Disappearance Curves and Single-Breath D_{LCO} from Consecutive Samples during Slow Expiration

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KIKUCHI, R., SASAKI, T. and TAKISHIMA, T. *Disappearance Curves and Single-Breath D_{LCO} from Consecutive Samples during Slow Expiration.* Tohoku J. exp. Med., 1982, **136** (4), 419–431 — The disappearance of CO in three expirates consecutively sampled after various breath holding periods was measured in seven normal subjects. The CO disappearance curves in the three expired portions were nearly linear and parallel each other, but the curves for later sampled expirates were lower, and even those for earlier sampled expirates passed under unity at time zero when extrapolated on a logarithmic graph of relative expired alveolar CO concentration against breath holding time. The slope of each disappearance curve shows “the true diffusing capacity at breath holding lung volume”, eliminating an effect of rapid CO absorption at a lower lung volume during expiration. This effect is considered the cause of the downward shift of the decay curve, and the shift is magnified in slow expiration. In addition, the three parallel lines indicate a homogeneous distribution of D_L/V_A within the lungs. Subsequently D_{LCO} in conventional calculations had greater values in shorter breath holding time and later sampling. — — carbon monoxide disappearance curve; single-breath D_{LCO} ; breath holding time; sampling portion; distribution of D_L/V_A

Many researchers have reported that the value of conventional single-breath D_{LCO} decreases as breath holding time increases (Forster et al. 1954; Ogilvie et al. 1957; Cadigan et al. 1961; Kanagami et al. 1961) and others have reported that it increases when measured from gas samples late in expiration, even in normal lungs (Ogilvie et al. 1957; Marshall 1958; Spicer et al. 1962; Wagner et al. 1971; Newberg et al. 1973). These results have been tentatively interpreted as indicating an unequal distribution of both ventilation and diffusing capacity within the lungs. However, if lung emptying is done sequentially from a base which has much pulmonary capillary blood volume, to an apex with less pulmonary capillary blood volume, D_{LCO} can be expected to decrease in later samples.

Single-breath D_{LCO} has been measured also from the slope of the CO disappearance curve. (Forster et al. 1954; Johnson et al. 1960; Spicer et al. 1962; Finley et al. 1974; Gary et al. 1979). Originally it was uncertain if the value from each method was not different physiologically.

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Spicer et al. (1962) observed two parallel alveolar CO disappearance curves from early and late expired samples, and the curves for the late samples positioned below those for the early samples. They suggested that D_L/V_A were distributed homogeneously throughout the lungs and the displacement of the disappearance curves could be explained by errors in estimation of the time the gas spends in the alveoli and by an increased rate of CO disappearance caused by reduced alveolar volume during expiration.

Recently, Wagner et al. (1971) using a stable isotope of CO ($C^{18}O$) and a mass spectrometer and Cotton et al. (1979) developing a rapidly responding infrared CO analyzer, analyzed D_{LCO} by using a method comparable to the conventional single-breath technique. Both reported this value remained nearly constant despite different sampling portions, various breath holding times, and the expired flow rate when corrections were made by using time-weighted mean alveolar volume, considering volume and volume change during inspiration and expiration.

The above two reports suggest that a difference in each method exists in the diffusion process during alveolar volume change, especially during expiration.

Spicer et al. (1962) inferred furthermore that the inherent errors in the conventional method might have originated from the displacement of the CO disappearance curves. This is also very important for clinical single-breath D_{LCO} measurements. Therefore, the mechanism and magnitude of displacement of the CO disappearance curves must be clearly understood.

In the present experiments we refined the method of Spicer et al. and examined the CO disappearance curves in detail from consecutive samples during slow expiration, and analyzed the physiological difference between the pulmonary CO diffusing capacity in CO disappearance curves and that in the conventional measurement.

MATERIALS AND METHODS

Subjects

We studied seven healthy non-smoking laboratory workers trained in respiratory maneuvers. Anthropometric and spirogram data appear in Table 1.

Apparatus and procedure

A schematic diagram of equipment and experimental procedure are shown in Fig. 1. The apparatus used for collecting the alveolar samples consisted of four 2-liter sampling bags (A, B, C, D, in Fig. 1) each equipped with a solenoid valve. The solenoid valves were connected to a potentiometer functioning with a bell and control unit that enabled a similar quantity of expirate to be sampled sequentially in four bags. The first expirate was collected in bag A which was placed innermost so that dead space air during expiration was removed and this sample was discarded from the measurement. The difference between the amount programmed by the control unit and the actual amount collected in each bag was confirmed with a syringe as being less than 20 ml.

Prior to the experiment, forced vital capacity (FVC) of the subjects was measured, and then the control unit connected to the potentiometer was adjusted so that one fourth of FVC could be sampled in each bag. The four sampling bags and the dead space of expiration were washed by air. The bags were emptied by suction and closed off before each test. Bag E and the conducting tube of inspiration were previously filled with a test

TABLE 1. *Anthropometric and spirogram data on normal subjects*

Group	Subject No.	Age (years)	Sex	Weight (kg)	Height (m)	VC*	FEV _{1.0} /VC†
Normal	1	32	M	67.0	1.73	116	91.0
	2	28	M	63.0	1.72	92.0	96.8
	3	29	M	68.0	1.71	91.4	82.4
	4	28	M	60.0	1.68	95.8	93.5
	5	26	M	67.0	1.67	104.9	84.2
	6	29	M	60.0	1.69	95.4	94.9
	7	28	M	67.0	1.82	106.8	82.4
Mean±s.d.		28.6±1.81		64.6±3.50	1.70±0.02	100.3±9.15	89.3±6.18

*VC=vital capacity expressed as percentages of the values predicted from Cotes et al. (1975).

†FEV_{1.0}/VC=forced expired volume in 1 sec expressed as percentages of vital capacity.

Fig. 1. Schematic diagram of equipment and experimental procedure for measurement of breath holding $D_{\text{I,CO}}$. Expirates were sampled first in innermost bag A, next in bags B, C and D, consecutively.

gas. The dead space of the apparatus was about 10 ml during inspiration, whereas it was 55 ml from the mouth piece to the site of valve D and 25 ml between each two adjacent valves (A-B, B-C, C-D) during expiration. The five-way tap was turned "to air" and the seated subject, wearing a nose clip, expired to his residual volume (RV). Then the tap was turned on and the subject rapidly inspired a mixture containing 0.3% CO, 1.65% Ne, 21% O₂, and the balance N₂ from a balloon (E), held his breath for a prescribed time, and then expired until RV was again reached. Contrary to rapid inspiration, the expired flow rate was rather slow (about 0.6 liter/sec in normal) because of the relatively small orifice of the solenoid valve (ϕ 12 mm). The expirate was sampled consecutively in each bag, A, B, C, D. This was repeated every 15 min varying the breath holding time which was timed with a stop watch from the start of inspiration to the beginning of expiration. Breath holding time, varying in five grades, was from about 3 to about 20 sec. The subjects were

closely regulated by means of kymograph tracing to insure an equal amount of FVC in each breath holding trial; cases in which FVC varied over 20% were excluded. To reduce errors originating from back diffusion of CO from venous blood, each breath holding time was chosen at random. Gas concentrations, namely, CO and Ne of each bag B, C, D and inspired gas were promptly measured by a gaschromatograph (Hitachi 063, Tokyo). The linearity of the gaschromatograph was verified against each gas. It was confirmed that there was no leakage from the bag by measuring the sampling gas in each bag several times.

Analysis

CO disappearance curves were obtained by plotting five values of logarithm F_{AICO} divided by F_{A0CO} ($\ln F_{AICO}/F_{A0CO}$) against breath holding times in each sampling portion. F_{AICO} represents the expired alveolar CO concentration sampled after breath holding. F_{A0CO} , assumed initial alveolar CO concentration was calculated from the inspired CO concentration F_{ICO} multiplied by Ne dilution ratio ($F_{A0CO}=F_{ICO} \cdot F_A/F_I$). Breath holding time was measured from the start of inspiration to the beginning of each sample collection, retrospectively, from the kymograph tracing. The slope of the CO disappearance decay was derived from fitting a straight line to each five-point data by the least square method, which would be tentatively equal to D_L/V_A (Forster et al. 1954; Johnson et al. 1960), and then D_{LCO} was calculated by multiplying mean alveolar volume ($\bar{V}_A$) at five breath holding trials. Alveolar volume, V_A in ml (STPD) was calculated from the inspired volume (V_I) divided by Ne dilution ratio ($V_A=V_I \cdot F_I/F_A$) in each sample.

Namely

$$D_{LCO} = (\text{the slope of CO disappearance curve}) \times V_A$$

$$\bar{V}_A = \frac{1}{5} \cdot \{V_A(3'') + V_A(5'') + V_A(10'') + V_A(15'') + V_A(20'')\}$$

In addition D_{LCO} was calculated by the conventional method of Forsters et al. (1954) from the following equation in each sampling portion of each breath holding time (conventional D_{LCO} , D_L/A_A).

$$D_{LCO} = \frac{V_A \times 60}{P_B - 47} \times \frac{1}{\Delta t} \cdot \ln \frac{F_{A0CO}}{F_{AICO}}$$

(P_B , barometric pressure; Δt , breath holding time)

RESULTS

In Fig. 2 are shown logarithmic disappearance curves of relative expired alveolar CO concentrations to assumed F_{A0CO} in samples B, C and D. The logarithmic disappearance curves for three samples (B, C, D) were nearly linear over the time period studied, and the later the sampling portion, the lower the regression line in all subjects. Furthermore, even the lines for earlier sampled expirates passed under unity at time zero when extrapolated on the graph. Table 2 shows the slope of CO disappearance curves by the least square method and the values of D_{LCO} .

Each slope, D_{LCO} calculated from the slope of the regression line of CO decay was not significantly different among different sampling portions in any subject.

Average values of conventional D_L/V_A , D_{LCO} and breath holding times (t) in normal subjects are presented in Table 3.

In the upper and lower panels of Fig. 3 the relation of each conventional D_L/V_A , D_{LCO} in early samples (B) and late samples (D) to the breath holding time is shown.

TABLE 2. Slope and D_{LCO} from CO disappearance curve

Subject	B sample			C sample			D sample		
	Slope	r	$\bar{V}_A$	D_{LCO}	Slope	r	$\bar{V}_A$	D_{LCO}	D_{LCO}
1	4.48	0.990	5.29	23.7	4.40	0.893	5.30	23.3	24.0
2	6.46	0.992	4.64	30.0	5.79	0.986	4.82	27.9	30.6
3	6.29	0.998	3.82	24.0	6.19	0.993	3.84	23.8	25.0
4	8.24	0.996	4.62	38.1	8.06	0.998	4.70	47.9	35.3
5	5.45	0.984	4.20	22.9	5.37	0.974	4.21	22.6	23.2
6	5.87	0.991	4.22	24.8	6.04	0.995	4.24	25.6	24.7
7	5.27	0.997	5.12	27.0	5.44	0.997	5.11	27.8	25.8
Mean $\pm$ s.d.	6.00 ± 1.18	0.993 ± 0.005	4.56 ± 0.52	27.3 ± 5.44	5.89 ± 1.11	0.989 ± 0.009	4.60 ± 0.53	26.9 ± 5.25	26.9 ± 4.39

Slope=slope of CO disappearance curve derived from fitting a straight line by the least square method.
 r =regression coefficient.
 $\bar{V}_A$ =mean alveolar volume (l, STPD) by various breath holding times.
 D_{LCO} =single-breath diffusing capacity from the slope of CO disappearance curve ($\text{ml}\cdot\text{min}^{-1}\cdot\text{Torr}^{-1}$, STPD).

TABLE 3. D_L/V_A and D_{LCO} by conventional Forster's method

	B sample			C sample			D sample		
	t	D_L/V_A	D_{LCO}	t	D_L/V_A	D_{LCO}	D_L/V_A	D_{LCO}	D_{LCO}
3"	4.15 $\pm$ 0.34	7.96 $\pm$ 1.29	36.5 $\pm$ 7.18	5.81 $\pm$ 0.32	9.54 $\pm$ 1.34	44.3 $\pm$ 8.54	7.58 $\pm$ 0.42	1.09 $\pm$ 1.48	52.0 $\pm$ 10.5
5"	6.51 $\pm$ 0.46	7.42 $\pm$ 0.97	33.7 $\pm$ 5.17	8.18 $\pm$ 0.37	8.45 $\pm$ 0.89	33.8 $\pm$ 5.68	9.93 $\pm$ 0.32	9.67 $\pm$ 0.90	44.8 $\pm$ 5.70
10"	11.2 $\pm$ 0.42	7.00 $\pm$ 0.91	31.2 $\pm$ 4.68	13.0 $\pm$ 0.38	7.83 $\pm$ 1.14	35.2 $\pm$ 5.50	15.2 $\pm$ 0.82	8.67 $\pm$ 0.83	39.6 $\pm$ 5.17
15"	16.1 $\pm$ 0.57	6.67 $\pm$ 1.25	30.8 $\pm$ 5.85	17.8 $\pm$ 0.68	6.94 $\pm$ 1.28	32.2 $\pm$ 6.56	19.8 $\pm$ 0.99	7.74 $\pm$ 1.12	36.3 $\pm$ 5.28
20"	20.4 $\pm$ 1.13	6.46 $\pm$ 1.03	29.6 $\pm$ 5.35	22.3 $\pm$ 1.35	6.91 $\pm$ 1.01	31.9 $\pm$ 5.36	23.7 $\pm$ 2.22	7.34 $\pm$ 0.83	34.1 $\pm$ 4.53

All data represent average values $\pm$ s.d.
 t =breath holding time (sec) calculated from the start of inspiration to the beginning of each sample collection.
 D_L/V_A ="permeability" from conventional method.
 D_{LCO} =single-breath diffusing capacity from conventional method ($\text{ml}\cdot\text{min}^{-1}\cdot\text{Torr}^{-1}$, STPD).

Fig. 2. Carbon monoxide alveolar disappearance curves in the three sampling portions of expired air. Each point represents an average value. Each line represents a linear regression by the method of least squares. Open circles correspond to B sample (early), triangles to C sample (later), closed circles to D sample (latest).

Fig. 3. The relation of D_L/V_A by conventional method to the breath holding time (upper panel), and the relation of D_{LCO} by conventional method to the breath holding time (lower panel). Each point represents average value $\pm$ s.e., but all data are calculated as percent value against D_L/D_A of B sample at a 10 sec breath holding time for each subject. Circles correspond to D sample (latest), triangles to B sample (early).

Values in these figures were plotted as percent values against those of sample B at 10 sec breath holding time maneuver.

According to conventional calculations, D_L/V_A and D_{LCO} of later samples were significantly larger than those of earlier samples ($p < 0.01$) in all subjects, and in each breath holding time. Both D_L/V_A and D_{LCO} decreased as the duration of

breath holding increased. This trend was significant ($p < 0.01$) and became exaggerated in later samples.

DISCUSSION

We carefully re-examined the experiments of Spicer et al. (1962). The expirates were sampled automatically rather than manually, and at three portions rather than two. The CO disappearance curves were nearly linear ($r = 0.925 \sim 0.999$) regardless of sampling portion and parallel to each other, but the curves for later sampled expirates were lower as the findings of Spicer et al. (1962), with an exception that even the uppermost curve positioned below unity when extrapolated to zero time, whereas Spicer et al. (1962) found that the intercept could appear above or below unity.

What causes the displacement of the CO disappearance curve in later samples? Spicer et al. (1962) suggested that it was primarily due to the "first-in last-out" theory (Fowler 1952) that the later samples spent more time exposed to capillary blood than the calculated time of breath holding indicated, and secondly due to the accelerating effect of reducing lung volume on the fall in alveolar CO concentration during expiration. Jones and Meade (1961) also referred the displacement of the CO disappearance curve to an error in estimating time and they stated that they could eliminate such errors by fixing the time of onset of CO uptake at three-tenths of the inspired volume. However, calculating a presumed time in which each CO disappearance curve would pass through unity ($\ln F_{AiCO}/F_{A0CO} = 1$), our average results showed -1.73 sec in sample B, -3.96 sec in sample C, and -7.17 sec in sample D. For example, in the 10 sec breath holding time maneuver each sampling end time, calculated from the start of inspiration to the end of each sampling, was 12.9 sec in sample B, 15.0 sec in sample C, and 17.1 sec in sample D. The absolute value of the presumed time plus the sampling start time (for 10 sec breath holding time – Table 3) was always greater than the above sampling end time in the case of sample C in each of the subjects, and was especially so in sample D. In addition, similar results were obtained in other breath holding time maneuvers. Therefore, the displacement of CO disappearance curves cannot be explained only by the "first-in last-out" theory or errors in estimating breath holding time.

Facilitating effect of CO absorption during expiration

We have also presumed that the carbon monoxide absorption rate during expiration differed appreciably from the rate during breath holding as assumed in the simple equation used in the calculation of D_{LCO} . If the rate of CO absorption from the alveoli increases during expiration and collection of the alveolar samples as suggested by Spicer et al. (1962), the displacement of the CO disappearance curve is self-explanatory.

In panel A of Fig. 4 single breath maneuvers at two periods of breath holding (t_1 , t_5) are shown, assuming a single compartment lung model which has increasing D_L/V_A at a lower lung volume. In addition, panel B of this figure shows modified

Fig. 4. Analysis of CO disappearance curves of different sampling portions in a single compartment lung model. Panel A shows single breath maneuver at two breath holding times (t_1 , t_5). Expirates are sampled at three portions in each breath holding trial. Panel B shows a modified single breath maneuver in which inspiration and expiration are assumed to be of infinite speed. Following a prior sampling the second, third and fourth samplings are taken after a short breath holding time, and each short breath time is held for the duration of each sampling time in panel A, and then these processes are the same in each breath holding trial. In this model, D_L/D_A at each breath holding lung volume is assumed to be P_1 (at TLC), P_2 , P_3 and P_4 , respectively. In panel C open and closed marks show the alveolar sample in breath holding time t_1 and t_5 , respectively. The slope of each CO disappearance curve is equal to that of line binding the supposed relative CO concentration at TLC. CO disappearance curves of later samples place in a lower position. True alveolar samples at TLC cannot be obtained practically because of the problem of dead space and alveolar volume change.

single breath maneuvers in which both inspiration and expiration are assumed to be infinitely rapid, and that second, third and fourth expirates among those sampled in each breath holding trial are sampled after short breath holding time following prior sampling. The processes are assumed to be the same in each breath holding trial, and the relative alveolar CO concentration of the later sample will be reduced to a constant fraction of the earlier sample because of a larger D_L/V_A in a lower lung volume. This should cause a down displacement of the later CO disappearance curve which remains parallel as seen in panel C of this figure.

In order to test this assumption we made two experiments. The first measured the CO disappearance curve at different lung volumes, namely, FRC and TLC. The second measured the CO disappearance curve at different expiring speeds, because such a lung volume effect on CO absorption must be more apparent at slower expiring speeds. The results of these two experiments are

shown in Figs. 5 and 6. Alveolar CO disappearance curves measured at FRC were steeper than those at TLC, and when slowly expired, the CO disappearance curves were positioned to the left of those for fast expiration in each sampling portion, and this trend was more apparent in later sampling portions. These results indicate increased D_L/V_A at lower lung volume and raise the possibility of a similar phenomenon occurring in actual lungs as discussed in Fig. 4. Howell et al. (1961) reported that lung inflation compressed the pulmonary vascular bed mainly in small vessels closely related to the alveoli. If such compression is released with expiration, CO absorption will be more rapid. Mittman and Burrows (1959) also reported on CO disappearance curves at TLC and FRC in two methods of breath holding and equilibration. In both techniques, steeper curves measured at FRC appeared in their figures.

Recently, Newth et al. (1977) demonstrated that despite prior breath holding time and lung volume there is no difference in D_{LCO} when measured in lung volume within each 10 percent decrement from 20 to 80% of the vital capacity during exhalation. Their "exhaled" diffusing capacity is similar to that calculated from the slope of the binding line between relative alveolar CO concentration of sample B and that of sample C and sample D during each expiration in Fig. 2. For the calculation of "exhaled" D_{LCO} , they used RV plus a mid-point volume of 10

Fig. 5. Carbon monoxide alveolar disappearance curves at different lung volumes, for FRC and TLC, in the seven normal subjects. Each point represents average value, and each line is obtained from the least square method. The slope of each line is steeper at FRC than at TLC in each sampling portion.

Fig. 6. The effect of expiring flow rate to carbon monoxide alveolar disappearance curves in three subjects (Nos. 4, 5, 6 on Table 1). Representation method is the same as in Fig. 5. Open circles correspond to cases of comparably high flow rate ($=0.60$ liter/sec); closed circles to a slow flow rate ($=0.34$ liter/sec).

percent decrements in the lung volume during expiration as V_A , and we must conclude from their results that D_L/V_A during expiration increases with reducing lung volume and remains constant despite prior breath holding time.

From the above discussion, only D_L/V_A and D_{LCO} calculated from CO disappearance curves would indicate true values at breath holding lung volume for the region where the sampled expirate comes from in the lungs since it eliminates "lung volume effect" although Spicer et al. (1962) did not refer to these points exactly.

The difference between our results and those of Spicer et al., namely the lower position of our CO disappearance curve, might originate in the slower expiring maneuver of our experiment, but the intercept where CO disappearance curve was projected back to zero time should be always below unity because of "lung volume effect" during sampling.

Comparison between CO disappearance curve and conventional methods

Many researchers have presented experimental results indicating that single breath diffusing capacity for CO increased with shorter breath holding times (Forster et al. 1954; Ogilvie et al. 1957; Cadigan et al. 1961; Kanagami et al. 1961)

and later sampling portions (Ogilvie et al. 1957; Marshall 1958; Wagner et al. 1971; Newberg et al. 1973). We obtained similar results in conventional analysis. Usually the difference in D_{LCO} is interpreted as uneven distribution of pulmonary diffusing capacity within the lungs.

Even the uppermost CO disappearance curve (for sample B) positioned below unity when extrapolated at time 0 in Fig. 2. Conventional D_{LCO} is generally calculated from the slope of the line, which corresponds to conventional $D_{\text{L}}/V_{\text{A}}$, binding the point (0.1), i.e., assumed initial alveolar CO concentration at time 0, with the other points ($t, \ln F_{\text{A}t\text{CO}}/F_{\text{A}0\text{CO}}$), that is, CO concentration sampled at time t . Therefore, the slope of such a line becomes steeper with shorter breath holding time, and this trend is exaggerated in later samples. As alveolar volume did not so change with the sampling portion or various breath holding time, conventional D_{LCO} also changed in the same direction. For example, the average values, comparing D_{LCO} from conventional method at 10 sec breath holding time with D_{LCO} from the slope of CO disappearance curve, are as presented below.

	D_{LCO} (disappearance curve method)	D_{LCO} at 10 sec (conventional method)
Sample B:	27.3	31.2 ml/min·mmHg
Sample C:	26.9	35.2
Sample D:	26.9	39.6

D_{LCO} from the disappearance curves were nearly constant, while D_{LCO} from the conventional method increased for later samples. We cannot rule out the effect discussed above from previous reports since most of the authors did not indicate expiratory flow or the CO disappearance curve.

Comparison with other previous reports by continuous CO analysis

Cotton et al. (1979) utilized a method comparable to the conventional single breath technique and reported that D_{LCO} increased when measured from gas sampled at progressively lower lung volumes, and this trend was exaggerated for shorter breath holding times and slower expired flow rate. Such tendencies were largely eliminated by using time-weighted mean alveolar volume and D_{LCO} remained nearly constant.

Their time-weighted mean alveolar volume at various lung volumes was smaller for shorter breath holding times and decreased more rapidly with reducing lung volume for lower expired flow rate. That means, in other words, that $D_{\text{L}}/V_{\text{A}}$ increases in later samples, and that trend is exaggerated for shorter breath holding times and slower expired flow rates, because D_{LCO} was constant. Quite the reverse is indicated, judging from the distribution of $D_{\text{L}}/V_{\text{A}}$ within the lungs because lung emptying is especially distinct at slower expired flow rates from the base of the lung where blood flow and diffusing capacity may be relatively greater to the apex where blood flow and diffusing capacity is relatively low. Their $D_{\text{L}}/V_{\text{A}}$ are like those of the conventional method and like mean values during breath holding and

expiration. Their results can be also interpreted from the view point of our experimental results in Figs. 2 and 6. Cotton et al. (1979) would choose a slope of the line binding unity and a sampling point on the CO disappearance curve as a value of D_L/V_A without taking rapid CO uptake during expiration into consideration. As in our analysis of the conventional method, such a slope, namely their D_L/V_A , increases when measured from gas sampled at progressively lower lung volumes and becomes exaggerated for shorter breath holding times and for slower expired flow rates. As their time-weighted mean alveolar volume changed in the opposite direction of their D_L/V_A , the values of D_{LCO} became constant, whereas in the conventional method, breath holding lung volume (TLC) is used as alveolar volume (V_A) for the calculation of D_{LCO} neglecting alveolar volume change.

Consideration of experimental validity

It is important to make the sampling volume and sampling position the same in each various breath holding trial because they directly affect the CO disappearance curve. Therefore, we used an automatic sampling device rather than a manual sampling device.

In our experiment, the expired flow rate was rather slow (0.6 liter/sec) but we thought it was better to empty the lung sequentially from base to apex and to analyze the effect of the expiration phase. Moreover, since patients with severe obstructive impairment expire as slowly as in our experimental situation, and even normal persons cannot expire infinitely fast, our study focuses on what is necessary and appropriate.

Resistance originating in the orifice of the solenoid valve may make intrathoracic pressure increase during expiration, which depresses the rate of CO absorption (Smith and Rankin 1969). Such an effect should be larger in later samples which are exposed to the pressure for a longer time; subsequently it will be considered that the later the sampling portion, the higher the displacing effect against the CO disappearance curve in Fig. 2 will occur. Actually, however, as the CO disappearance curves in later samples positioned below those of earlier samples, the facilitating lung volume effect during expiration mentioned above is functioning, surpassing that of the pressure effect.

In our apparatus, dead space volume during expiration was larger, which may raise some questions. Inside this dead space turbulent gas mixing is also anticipated, in addition it may be that dead space contributes to sample B or even sample C. However, since expiring speed was so slow and the initial one-fourth expirate (sample A) was discarded, most of dead space would be removed. If the above contribution of dead space would occur, sample B or sample C would be diluted by dead space air because the expiration side was washed by air before each breath holding trial, but the slope or position of the CO disappearance curve will not change as both expired CO and Ne are similarly diluted. Turbulent mixing would close the position of the CO disappearance curves in each sample.

If sample D, drawn from the last one-fourth of expiration contains a closing

volume artifact as observed by Newth et al. (1977), then the position of the CO disappearance curve would be shifted. But the slope of the CO disappearance curve of sample D represents the CO permeability at breath holding lung volume for the region where sample D comes from as mentioned in previous paragraphs, irrespective of closing volume phenomenon during expiration.

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