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Research Article

A MODIFICATION OF THE KROGH CARBON MONOXIDE BREATH HOLDING TECHNIQUE FOR ESTIMATING THE DIFFUSING CAPACITY OF THE LUNG; A COMPARISON WITH THREE OTHER METHODS [1]

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There are at present four methods for assessing the pulmonary diffusing capacity (D), one using O_2 (D_{O_2}) and three using CO (D_{CO}) namely, the O_2 method of Lilienthal, Riley, Proemmel, and Franke (1), the steady state CO method of Filley, MacIntosh, and Wright (2) and the CO breath holding technique of Krogh and Krogh (3, 4) as modified by some of the authors (5), all of which provide estimates of the pulmonary diffusing capacity, and finally the fractional CO uptake of Bates (6) which gives an index of pulmonary diffusing capacity. This is a report of a cooperative research effort to compare these methods as nearly simultaneously as possible on six patients and one normal subject. Measurements were made over the extremes of pulmonary diffusing capacity, from a patient with a severely decreased pulmonary diffusing capacity at rest (Subject 5) to a normal subject during exercise (Subject 1).

METHODS

A. Steady state D_{O_2} method

This method requires the simultaneous sampling of expired gas and arterial blood with the subject breathing first air and then a low concentration of O_2. Analysis of the alveolar-arterial P_{O_2} gradient under these two conditions makes possible the estimation of the mean alveolar-capillary P_{O_2} difference, which is divided into the O_2 uptake to give D_{O_2}. This technique has been adequately

described in detail before (1, 7, 8), the only significant alteration in previous procedure was in the gas mixtures breathed. For an initial period of equilibration of 5 to 12 minutes the subject inspired a predetermined O_2 and N_2 mixture from a compressed gas tank. At the end of this period, the patient was suddenly switched to inspire a gas mixture containing the same percentages of O_2 and N_2 and in addition approximately 0.1 per cent CO. After breathing this mixture for a further 1 to 2 minutes, the collection period was started as usual, (1). This procedure permitted simultaneous steady state estimates of D_{O_2} and D_{CO}, with a minimal total CO uptake. Although all but two of the seven individuals were studied during exercise, we do not know whether the values of D_{O_2} and steady state D_{CO} are *maximal diffusing capacities* (7) or not.

B. Steady state D_{CO} method

This procedure has been described in detail by Filley, MacIntosh, and Wright (2) and will not be fully discussed here. In general it consists of establishing steady state conditions while breathing a mixture containing 0.1 to 0.15 per cent CO, and measuring the inspired and expired CO and CO_2 concentrations, plus the arterial P_{CO_2}. From the ventilation and the CO concentrations, the CO uptake can be calculated. As in the method of Lilienthal, Riley, Proemmel, and Franke (1), arterial P_{CO_2} is considered equal to mean alveolar P_{CO_2} and, along with the values above, is used to calculate alveolar P_{CO}.[5] This value for effective alveolar P_{CO} is divided into the CO uptake to give D_{CO}. Although Filley, MacIntosh, and Wright used a 6-minute preliminary period breathing the CO mixture (2), Bates (6) and ourselves (9) found that 1 to 2 minutes sufficed to reach a sufficiently steady state under these circumstances. Analyses of CO concentration in the various gases were made with an infra red gas analyzer,[6] instead of by the chemical method used by Filley, MacIntosh, and Wright (2).

[5] A simple version of the equation used is:

$$\text{alveolar } P_{CO} = \text{inspired } P_{CO} - \frac{\text{arterial } P_{CO_2}}{\text{expired } P_{CO_2}}(\text{inspired } P_{CO} - \text{expired } P_{CO}).$$

[6] Liston-Becker Co., Stamford, Conn.

C. *Fractional CO uptake*

This is

$$\frac{\text{CO absorption in ml./min.}}{\text{CO inspired in ml./minute (corrected for instrumental dead space)}}.$$

The data were available from the determinations of D_{CO} by the steady state technique, and were not collected in a separate procedure as described by Bates (6).

D. *Breath holding technique for D_{CO}*

We regarded this study as an opportunity to compare the modified Krogh breath holding technique for measuring pulmonary diffusing capacity with the other methods and to obtain simultaneous rather than consecutive comparisons of D_{CO} steady state with D_{O_2}. We have previously published a detailed analysis of a modification of the Krogh breath holding technique, which utilizes 10 per cent He along with a small concentration of CO in the inspired mixture to permit correction for the effects of uneven distribution of inspired gas throughout the lung upon the calculation of D_{CO} (5). In addition to the effects of uneven inspired gas distribution upon the calculated D_{CO}, there is the effect of duration of breath holding: calculated D_{CO} decreases with the duration of breath holding (5). Nevertheless, it seemed worthwhile to determine whether careful standardization of the test might provide clinically useful values for D_{CO}. The desirability of developing a quick and repeatable clinical test with minimal discomfort to the patient justified the inclusion of the single breath procedure. We have arbitrarily chosen a 10-second period for breath holding because all the patients studied were able to hold their breath this long, because the resulting change in alveolar CO concentration was large enough to measure accurately, and because the values for D_{CO} at 10 seconds are reasonably comparable to those in the literature. Although a series of 5 to 9 estimations of D_{CO} was made on each individual at different periods of breath holding up to his limit, or to 60 seconds, only those values obtained with breath holding periods from 9 to 15 seconds
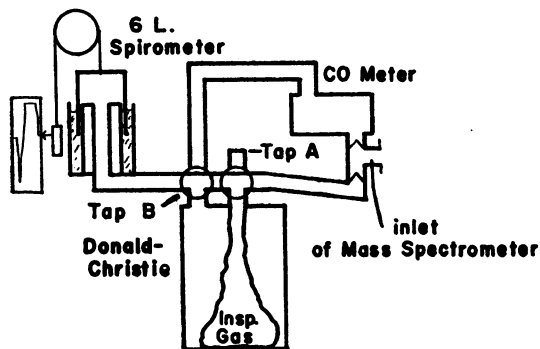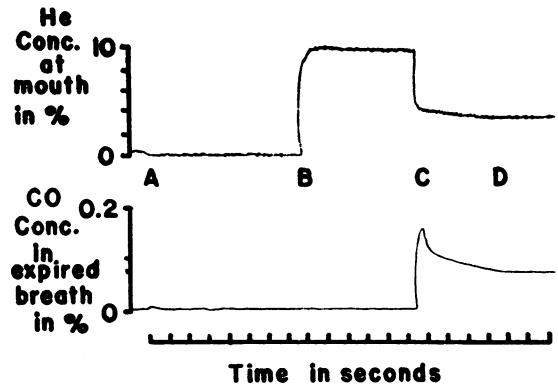

FIG. 2. RECORD FROM PATIENT 2 SHOWING THE He CONCENTRATION IN THE GASES AT THE MOUTH AND THE CO CONCENTRATION IN EXPIRED GAS AS USED IN THE DETERMINATION OF D_{CO} BY THE BREATH HOLDING TECHNIQUE

are included. A variation of the duration of breath holding between these particular limits caused changes in the calculated D_{CO} of less than 10 per cent judging by the individual graph of expired alveolar CO concentration versus time of breath holding. The range of 9 to 15 seconds represents extremes of actual breath holding times in inexperienced patients attempting to hold their breaths for the standard 10 seconds. The slow rate of expiration in some patients made the actual breath holding time greater than 10 seconds more often than it was less than 10 seconds.

The apparatus (Figure 1) and method of measuring the breath holding D_{CO} differed from that described previously (5) mainly in that the expired breath went directly through a low flow resistance sample chamber in the infra red CO analyzer and was not collected in a bag for later analysis. Concentration of He at the mouth was analyzed with a continuously sampling mass spectrometer.[7] Both the expired CO and He concentrations were recorded through DC amplifiers on a Brush magnetic pen ink writer system. Although the He concentration was recorded with a 90 per cent response time of 0.06 second, the slower response time of the infra red analyzer (90 per cent in 0.4 second) combined with the lag in time for the washout of the actual analysis chamber (25 ml.) and associated tubing (100 ml.) made it difficult to compare the two records while expiration was in progress. Therefore, the He and CO concentrations were compared at the end of expiration, after the infra red CO analyzer had had time to respond completely to the expired gas that remained in the chamber.

In performing the breath holding D_{CO} determination the patient sat quietly wearing a nose clip. At the end of a normal expiration, he took the mouthpiece in his mouth, expired to his residual volume, and then inspired, as rapidly as possible, a maximal volume from the bag of the Donald-Christie apparatus which contained a

FIG. 1. DIAGRAM OF THE APPARATUS USED TO MEASURE D_{CO} BY THE BREATH HOLDING TECHNIQUE

Tap A and Tap B were used in making up gas mixtures.

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[7] Consolidated Engineering Corp., Pasadena, California.

mixture of 0.3 per cent CO, 10 per cent He, 20 per cent O_2 and 70 per cent N_2. He held his breath for approximately 10 seconds and then expired, again as rapidly as possible, a volume of 1 liter or more.

A record of He concentration at the mouth and expired CO concentration is shown in Figure 2. At the beginning of the record there is a slight contamination of He in the mouth piece which decreases to zero with the start of patient's expiration (A). At B, the patient started to inspire and the He concentration at the mouth rose rapidly to 10 per cent. Six seconds after the start of inspiration the patient started to expire (C) and the He concentration at first fell abruptly to 4.4 per cent and then continued to fall throughout the rest of the expiration in a downward sloping "alveolar plateau." At D, 9.8 seconds after the start of inspiration, the patient completed his expiration; the He concentration remained constant thereafter, and the CO record leveled off less than one second later. The output of the CO meter between A and B, when normal expired gas is in the analysis chamber, is considered zero CO concentration as the patient had previously inspired no CO. The water vapor and CO_2 in the expired gas at this point provide an automatic correction for the interference of these gases with the CO analysis of the expired alveolar sample at D. During inspiration (B) no CO appears in the analyzer because of the valves in the mouthpiece. At C, expired gas containing CO is blown through the infra red analyzer, the initial peak being inspired gas from the respiratory and instrumental dead spaces. At D, the final expired gas is trapped in the analyzer. This CO concentration and the corresponding final expired alveolar He concentration are used to calculate the pulmonary diffusing capacity.

In the example illustrated in Figure 2, the concentration of inspired CO was 0.302 per cent (dry) and of inspired He 10 per cent (dry). At point D, the final CO concentration was 0.069 per cent (BTS) and that of He was 3.52 per cent (BTS). Since He is practically insoluble in the blood relative to CO, the decrease in the He concentration from 10 per cent in the inspired gas to 3.52 per cent in the expired alveolar gas is entirely a result of its dilution by the residual gas in the lung at the start of inspiration. The inspired CO concentration must have been decreased by dilution alone to precisely the same degree as the inspired He. Furthermore, the gas within those regions of the lung where significant CO absorption takes place, must be mixed within at least a tenth of a second after inspiration (10). Therefore, the initial CO concentration, before any absorption into the blood had taken place, of that particular aliquot of the expired gas which was in the infra red analyzer chamber at the end of expiration, must have been (3.52/10.0) $\times$ 0.302 per cent CO, or 0.106 per cent BTS. This calculation of the initial CO concentration in a particular expired gas sample will be true whether the inspired gas was evenly or unevenly distributed and whether the expired sample came from one or a great many different alveoli. According to the equation of

Krogh and Krogh (3),

$$D_{CO} = \frac{\text{alveolar volume (STPD)} \times 60}{\text{time in seconds} \times (B\text{-}47)} \times Ln \left[\frac{\text{initial expired alveolar CO concentration}}{\text{final expired alveolar CO concentration}} \right]. \quad (2)$$

The alveolar volume equaled the sum of the volume inspired (1280 ml. STPD) plus residual volume (3780 ml. STPD, by the method of Darling, Cournand, and Richards [11]) or 5060 ml.: B-47 was 713 mm. Hg: the time of breath holding, actually the time from the start of inspiration until the end of the expiration, was 9.8 seconds. Thus the breath holding D_{CO} equaled

$$\frac{5,060 \times 60}{9.8 \times 713} Ln \, [0.106/0.069] = 18.5 \text{ ml./min./mm. Hg.}$$

These measurements of D_{CO} by the breath holding method were made the same day as the measurements of D_{O_2} and the steady state D_{CO} in the majority of patients.

It can be seen in Figure 2 that the expired alveolar He concentration decreases during the course of a single expiration, which means that the proportion of inspired gas in the expired alveolar gas decreases during the expiration. In the technique originally described by Krogh (4), two alveolar samples were collected, the initial one from the first part of an expiration, and the other from a later part of the same expiration, after an intervening breath holding period of 10 seconds' duration. Since the difference between the CO concentrations of the two samples was considered wholly a result of diffusion of CO into the blood, an incorrect value of D_{CO} was obtained.[8] We avoid this particular difficulty by using the dilution of the inspired He to calculate the initial CO concentration of the alveolar sample.

E. *Other pulmonary function studies*

Determinations of lung volumes, including residual volume by the method of Darling, Cournand, and Richards (11), maximum breathing capacity, per cent of the vital capacity expired in one second, uneven ventilation estimated by the method of Cournand, Baldwin, Darling, and Richards (12) and by the single breath test of Comroe and Fowler (13), were also carried out on each patient.

SUBJECTS

The subjects were chosen so as to give as large a range of values for diffusing capacity of the lungs as possible. Pt. 1 was a healthy young male with no evidence of pulmonary disease. Pt. 2 had a history, physical findings and X-ray examination compatible with a

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[8] Since the apparent rate of disappearance of CO is greater than the actual, under these circumstances, the Krogh technique will lead to an overestimate of D_{CO}. If we had used the two-stage expiration technique in the example given above, we would have obtained a D_{CO} of approximately 24 ml. per min. per mm. Hg, instead of 18.5 ml. per min. per mm. Hg.

TABLE I

Results of pulmonary function studies *

Subject	Diagnosis	Sex	Age yrs.	Height inches	Weight pounds	Surface area M²	VC ml.	IC ml.	ER ml.	RV ml.	TLC ml.	RV/TLC %†	MBC L./min./M²	%VC in 1 sec. %‡	Alv. N₂ after 7 min. %§	N₂ single breath test %||
1	Normal	M	27	66	160	1.81	4,260 (4,250)	2,900 (3,380)	1,380 (845)	1,592 (1,070)	5,852 (5,320)	27	107 (72)	88	0.4	0.6
2	Emphysema	M	54	64⅔	185	1.89	1,440 (3,940)	910 (2,995)	577 (985)	4,590 (1,380)	6,130 (5,320)	75	13 (58)	30	<2.5	10.5
3	Silicosis	M	42	74	172	2.02	4,640 (4,750)	2,980 (3,800)	1,140 (950)	5,127 (1,420)	9,767 (6,190)	52	64 (86)	73	0.9	2.6
4	Chronic bronchitis	M	63	66	107	1.52	1,735 (3,860)	1,350 (2,895)	566 (905)	4,397 (1,740)	6,132 (5,600)	72	38 (54)	47	2.7	7.4
5	Sarcoidosis	F	45	66	140	1.66	1,485 (2,925)	1,155 (1,950)	333 (975)	1,719 (895)	3,204 (3,810)	54	57 (50)	86	<2.5	3.0
6	Sarcoidosis	F	39	66	134	1.67	1,685 (3,350)	1,425 (2,230)	267 (1,120)	1,926 (1,030)	3,611 (4,380)	53	29 (53)	79	<2.5	
7	Mitral commissurotomy (postoperative)	M	41	68	196	2.00	4,270 (4,320)	3,140 (3,456)	1,112 (864)	2,042 (1,320)	6,312 (5,640)	32	78 (65)	71	1	1.3

* Figures in brackets are predicted normal values.
† Normal value <30 per cent.
‡ Normal value >83 per cent.
§ Normal value <2.5 per cent.
|| Normal value <2.0 per cent.

Symbols for lung volumes: VC is vital capacity; IC is inspiratory capacity; ER is expiratory reserve; RV is residual volume; TLC is total lung capacity; MBC is maximum breathing capacity.

diagnosis of pulmonary emphysema, which was borne out by his pulmonary function tests. He also had a good deal of bronchospasm and, in fact, it was found necessary to use bronchodilators to make him comfortable during the diffusion capacity measurements. Pt. 3 had a history of exposure to silica, complained of dyspnea, and had physical and X-ray findings associated with silicosis. Pt. 4 had a long history of chronic bronchitis and physical and X-ray findings compatible with that diagnosis. Pts. 5 and 6 both had sarcoidosis, with the typical history and chest X-ray findings associated with that disease, and fitted the syndrome of "alveolar-capillary" block. Pt. 7 although he had had a mitral commissurotomy about 6 months prior to these studies, was essentially normal as far as his pulmonary function was concerned.

RESULTS

The vital statistics and pulmonary function studies on each subject are in Table I. D_{O_2}, steady state D_{CO}, fractional CO uptake, D_{CO} breath holding, and pertinent data for each subject are presented in Table II. The reader is referred to the published discussions of these methods (1, 2, 5–8) for a detailed explanation. There are two different values for D_{CO} steady state and fractional CO uptake to compare with each value of D_{O_2}, because it is possible to get the CO values while breathing both the air and the low O_2 mixtures which are necessary for each value of

D_{O_2}. These data are also shown in Figure 3; average D_{CO} breath holding, D_{CO} steady state and fractional CO uptake measurements are plotted on the ordinates against the D_{O_2} values on the common abscissa.

DISCUSSION

A. Comparisons of D_{O_2}, D_{CO} steady state, D_{CO} breath holding and fractional CO uptake

The relations between the different estimates of pulmonary diffusing capacity are best seen in Figure 3. The lowest graph is a plot of D_{CO} breath holding against D_{O_2}. There is a definite increase in D_{CO} breath holding as D_{O_2} rises. The dashed lines in the lower two graphs represent the relation that would hold between D_{CO} and D_{O_2} if the only difference between the two measurements lay in the different molecular weights and solubilities of O_2 and CO and if the pulmonary membrane were chiefly saline (3). There are, however, many differences in the physiologic bases of D_{CO} breath holding and D_{O_2}. For example, if the standardized time of breath holding were taken to be 20 seconds instead of 10, D_{CO} breath holding would decrease (5), as noted earlier in this paper. In addition, recent work of Pappenheimer, Renkin, and Borrero (14) suggests that the lipid solubility of gases may be the most important fac-

TABLE II *

Simultaneous D_{O_2}, D_{CO} steady state and fractional CO uptake measurements, and resting D_{CO} breath holding measurements on seven individuals

Subject	Exercise		$F_{I_{O_2}}$ %	$\dot{V}_E$ L.(STPD)/ min.	Resp. rate per min.	$\dot{V}_{O_2}$ ml.(STPD)/ min.	R	Pa_{CO_2} mm. Hg	$P^eA_{O_2}$ mm. Hg	Pa_{O_2} mm. Hg	$P^eA_{\overline{O_2}} - \overline{P}_{CO_2}$ mm. Hg
	Rate m.p.h.	Grade %									
1	2.5	6	20.93	32.9	44	1,469	0.81	38	102	92	17
	2.5	6	12.72	34.6	46	1,096	0.80	35	47	38	17
2	0	0	20.93	10.2	19	337	0.74	45	94	78	13
	0	0	13.97	9.8	16	282	0.90	43	51	44	13
3	1.3	6	20.93	34.5	26	1,342	0.89	39	104	92	25
	1.3	6	12.72	48.6	35	1,405	0.95	34	54	37	25
4	1.3	6	20.93	30.0	34	956	0.80	46	93	75	26
	1.3	6	13.97	25.6	30	619	0.91	36	59	42	26
5	1	0	20.93	24.2	32	604	0.80	35	105	60	43
	1	0	13.97	35.8	50	510	1.02	28	70	31	43
6	0	0	20.93	13.2	40	301	0.88	41	102	86	21
	0	0	13.97	13.3	46	230	0.89	36	59	46	21
7	1.5	0	20.93	21.0	13	961	0.85	37	108	95	15
	1.5	0	12.72	22.3	12	952	0.78	30	54	49	15

TABLE II—*Continued*

Subject	V_D/V_T %	$\dot{Q}_{VA}/\dot{Q}_T$ %	D_{O_2} ml. min.×mm. Hg	F_{ICO} %	CO uptake ml./min.	P^*_{ACO} mm. Hg	Fractional CO uptake %	D_{CO} steady state ml. min.×mm. Hg	D_{CO} breath holding at rest, breathing air ml. min.×mm. Hg
1	24	3	65	0.092	12.6	0.24	46	52	37, 41
	43			0.089	11.2	0.17	40	66	
2	54	8.5	22	0.094	3.4	0.05	43	71	13, 13, 18
	50			0.107	3.2	0.20	36	16	
3	32	4	56	0.098	10.7	0.34	34	32	20, 31
	38			0.088	12.2	0.30	30	41	
4	50	8	24	0.148	10.0	0.46	25	22	12
	47			0.098	6.7	0.26	29	26	
5	53	20	12	0.095	2.7	0.47	13	6	7, 7, 8, 9
	60			0.088	2.0	0.50	7	4	
6	54	6.5	11	0.138	4.1	0.34	28	12	14
	53			0.083	2.9	0.13	36	22	
7	21	3.5	63	0.154	16.7	0.35	54	48	22
	17			0.067	7.3	0.17	50	42	

* F_{IO_2} is dry inspired O_2 concentration in %.

$\dot{V}_E$ is expired minute ventilation in liters STPD/min.

V_{O_2} is the O_2 consumption in ml. STPD/min.

R is the respiratory exchange ratio.

Pa_{CO_2} is the arterial CO_2 tension in mm. Hg measured directly.

$P^*A_{O_2}$ is the calculated effective alveolar O_2 tension in mm. Hg.

Pa_{O_2} is the arterial O_2 tension in mm. Hg measured directly.

$P_{C_{O_2}}$ is mean capillary O_2 tension in mm. Hg.

V_D/V_T is dead space volume/tidal volume in %: normally less than 25%.

$\dot{Q}_{VA}/\dot{Q}_T$ is the "physiological" shunt of mixed venous blood across the lung over the total capillary blood flow in %. Called "percentage venous admixture." Normally less than 6%.

D_{O_2} is pulmonary diffusing capacity for O_2 in ml. STPD/min./mm. Hg.

F_{ICO} is dry inspired CO concentration in %.

P^*_{ACO} is the calculated effective alveolar CO tension in mm. Hg corrected for the increase in COHb during the experiment.

D_{CO} is pulmonary diffusing capacity for CO, either steady state or breath holding, in ml. STPD/min./mm. Hg.

tor in their diffusion through the pulmonary membrane, which would change the theoretical relation between D_{CO} and D_{O_2}. Patients 2 and 6 were studied at rest both for D_{CO} breath holding and D_{O_2}. Patients 4 and 5, whose D_{CO} breath holding was measured at rest and whose D_{O_2} during exercise, had diagnoses of long standing chronic bronchitis and sarcoidosis, respectively. It is apparent that these patients were not able to increase their pulmonary diffusing capacity on exercise (7) to the same extent as normal subjects, and for this reason there is less difference between the breath holding values determined at rest and the D_{O_2} values obtained during exercise. Patients 1, 3, and 7 on the other hand, who were considered to be in better physical condition than the others, did presumably increase their D_{O_2} on exercise which displaced their points to the right. It should be noted that the D_{O_2} measurements on relatively healthy individuals, are more successful technically when done during exercise. At the time this study was done, we were not able to make D_{CO} breath holding measurements during exercise. Presumably, D_{CO} breath holding would have increased with exercise, displacing the values for patients 1, 3, and 7 upwards, as more recent studies have shown (15). All in all the findings are not unreasonable in view of the circumstances under which they were obtained.
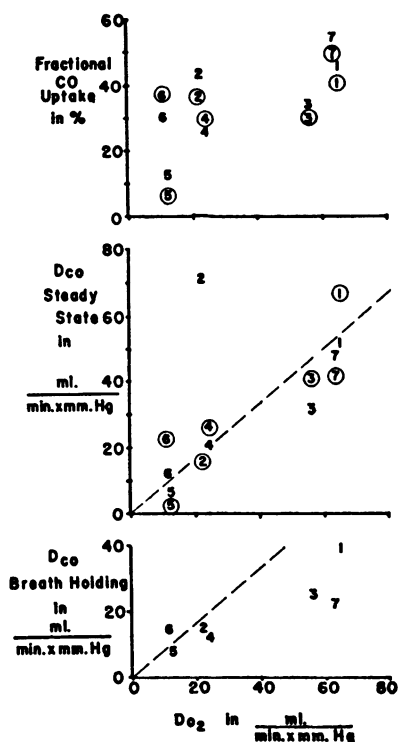
The middle graph in Figure 3 is a plot of simul-

FIG. 3. FRACTIONAL CO UPTAKE, DCO STEADY STATE AND DCO BREATH HOLDING PLOTTED AGAINST D_{O_2}

The numbers refer to the particular subject; a circle about a number indicates that value was obtained breathing an O_2 concentration less than 20.93 per cent. The dashed lines are the theoretical relationships between the pulmonary diffusing capacity for O_2 and that for CO; $D_{O_2} = 1.23 \, D_{CO}$. Individuals 1, 3, 4, 5, and 7 were studied during exercise, except for the D_{CO} breath holding which was measured at rest in all subjects. Individuals 2 and 6 were studied in rest in all tests.

taneous measurements of D_{CO} steady state against D_{O_2}. In this comparison, the level of exercise was obviously the same during both tests. These two methods are analogous in that both are performed during a steady state and, in both, alveolar tensions (P_{O_2} or P_{CO}) are calculated from the Bohr equation, assuming that arterial P_{CO_2} is a good estimate of mean alveolar P_{CO_2}. The value of D_{CO} steady state is sensitive to errors in arterial P_{CO_2} particularly when the fractional uptake of CO is high, and to errors in inspired and expired CO analysis, particularly when the ratio of dead space to tidal volume is high. A summation of such errors presumably accounts for the very high value recorded for D_{CO} in Patient 2 when breathing CO in room air. In all other instances

the correspondence between the two methods is as good as could be expected in view of known limits of analytical accuracy.

The topmost graph in Figure 3 is a plot of fractional CO uptake against D_{O_2}. Although there is a general tendency for fractional uptake to rise with increasing values of D_{O_2}, there is a considerable amount of scatter. This is probably due to the fact that fractional CO uptake decreases with increasing alveolar ventilation as well as decreasing with a lowered pulmonary diffusing capacity (5); in these studies the alveolar minute ventilation varied significantly. It is possible to calculate a value of D_{CO} from the fractional CO uptake if alveolar ventilation is known (16). This in effect corrects fractional CO uptake for variations in alveolar ventilation.

B. Critique of the measurement of D_{O_2}

The accuracy of D_{O_2} estimation by the Lilienthal-Riley technique has been discussed before (7). The method is inaccurate unless the end gradient, or mean alveolar to end capillary blood P_{O_2} difference is 6 mm. Hg or more. Since both exercise and 'alveolar-capillary block' cause this gradient to rise, the circumstances of the present studies were to the advantage of the method. If the gradients are sufficiently large, the estimate of D_{O_2} by this method appears to be good in health or in disease. The rate of combination of O_2 with intracorpuscular Hb, although faster than that for CO, interferes with the uptake of O_2 by the blood in the lungs in an analogous manner (17). The degree of underestimation of D_{O_2} because of this theoretical error is not at present known.

C. Critique of the measurement of D_{CO} steady state and breath holding

The presence of a CO tension in the capillary blood will interfere with the diffusion of CO into blood and if it is not allowed for, will produce an apparent underestimate of the true pulmonary diffusing capacity (17). There are two reasons for the existence of a significant capillary blood P_{CO}, the first of which is the presence in the blood of COHb in a large enough concentration to have a significant P_{CO} in equilibrium with it. Although the blood COHb was not analyzed in these studies, the patients were all non-smokers, so their ini-

tial mixed venous COHb would have been expected to lie between 0 and 5 per cent saturation (18). The effect of this COHb was ignored in both steady state and breath holding D_{CO} measurements. During the steady state procedure there was, in addition, a significant increase in mixed venous COHb, which can be calculated from the total CO uptake and an assumed blood volume. The values of alveolar P_{CO} used to calculate D_{CO} steady state in Table II have been corrected by subtracting from the actual alveolar P_{CO} the tension of CO in equilibrium with the average mixed venous COHb during the test at the mean capillary P_{O_2}. The calculation of D_{CO} breath holding needs no correction for blood COHb except in exceptional circumstances because: (a) the total amount of CO absorbed in the procedure is about a quarter of that taken up in the steady state D_{CO} measurement; (b) in addition, the alveolar P_{CO} is much higher; in the example of the calculation of breath holding D_{CO} of Patient 2 cited above, compare the alveolar CO concentration of 0.106 per cent initially, falling to 0.069 per cent at the end of 10 seconds, with the steady state alveolar CO concentration in Table II of 0.2 mm. Hg/713 or 0.028 per cent CO.

The second cause of a CO tension in the capillary blood during its passage through the alveolus is the slowness of the reaction of CO with intracellular Hb which prevents its being bound in the blood and allows it to pile up in solution in the plasma. This problem does in fact exist (17), but will result in an underestimate of D_{CO} by the same proportion in either the breath holding or steady state methods of estimating D_{CO}. The rate of combination of CO and Hb decreases as the blood O_2 tension rises. Therefore, measurements of D_{CO} by either breath holding or steady state methods must be done at a relatively constant alveolar P_{O_2} (actually constant capillary blood P_{O_2}) if the test is to be standardized. In the present studies all the breath holding tests were done under conditions which could reasonably be expected to produce an alveolar P_{O_2} of about 100 mm. Hg. On the other hand, the steady state D_{CO} measurements on each subject were made with two different alveolar P_{O_2} levels: 100 mm. Hg and 55 mm. Hg. At the lower O_2 tension, the rate of combination of CO and Hb should have been greater; as a result the capillary P_{CO} should have been less and

the calculated steady state D_{CO} should have been greater. However, the calculated value of steady state D_{CO}, while breathing a low O_2 mixture, was greater than that when breathing air in only 4 out of 7 instances (Figure 3 and Table II). Since other data definitely show an increase in calculated D_{CO} by either steady state or breath holding techniques when the alveolar P_{O_2} is decreased (5, 9), the failure of the present measurements to show a similar change must probably be accounted for by the presence of analytical inaccuracies which conceal this effect. It must be recognized that the determinations of diffusing capacity reported in this paper put considerable strain on the analytical techniques which were available.

In the breath holding technique the calculation of the initial CO concentration in the expired alveolar gas depends upon the assumption that the inspired CO-He mixture is completely mixed with alveolar gas before significant absorption of CO into the blood takes place. The validity of this assumption is in turn based on (a) calculations of the rapidity with which diffusion mixes gases in the finer divisions of the respiratory tree (10), and (b) the fact that the expired alveolar CO concentration, when measured after various intervals of breath holding in normal subjects and plotted against time, back-extrapolates to the initial CO concentration as calculated on the basis of this assumption. Expired alveolar gas samples were obtained at various durations of breath holding in the present subjects (these data are not all included) and observation (b) is also true for them. Therefore, it appears valid to calculate the initial CO concentration in the collected expired alveolar sample from the dilution of the inspired He concentration, but the question may arise as to whether the sample derives from alveoli which have local diffusing capacities which are representative of the pulmonary diffusing capacity of the lung as a whole. D_{CO} breath holding (measured over a 10-second period) in normal subjects and in a limited number of patients with sarcoidosis and emphysema, varies less than 10 per cent when the expired alveolar sample analyzed is from the early or late parts of the expiration (15). Since the early parts of the expired alveolar gas come from different alveoli than the later parts (19), this suggests that the different parts of the expired

alveolar gas are reasonably representative in respect to the total pulmonary diffusing capacity.

The steady state D_{CO} method and D_{O_2} method for measuring pulmonary diffusing capacity are both subject to theoretical errors if pulmonary diffusing capacity is not distributed evenly throughout the lung in relation to alveolar ventilation (16), just as the breath holding D_{CO} estimate is subject to error if pulmonary diffusing capacity is not distributed throughout the lung evenly with respect to alveolar volume. In comparing D_{CO} breath holding with either D_{O_2} or D_{CO} steady state it must be borne in mind that conditions are not strictly comparable during the tests. The breathing maneuvers performed in the breath holding test may alter the state and number of the perfused capillaries and may do so to a different degree in the different subjects. Although the D_{CO} breath holding could not have been expected to be exactly equal to D_{CO} steady state or to D_{O_2}, particularly when the latter were measured during exercise, one of the purposes of this investigation was to determine whether it provides a convenient and reliable means of evaluating the state of the diffusing surface in patients. The results are sufficiently encouraging to warrant a more extensive investigation of the method.

SUMMARY AND CONCLUSIONS

Measurements of D_{CO} were made at rest on seven subjects by a modification of Krogh's breath holding technique. These findings were compared to simultaneous steady state measurements of D_{O_2} by the Lilienthal-Riley technique, of D_{CO} by the Filley technique and of fractional CO uptake as described by Bates. The latter methods were applied during exercise in five of the seven subjects. All but one of the subjects were hospital patients and together they provided a wide range of values for pulmonary diffusing capacity.

It is concluded for the present that the methods for the estimation of steady state D_{O_2} and D_{CO} provide similar values when allowance is made for the differences in physical characteristics between the two gases. Estimates obtained by the CO breath holding technique at rest show directional changes which are comparable to those obtained by the other methods during exercise, although the absolute values are lower. The frac-

tional CO uptake, when uncorrected for changes in ventilation rate, is less reliable.

The optimum conditions for each method vary, and further studies of the advantages and limitations of each are required.

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