This effect varied with the duration of hypercapnia. When 10 per cent CO_2 was added to the mixture inspired, D_{LCO} increased 5 per cent after 10 seconds' breath-holding and 12 per cent after 50 seconds' breath-holding. When 7 per cent CO_2 was breathed for 10 minutes beforehand, D_{LCO} increased about 24 per cent. We have no data on the effect of added CO_2 on the rebreathing D_{LCO}, but the concentration of CO_2 in the rebreathing bag after 45 seconds (6 to 7 per cent) is about that to be expected after holding a breath containing 10 per cent CO_2 for 10 seconds.

TABLE V

Oxygen consumption during rebreathing

Subject	$\dot{V}_{O_2}$* Basal	$\dot{V}_{O_2}$ Rebreathing
W. G.	208	280
M. R.	221	443
J. C.	208	317
S. H.	185	369
J. McG.	260	599
J. N.	273	425
Average	225.8	405.5

* $\dot{V}_{O_2}$, oxygen consumption in milliliters STPD per minute. Basal V_{O_2} calculated on basis of body size.

Results in normal subjects

Our data, which show an average D_{LCO} of 27.3 ml. per mm. Hg × min. for 36 normal men and 19.7 ml. per mm. Hg × min. for 11 normal women, agree reasonably well with those of Kruhøffer (12) who found that the average D_{LCO} of 10 normal men, rebreathing $C^{14}O$, was 27 ml. per mm. Hg × min. and the average D_{LCO} of five normal women was 22 ml. per mm. Hg × min. Like Kruhøffer we found that D_{LCO} increases with height.

The rebreathing D_{LCO} is well correlated with the single breath D_{LCO} (Figure 7) and, like the single breath D_{LCO}, increases with body surface area, height and weight (9). Application of our regression equations and those of Ogilvie and associates (9) to subjects of the same physical parameters gives higher values for the single breath D_{LCO}, *e.g.*, for a subject of 1.73 $M.^2$ body surface area the predicted single breath D_{LCO} would be 25.8 ml. per mm. Hg × min. and the predicted rebreathing D_{LCO} would be 23.3 ml. per mm. Hg × min. Similarly, in Figure 7 values for the single breath D_{LCO} are, in general, higher than for the rebreathing D_{LCO} in the same individual. These differences, however, do not reach the level of statistical significance.

The D_{LCO} for normal females in our series is less than that for normal males. This is usually attributed to the smaller body size of women (1). Yet when our regression equations are applied to

normal females, the predicted values are higher than those actually found. Thus, the average body surface area for our female subjects was 1.66 M.^{2}, the average D_{LCO}, 19.7 ml. per mm. Hg × min. and the predicted D_{LCO}, 21.6 ml. per mm. Hg × min. Similarly, in the data of Ogilvie and co-workers (9) the average body surface area for women was 1.71 M.^{2}, the average D_{LCO}, 24.0 ml. per mm. Hg × min. and the predicted D_{LCO}, 25.4 ml. per mm. Hg × min. These differences are suggestive rather than statistically significant.

The rebreathing D_{LCO} is irregularly higher than the steady state D_{LCO} in the five normal subjects in our data (Figure 8).

Results in patients

Of our 19 patients with emphysema (Table III), in only five (N. F., E. N., J. C., G. V., W. F.) was the rebreathing D_{LCO} decreased a third or more below the predicted value based on body surface area (or weight). Thus, in 14 of these patients there was no marked impairment of diffusion. Kjerulf-Jensen and Kruhøffer (26), using a $C^{14}O$ rebreathing method, report similar findings. Only four of their 12 emphysematous patients had a D_{LCO} a third or more below the predicted value. In 10 of our emphysematous patients, D_{LCO} was also measured by the single breath technique. Only three of these (S. P., G. V. and W. F.) had a value for D_{LCO} less than two-thirds of the predicted value. Marshall (20), also using the single breath technique, found that the D_{LCO} was decreased by a third or more in only three of 11 emphysematous patients.

These findings must be contrasted with those of Ogilvie and associates (9), using the single breath technique; Bates, Knott and Christie (24), using the alveolar sample steady state technique and Donald, Renzetti, Riley and Cournand (27), using the steady state oxygen method. All of these investigators concluded that diffusion was usually impaired in emphysema.

When the same method gives conflicting results [*cf.* the results of Marshall (20) and of Ogilvie and associates (9)], the explanation presumably lies in the selection of patients. As far as the differences among the methods are concerned, we have set forth above our reasons for believing that the rebreathing D_{LCO} gives reasonably valid results in emphysematous patients despite the fact that ventilation is grossly uneven in this disease. Marshall (20) has presented evidence that the alveolar sample steady state D_{LCO} gives erroneously low values in emphysema and Kjerulf-Jensen and Kruhøffer (26) have criticized the use of the Bohr integration for determining mean capillary pO_2 in the steady state oxygen method (2).

D_{LCO} in our four patients with sarcoid varied from markedly impaired (D. B. and M. B.) to normal (J. S.). Impairment of diffusion in sarcoid was first demonstrated by Austrian and co-workers (28). Williams (29) has emphasized the frequent occurrence of normal pulmonary function in sarcoid despite widespread pulmonary involvement by X-ray.

Other authors using different techniques have reported results similar to ours in interstitial fibrosis (28), radiation fibrosis (30), anemia (31), polycythemia (31) and the "Pickwickian" syndrome (32).

SUMMARY

1. This paper describes a rebreathing method for the measurement of pulmonary diffusing capacity for carbon monoxide (D_{LCO}) using stable CO and continuous analysis.

2. The fall of CO concentration in the rebreathing bag was apparently exponential for at least 15 seconds in 571 of 578 studies. In 80.4 per cent of these studies, this exponential decrease began in the first 10 seconds of rebreathing.

3. In four normal subjects calculated D_{LCO} was independent of increases in apparatus dead space. This would be true if the rate of rebreathing were infinitely fast.

4. In patients with emphysema calculated D_{LCO} decreases as apparatus dead space is increased. This would occur if the rebreathing system were ventilated breath by breath at a finite rate.

5. In four normal subjects the rebreathing D_{LCO} decreased sharply when the total volume of the lung-bag system was decreased from near total lung capacity to approximately functional residual capacity.

6. In 47 normal subjects the rebreathing D_{LCO} was significantly correlated with height, weight, body surface area and vital capacity.

7. There is a high correlation between rebreathing and single breath measurements of

D_{LCO} in normal subjects and patients without airway obstruction. There is a less striking correlation between these two methods in patients with emphysema.

8. The residual volume determined by the rebreathing method is significantly correlated with that measured by the closed circuit helium method in both normal subjects and patients.

9. An empiric argument is presented for the approximate validity of the rebreathing method in patients with uneven ventilation and slow mixing in whom the rate of rebreathing is finite.

10. The rebreathing method is rapid, simple and gives a simultaneous measurement of D_{LCO} and residual volume.

11. The rebreathing D_{LCO} was definitely decreased (two-thirds or less the predicted value) in only five of 19 patients with emphysema.

APPENDIX I

The diagnosis of emphysema in patients H. K. through J. C. (Table III) was based on: *1)* a low one second vital capacity and/or maximum breathing capacity, *2)* an increased residual volume and *3)* slight or no improvement in these tests after bronchodilators. All patients were dyspneic and their physical findings and X-rays were compatible with chronic obstructive emphysema. Of these patients, complicating diseases were present in the following: S. P. (aneurysm of the abdominal aorta, carotid artery thrombosis), J. D. (peptic ulcer), N. F. (rheumatoid arthritis), E. N. (myocardial infarct), C. S. (peptic ulcer) and J. C. ("coin lesion" right lung). The chest X-ray of W. M. showed a large bulla in the left lower lung and the chest X-ray of W. F. showed a large bulla in the left upper lung field. Patients D. B. through J. S. had widespread, soft, patchy densities on X-ray of the chest. In each, either a scalene node (D. B., J. S.) or a lung biopsy (W. S., M. B.) was compatible with sarcoid histologically. D. B. and M. B. were dyspneic while W. S. and J. S. were working full time. W. Se. had relentlessly progressive dyspnea for two years. Chest X-ray revealed diffuse fibrotic infiltration. E. J. received Co^{60} therapy after radical removal of carcinoma of the left breast. At the time of these studies she had a severe cough and a soft tissue density was present in the apical and infraclavicular portions of the left lung field. A. P. had widespread miliary calcific densities on chest X-ray. He was severely dyspneic. Histoplasmin and tuberculin skin tests were positive, but cultures for tuberculosis were negative. R. J. was diabetic and had a hemolytic anemia of unknown cause. His hemoglobin at the time of study was 8.0 Gm. per cent. W. Sh. was anemic because of bleeding from a small bowel tumor. Her hemoglobin was 6.9 Gm. per cent. The hemoglobin in S. S. was 18.6 Gm. per cent. G. G. had exudative lesions in both upper lung fields. Sputum smear was positive for tuberculosis. L. D. was somnolent, dyspneic, weak and grossly obese. H. B. had a markedly increased anteroposterior diameter of the chest with dorsal kyphosis. The lung fields showed increased radiolucency. F. H. was hypertensive (190/90 mm. Hg) and had been in congestive heart failure twice before. He had a lesion of the left mid-lung field that proved to be carcinoma at thoracotomy. J. Sh. had the classic murmur of mitral stenosis. She had several episodes of acute pulmonary edema.

APPENDIX II

During the first breath of a rebreathing experiment the tidal volume (V_T) of the subject flushes the dead space (V_D) with the concentration of CO in the bag (F_I) and brings $F_I(V_T - V_D)_i$ ml. of CO to the ith alveolus. This volume of CO is contained in the end inspiratory volume of the alveolus (V_{A_i}). Hence, the concentration of CO in the ith alveolus at the end of inspiration, which is assumed to be instantaneous, is $F_I \frac{(V_T - V_D)_i}{V_{A_i}}$. If full inspiration lasts t minutes, this concentration falls because the ith alveolus has a certain diffusing capacity for CO (D_i). The rate of fall of CO concentration is the power of e (base of the natural logarithm) $e^{-\frac{D_i\, Pb\, t}{V_{A_i}}}$, where Pb is the pressure of dry gas in the alveoli. On expiration, which is also assumed to be instantaneous, the ith alveolus contributes a fraction (ϕ_i) of the total ($V_T - V_D$). The concentration of CO in the rebreathing bag at end expiration (F_{s_1}) is then a mixture of the contribution of the apparatus dead space (V_{D_a}), the subject's dead space and what was expired from the alveoli. Thus,

$$F_{s_1} = F_I\left(\frac{V_D + V_{D_a}}{V_T + V_{D_a}} + \frac{V_T - V_D}{V_T + V_{D_a}} \sum_1^N \phi_i \frac{(V_T - V_D)_i}{V_{A_i}} e^{-\frac{Pb\, t\, D_i}{V_{A_i}}}\right).$$

During full expiration, which also lasts t minutes, the concentration of CO remaining in the ith alveolus falls by the power $e^{-\frac{D_i\, Pb\, t}{(V_A - V_T)_i}}$. On the second breath the concentration of CO in the dead space is F_{s_1}. The ith alveolus receives first a volume (V_{D_i}) of the mixed alveolar gas in the dead space at the end of expiration (the "alveolar" part of the equation for F_{s_1}) and then a volume $(V_T - V_D)_i$ of the gas in the bag. Both of these moieties are mixed with the gas remaining in the alveoli during expiration to give the concentration of CO at the end of the second inspiration. During a full inspiration of t minutes, CO again diffuses out of the alveolus as explained above. The concentration of CO in the bag at the end of the second expiration (F_{s_2}) is:

$$F_{s_2} = F_{s_1}\frac{V_D + V_{D_a}}{V_T + V_{D_a}} + \frac{V_T - V_D}{V_T + V_{D_a}} \sum_1^N \phi_i \left[F_I\left(\frac{V_{D_i}}{V_{A_i}} \sum_1^N \phi_i \frac{(V_T - V_D)_i}{V_{A_i}} e^{-\frac{D_i\, Pb\, t}{V_{A_i}}}\right)\right.$$
$$\left. + F_I\left(\frac{(V_A - V_T)_i}{V_{A_i}} \frac{(V_T - V_D)_i}{V_{A_i}} e^{-\frac{D_i\, Pb\, t}{V_{A_i}}} e^{-\frac{D_i\, Pb\, t}{(V_A - V_T)_i}}\right) + F_{s_1}\frac{(V_T - V_D)_i}{V_{A_i}}\right] e^{-\frac{D_i\, Pb\, t}{V_{A_i}}}.$$

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REFERENCES

1. Forster, R. E. Exchange of gases between alveolar air and pulmonary capillary blood. Pulmonary diffusing capacity. Physiol. Rev. 1957, **37**, 391.
2. Riley, R. L., Cournand, A., and Donald, K. W. Analysis of factors affecting partial pressures of oxygen and carbon dioxide in gas and blood of lungs: Methods. J. appl. Physiol. 1952, **4**, 102.
3. Bates, D. V. The measurement of the pulmonary diffusing capacity in the presence of lung disease. J. clin. Invest. 1958, **37**, 591.
4. Roughton, F. J. W., Forster, R. E., and Cander, L. Rate at which carbon monoxide replaces oxygen from combination with hemoglobin in solution and in the red cell. J. appl. Physiol. 1957, **11**, 269.
5. Roughton, F. J. W., and Forster, R. E. Relative importance of diffusion and chemical reaction rates in determining the rate of exchange of gases in the human lung. J. appl. Physiol. 1957, **11**, 290.
6. Lewis, B. M., Lin, T., Noe, F. E., and Komisaruk, R. The measurement of pulmonary capillary blood volume and pulmonary membrane diffusing capacity in normal subjects; the effects of exercise and position. J. clin. Invest. 1958, **37**, 1061.
7. McNeill, R. S., Rankin, J., and Forster, R. E. The diffusing capacity of the pulmonary membrane and the pulmonary capillary blood volume in cardiopulmonary disease. Clin. Sci. 1958, **17**, 465.
8. Krogh, M. Diffusion of gases through lungs of man. J. Physiol. (Lond.) 1914, **49**, 271.
9. Ogilvie, C. M., Forster, R. E., Blakemore, W. S., and Morton, J. W. A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. J. clin. Invest. 1957, **36**, 1.
10. Filley, G. F., MacIntosh, D. J., and Wright, G. W. Carbon monoxide uptake and pulmonary diffusing capacity in normal subjects at rest and during exercise. J. clin. Invest. 1954, **33**, 530.
11. Bates, D. V., Boucout, N. G., and Dormer, A. E. The pulmonary diffusing capacity in normal subjects. J. Physiol. (Lond.) 1955, **129**, 237.
12. Kruhøffer, P. Studies on the lung diffusion coefficient for carbon monoxide in normal human subjects by means of $C^{14}O$. Acta physiol. scand. 1954, **32**, 106.
13. Sjostrand, T. A. A method for the determination of carboxy-hemoglobin concentrations by analysis of the alveolar air. Acta physiol. scand. 1948, **16**, 201.
14. McMichael, J. A. A rapid method of determining lung capacity. Clin. Sci. 1939, **4**, 167.
15. Van Slyke, D. D., and Sendroy, J., Jr. Line charts for graphic calculations by Henderson-Hasselbalch equation, and for calculating plasma CO_2 from whole blood content. J. biol. Chem. 1928, **79**, 781.
16. Peters, J. P., and Van Slyke, D. D. Quantitative Clinical Chemistry (Methods). Baltimore, Williams and Wilkins, 1943, vol. 2, p. 321.
17. Forster, R. E., Fowler, W. S., and Bates, D. V. Considerations on the uptake of carbon monoxide by the lungs. J. clin. Invest. 1954, **33**, 1128.
18. Lewis, B. M., Hayford-Welsing, E. J., and McGinty, J. D. Helium mixing and carbon monoxide absorption during rebreathing. Fed. Proc. 1959, **18**, 91.
19. Fowler, W. S. Intrapulmonary distribution of inspired gas. Physiol. Rev. 1952, **32**, 1.
20. Marshall, R. A. A comparison of methods of measuring the diffusing capacity of the lungs for carbon monoxide. Investigation by fractional analysis of the alveolar air. J. clin. Invest. 1958, **37**, 394.
21. Forster, R. E., Fowler, W. S., Bates, D. V., and Van Lingen, B. The absorption of carbon monoxide by the lungs during breathholding. J. clin. Invest. 1954, **33**, 1135.
22. Aub, J. C., and DuBois, E. F. The basal metabolism of old men. Arch. intern. Med. 1917, **19**, 823.
23. Cournand, A., Richards, D. W., Bader, R. A., Bader, M. E., and Fishman, A. P. The oxygen cost of breathing. Trans. Ass. Amer. Phycns 1954, **67**, 162.
24. Bates, D. V., Knott, J. M. S., and Christie, R. V. Respiratory function in emphysema in relation to prognosis. Quart. J. Med. 1956, N.S. **25**, 137.
25. Rankin, J., McNeill, R. S., and Forster, R. E. Influence of hypercapnia on pulmonary diffusing capacity for CO in man. Physiologist 1957, **1**, 68.
26. Kjerulf-Jensen, K., and Kruhøffer, P. The lung diffusion coefficient for carbon monoxide in patients with lung disorders, as determined by $C^{14}O$. Acta med. scand. 1954, **150**, 395.
27. Donald, K. W., Renzetti, A., Riley, R. L., and Cournand, A. Analysis of factors affecting concentrations of oxygen and carbon dioxide in gas and blood of lungs: Results. J. appl. Physiol. 1952, **4**, 497.
28. Austrian, R., McClement, J. H., Renzetti, A. D., Jr., Donald, K. W., Riley, R. L., and Cournand, A. Clinical and physiological features of some types of pulmonary diseases with impairment of alveolar-capillary diffusion. Amer. J. Med. 1951, **11**, 667.
29. Williams, M. H., Jr. Pulmonary function in Boeck's sarcoid. J. clin. Invest. 1953, **32**, 909.
30. Stone, D. J., Schwartz, M. J., and Green, R. A. Fatal pulmonary insufficiency due to radiation effect on the lung. Amer. J. Med. 1956, **21**, 211.
31. Rankin, J., McNeill, R. S., and Forster, R. E. Diffusion characteristics of pulmonary membrane and capillary bed in various diseases of lungs and cardiovascular system. J. clin. Invest. 1957, **36**, 922.
32. Burwell, C. S., Robin, E. D., Whaley, R. D., and Bickelmann, A. G. Extreme obesity associated with alveolar hypoventilation. A Pickwickian syndrome. Amer. J. Med. 1956, **21**, 811.