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Research Article

THE ABSORPTION OF CARBON MONOXIDE BY THE LUNGS DURING BREATHHOLDING[1]

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In 1915, M. Krogh reported the use of carbon monoxide in an ingenious technique for the measurement of the pulmonary diffusing capacity in man (1). In her method a maximal inspiration of a gas mixture containing CO was made from residual volume and followed immediately by an expiration of at least one liter of gas. The breath was held at the remaining volume for 6 to 10 seconds, and then a maximal expiration was made. The terminal volumes of the two expirations were considered to be alveolar gas and were analyzed for CO concentration. M. Krogh assumed the CO concentration decayed exponentially and derived the following equation to describe this decay.

$$F_A = F_{A_0} \exp\left(-\frac{DPbt}{V_A}\right) \qquad (1)$$

F_A is alveolar concentration of CO (dry) at time t (Krogh's final sample); F_{A_0} is the alveolar concentration (dry) at time zero (Krogh's initial sample); "exp" is e, the base of the natural logarithms, raised to the power contained in the brackets following; D is the pulmonary diffusing capacity for CO in ml. STPD/mm. Hg × sec.; V_A is the total alveolar gas volume in ml. STPD during the period of breathholding, obtained from a spirometer tracing and from measurement of functional residual capacity; t is time in seconds between the delivery of the two gas samples; and Pb is the total barometric pressure minus 47 mm. Hg. The pulmonary diffusing capacity (D) was calculated by substituting the values obtained for F_A, F_{A_0}, t, Pb and V_A in a rearrangement of Equation *1*.

In order to derive Equation *1*, Krogh had to make the following major assumptions: (a) that the CO concentration in the first sample was representative of all alveolar gas; and (b) that the CO tension in the pulmonary capillary[5] plasma (P_c) was negligible. Although her technique involves delivering two expired alveolar samples, these are actually parts of the same expiration, being separated by the breathholding period. It is now known that these two alveolar samples would not have the same initial CO concentration (2). Furthermore, Roughton has indicated that P_c may not be negligible (3), casting doubt on the validity of the second assumption. Therefore, it was decided to reinvestigate the disappearance of CO from the human lung during breathholding following a single inspiration of a gas mixture containing CO. Recently developed physical methods of gas analysis, in particular mass spectrometry and infrared absorption techniques, were used throughout.

At first Krogh's experiments were repeated as she described them (1) and values for the pulmonary diffusing capacity were obtained which agreed with those she reported. However, the calculated value of the pulmonary diffusing capacity varied with the length of time the breath was held, which is incompatible with Equation *1*. In order to investigate this phenomenon further, the experimental procedure was modified. Only one expired sample was obtained, and the initial CO concentration (F_{A_0}) was computed from the dilution of an insoluble gas (He) contained in the inspired mixture. This obviated the necessity of comparing CO concentration in two different alveolar gas samples, and thereby eliminated the need for Krogh's first premise above. The breath was held for different lengths of time, and when the resulting expired alveolar CO concentrations

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[5] The term "pulmonary capillary" refers to that part of the pulmonary vascular bed which is exposed to alveolar gas.

were suitably corrected for variations in the dilution of the inspired mixture, a plot of log F_A against time was obtained. This plot of log F_A against the duration of breathholding was not a straight line in the seven subjects studied, as Krogh had assumed. Some pertinent theoretical considerations of these results are presented in the accompanying paper (4).

METHODS

The concentration of CO was measured in an infrared gas analyzer.[6] It had an analysis chamber of 60 ml. capacity which required a 200 ml. sample to flush it completely. The response of the instrument to a sudden change in CO concentration was 90 per cent complete in 0.4 second. Background noise was equivalent to from 0.0005 to 0.001 per cent CO. The calibration curve was alinear, so that the proportional precision varied with the CO concentration. Representative limits of overall error were ±0.005 per cent at 0.400 per cent CO and ±0.001 per cent at 0.010 per cent CO. The instrument was also sensitive to water vapor and carbon dioxide. Forty-seven mm. Hg of water vapor gave a response equivalent to 0.002 per cent CO; this increased to 0.010 per cent CO over a matter of months, as the detector unit aged. This response was additive to the CO deflection, so in practice either a zero correction was made for water vapor in the breath or the samples were dried with $CaCl_2$ before analysis. Five per cent CO_2 gave a response equivalent to 0.001 to 0.002 per cent CO. In experiments in which this constituted a significant error, a suitable correction was made.

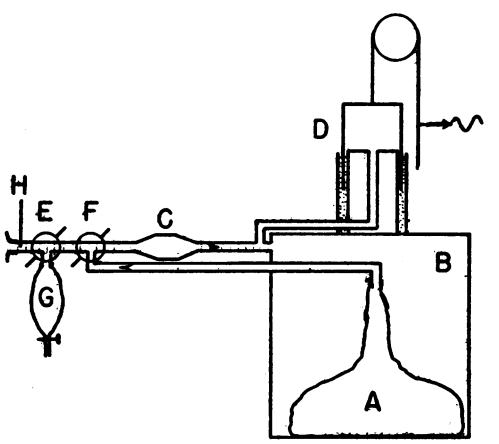

FIGURE 1

Arrangement of apparatus. (A) is a 300 litre plastic bag, (B) a 300 litre metal box, (C) a capacitance manometer resistance flow meter (Lilly), (D) a 6 litre spirometer, (E) and (F) are 2 cm. bore three way metal taps, (G) a 6 litre rubber sample bag, and (H) the inlet to the mass spectrometer sampling system. The circuit is fitted with unidirectional flow valves.

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

A CO mixture of known concentration was used to calibrate the infra red gas analyzer for each analysis.

Helium, nitrogen, oxygen, and carbon dioxide were analyzed with a single channel, continuously sampling mass spectrometer.[7] This instrument was precise to at least 1 per cent of full scale. The gas sample was drawn continuously into the spectrometer at a rate of 0.3 ml. per second. The 90 per cent response time was 0.12 second, of which 0.06 second represented the time the gas sample took to flow through the inlet system to the spectrometer. Since this inlet delay was constant, the useful 90 per cent response time was 0.06 second. The instrument was sensitive to 0.05 per cent N_2, 0.25 per cent He, 0.05 per cent O_2 and 0.04 per cent CO_2, and gave a linear output.

Respiratory flow rates were recorded with a capacitance manometer flowmeter (Lilly). The mass spectrometer and flowmeter outputs were recorded simultaneously by means of a double channel D.C. amplifier and pen motor[8] on a paper record running at either 5 or 25 mm. per second. Gas volumes were measured in either a 6 or 100 liter spirometer.

The diagram of the apparatus used is shown in Figure 1. Inspiration was made from a neoprene bag (A) contained in a sealed metal chamber (B). Expiration was made through the flowmeter (C) into the chamber (B). A 6 liter spirometer (D) recorded changes of total volume in the system. Two 2 cm. diameter three way taps (E and F) were provided. Tap E enabled the subject to breathe out the first liter of an expiration into the circuit, and to collect the remainder in a rubber bag (G) close to the mouthpiece. Tap F permitted closure of the inspiratory bag to prevent loss of the inspired gas. The inlet (H) to the mass spectrometer sampling tube was at the mouthpiece.

In an actual experiment the tubing from bag A to tap F was first flushed with the inspired gas, and the sampling bag evacuated and closed off. The inspired gas mixture was usually 0.3 per cent CO, 10 per cent He, 20 per cent O_2 and 70 per cent N_2. The seated subject, wearing a nose clip, stopped breathing at the end of a normal expiration, put the mouthpiece in his mouth and expired to his residual volume. He then rapidly inspired the desired volume of the gas mixture containing CO, held his breath for a predetermined time, and rapidly expired to residual volume, turning tap E to collect the sample after a liter or more of gas had been expired. Expiratory flow rate and He concentration were recorded simultaneously during this procedure. This discarded gas volume was considered sufficient to wash out the respiratory dead space, so that the collected sample was "alveolar gas." This sample was then analyzed for CO. Its average He concentration as a fraction of that inspired could be obtained from the records taken during the experiment. The time of breathholding was measured from the start of expiration of the sample, as indicated by the flowmeter. This experiment was repeated with the breath held for different periods of time such as 60, 10, 50, 20, 40, 30 seconds in that order,

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

[8] Brush Development Co., Cleveland, Ohio.

so that the relation between mixed expired alveolar CO concentration and time could be plotted.

Helium was added to the inspired gas mixture to provide a method of obtaining the alveolar CO concentration at time zero without collecting two gas samples as Krogh (1) did. The mixed expired alveolar CO concentration at time zero in the collected sample (F_{AEO})[9] was computed from the data by assuming that He was insoluble in blood and tissue, and that CO was not absorbed by blood or tissue to any significant extent until the inspired and residual gases were mixed. The validity of these assumptions is discussed later. The mixed expired alveolar concentration of CO at time zero could therefore be calculated as follows:

$$\overline{F_{AEO}} = \frac{\overline{F_{AE_{He}}}}{F_{I_{He}}} \times F_I \qquad (2)$$

Where: $\overline{F_{AEO}}$ = Calculated dry CO concentration of the collected sample at time zero.[10]

$\overline{F_{AE_{He}}}$ = Mixed expired alveolar He concentration dry.

F_I = Inspired CO concentration dry.

$F_{I_{He}}$ = Inspired He concentration dry.

The original alveolar concentration of CO calculated as described above varied a great deal because of differing inspired concentrations of CO and differing relations between inspired and residual gas volumes, in spite of conscious effort to maintain them constant. For convenience in plotting, all values of $\overline{F_{AEO}}$ were adjusted to an initial alveolar concentration of 0.2 per cent CO dry. That is, any value of $\overline{F_{AE}}$ was multiplied by the fraction

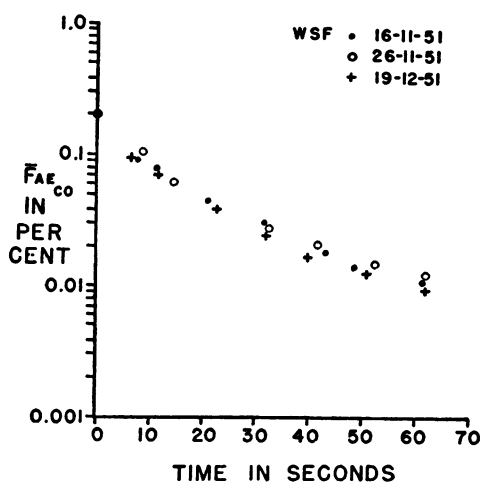

FIG. 2. REPEATED CO DISAPPEARANCE CURVES IN THE SAME SEATED SUBJECT ON DIFFERENT DAYS

Inspired gas mixture contained 20% O_2. Results typical of those on seven normal men.

[9] This terminology is discussed in the previous paper (4).

[10] The duration of delivery of the collected sample will be ignored at this point.

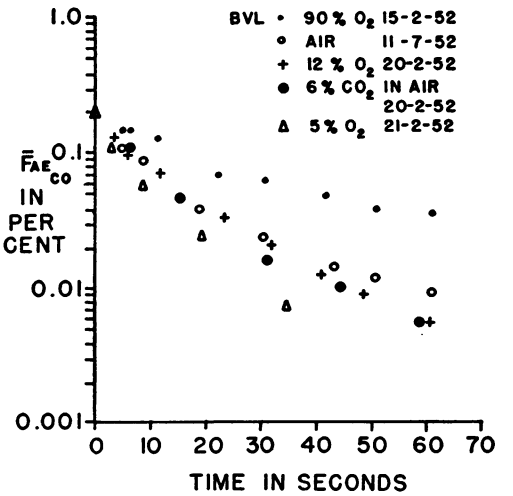

FIG. 3. THE EFFECT OF ALTERING THE O_2 AND CO_2 TENSIONS IN THE INSPIRED GAS MIXTURES UPON THE CO DISAPPEARANCE CURVE IN THE SEATED SUBJECT

Results typical of four normal men.

$\frac{0.2 \text{ per cent CO}}{\overline{F_{AEO}}}$ before it was plotted. The justification for this operation is given in Equation 5 of Reference 4.

Assuming an alveolar gas volume of 4,000 ml., a pulmonary blood flow of 6,000 ml. per minute, a volume of watery tissue exposed to the alveolar gas of 1,000 ml. (5), and a partition coefficient for He at 37° of 0.0098 (6), the He dissolved in the blood and tissues in a minute would be less than $\frac{(1{,}000 + 6{,}000)}{4{,}000} \times .0098$ or approximately 2 per cent of the He in the alveolar gas. For the purposes of the present experiments this error can be neglected, and the He can be assumed insoluble in blood and tissue.

RESULTS

Figure 2 shows three complete series of experiments on one subject on different days inspiring a CO-He mixture in air. It is clear that CO does not disappear exponentially from the alveoli during breathholding. The curvature is typical of 28 separate series of experiments on seven seated normal men 29 to 37 years of age.

The effect of varying the alveolar pO_2 was also tested because present information on the kinetics of the CO + Hb reaction predicts that lowering the alveolar O_2 tension would increase the rapidity of disappearance of CO from the lung (7). Roughton (3) has suggested that a significant capillary CO tension exists when breathing 20 per cent O_2, but that the true pulmonary diffusing capacity might be obtained in spite of this by observing the diffusing capacity at de-

creasing alveolar O_2 concentrations and extrapolating to a point where it might be expected that the capillary pCO was negligible. In order to investigate these points the effect of varying the alveolar O_2 tension on the CO uptake was studied in four normal men and typical results are shown in Figure 3. The experiments were carried out in the same manner as those described above except that, after breathing air, the subject inspired a gas mixture containing 0.3 per cent CO plus either 90 per cent O_2 and 10 per cent He; 12 per cent O_2, 78 per cent N_2 and 10 per cent He; or 5 per cent O_2, 85 per cent N_2 and 10 per cent He. As predicted (7) when alveolar oxygen concentration decreased, the rate of CO disappearance increased. This effect did not reach an obvious maximum with decreasing alveolar O_2 tensions nor was it possible to extrapolate the data to lower alveolar O_2 tensions. There was a significant difference in the CO uptake at different alveolar O_2 tensions even with breathholding for only 3 to 5 seconds.

The effect of adding 6 per cent CO_2 to the inspired mixture (6 per cent CO_2; 19 per cent O_2; 10 per cent He; approximately 0.3 per cent CO, 65 per cent N_2) is also presented in Figure 3. There is a tendency for the alveolar CO concentration after about 20 seconds to be lower when 6 per cent CO_2 is added to a given inspired mixture. This was typical of the four subjects studied.

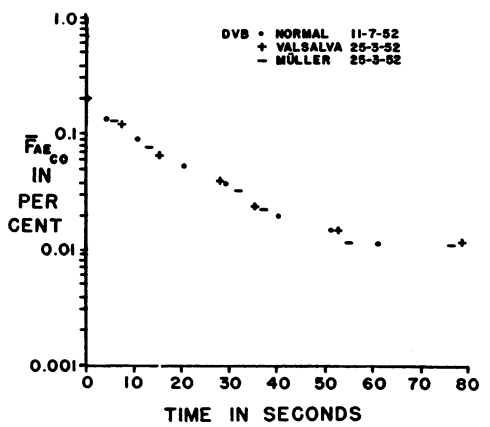

FIG. 4. THE EFFECT OF ALTERING THE INTRAPULMONIC PRESSURE DURING BREATHHOLDING ON THE CO DISAPPEARANCE CURVES IN THE SEATED SUBJECT

Intrapulmonic pressure approximately +40 mm. Hg during Valsalva; −40 mm. Hg during Müller. Results typical of two normal subjects.

Since the intrapulmonic pressure during breathholding may vary considerably, and any changes in it might affect the shape of the CO disappearance curve, the effects of extremes of intrapulmonic pressure were investigated. CO disappearance curves were obtained in two normal males, the subject maintaining voluntary positive (Valsalva) or negative (Müller) intrapulmonary pressure with the glottis closed, during the period of breathholding. The pressures so obtained were estimated to be roughly plus and minus 40 mm. Hg, respectively. These maneuvers require a high degree of cooperation, but in the trained experimenters used, it seemed reasonable to assume that the intrapulmonary pressure varied more between these two states than it would during a single experiment under the usual breathholding conditions. A typical set of disappearance curves is shown in Figure 4 along with a normal curve, the data of which are given in Table I. There is no significant difference between the data.

Since M. Krogh found that the diffusing capacity for CO varied with lung volume, the CO disappearance curve was investigated at different total lung volumes. We did not find a significant increase in pulmonary diffusing capacity with increasing lung volume. These results will form the substance of a future report.

DISCUSSION

Because the initial slope of log $\overline{F_{AE}}$ plotted against time may be two to more than five times greater than the slope between 40 and 60 seconds, and since the pulmonary diffusing capacity (D) as calculated by Krogh (1) is proportional to the slope, D can vary greatly depending on the portion of the log $\overline{F_{AE}}$ curve used in the calculation. The longer the breath is held, the less the apparent D.

Another source of error in Krogh's technique arises because of the presence of non-uniform ventilation of the lung. The two alveolar samples she collected were really parts of one expiration interrupted by a period of breathholding. It is known that the first part of any expiration (corresponding to Krogh's initial sample) contains higher concentrations of the gases that were inspired in the preceding inspiration than the later parts of the expiration (Krogh's final sample) (2). Thus Krogh's early gas sample

TABLE I *

Exp.	a Time *secs.*	b Vol. of inspired gas *L. STPD*	c $\overline{F_{AE_{O_2}}}$ % *dry*	d He dilution ratio	e $\overline{F_{AE_O}}$ (k × d) % *dry*	f $\overline{F_{AE}}$ % *dry*	g $\overline{F_{AE}}$ adjusted to 0.2% initially $\left(F \times \frac{.2}{e}\right)$ % *dry*	h F_c % *dry*	i $\overline{F_{AE}}$ corr. for blood COHb (f-h) % *dry*	j $\overline{F_{AE}}$ corr. for blood COHb and adjusted to 0.2% initially $\left(i \times \frac{.2}{e}\right)$ % *dry*
1	60.6	3.82	15.0	0.80	0.264	0.014	0.011	0.0010	0.013	0.010
2	4.6	3.70	20.3	0.75	0.247	0.146	0.120	0.0017	0.144	0.117
3	51.2	3.40	16.5	0.82	0.270	0.022	0.016	0.0025	0.020	0.015
4	10.6	3.73	18.6	0.76	0.250	0.108	0.088	0.0032	0.105	0.085
5	40.2	3.84	16.4	0.77	0.254	0.028	0.022	0.0040	0.024	0.019
6	20.6	3.72	18.2	0.82	0.270	0.068	0.051	0.0048	0.063	0.049
7	29.0	3.55	17.3	0.76	0.250	0.046	0.037	0.0055	0.040	0.033

* Inspired gas contained 0.327 per cent CO (k), 22.6 per cent O_2, about 10 per cent He and the remainder N_2, all expressed as dry gas.

Column (a) is explained elsewhere in the text. (b) is self-explanatory. (c) $F_{AE_{O_2}}$ is the mixed expired O_2 concentration in the alveolar sample (dry). (d) The He dilution ratio equals mixed He concentration (dry) of the collected expired alveolar sample/inspired He concentration (dry), both in scale deflection as measured at the mouthpiece. (e) $\overline{F_{AE_O}}$ equals the CO concentration that was in the sample initially and is computed by multiplying the inspired CO concentration (0.327 per cent) times the He dilution ratio (d). (f) Collected sample analysis in per cent CO (dry). This has been corrected for an analytical error of +0.0015 per cent CO because of presence of CO_2. (g) The sample analysis adjusted to 0.2 per cent CO (dry) initially. (h) F_c (per cent dry) is the "average" concentration of CO in the gas in equilibrium with blood in the pulmonary capillary during each experiment. The mixed venous COHb concentration was estimated immediately before and after the series of experiments using Siösteen and Sjöstrand's technique (13). Using the Haldane relationship and assuming a mean alveolar O_2 concentration of 17.4 per cent (dry) during an experiment, these values were initially 0.0013 ml. CO/ml. blood: finally 0.0134 ml. CO/ml. blood. The concentration of CO in the gas in equilibrium with the capillary blood before and after the experiments is calculated from the CO concentration in the rebreathing bag, assuming F_c is proportional to F_{cO_2}. The values are: 0.0006 per cent initially; 0.0055 per cent finally. The average increment of F_c per experiment is 0.0007 per cent CO. The average F_c in each experiment can thus be computed. (i) The mixed expired alveolar CO concentration in per cent (dry) corrected for the pressure of COHb in the capillary blood by subtracting F_c (Col. h) from $\overline{F_{AE}}$ (Col. f). (j) The mixed expired alveolar CO concentration corrected for the presence of COHb in the capillary blood is now adjusted to an initial alveolar CO concentration of 0.2 per cent (dry) by multiplying by $\frac{0.2}{\text{initial CO con.}}$ or $\frac{0.2}{\text{Col. e}}$.

Comparison of figures in Column g and Column j shows that the correction applied for the mixed venous COHb content changes the final value for $\overline{F_{AE}}$ by only a small amount.

probably had more CO in it at time zero, before any CO was absorbed, than the final sample did. This fact would increase the difference in CO concentration between the two samples and thus would increase the calculated value for D. This type of error is minimized in the present experiments by comparing the CO concentration in a given sample at any time with the initial CO concentration in *that* sample, using He as an insoluble tracer. It must be emphasized that although unevenness of ventilation can alter the absolute concentration of CO in a given fraction of the expired breath, it cannot *per se* produce a curvature of the plot of the logarithm of CO concentration against time.

A number of possible causes of the non-linearity of the semi-log plot of the mixed expired alveolar CO concentration ($\overline{F_{AE}}$) will be discussed:

1. *Systematic error in measurement.* The possible error in the measurement of time must have been less than 1 second because inspiration and expiration together took less than 2 seconds and time measurements were made in all cases from the start of inspiration to the start of expiration. Inspection of Figure 3 shows that an error of one second could not straighten the curve appreciably.

The ratio $\frac{F_{I_{He}}}{\overline{F_{AE_{He}}}}$ was found to be independent of the length of time the breath was held within limits of measurement. Thus the only analysis that could have produced the upward concavity of the log $\overline{F_{AE}}$ plot was that of $\overline{F_{AE}}$ itself.

The analytical error in $\overline{F_{AE}}$ ranged from ±2 per cent early in the disappearance curve to a maximum of ±10 per cent in the 50 second region, as stated earlier. If this ±10 per cent error is applied to the data in Figure 3, in any order whatsoever in disregard of its probably random distribution, the slope can hardly be changed more than 25 per cent in any region. Since the slope varies two to five fold over the

curve, the analytical errors alone could not produce the curvature found.

2. *Saturation of tissues with dissolved CO.* If a significant fraction of the CO absorbed during the early part of the disappearance curve were dissolved in tissues exposed to the pulmonary gases, as these tissues became saturated with CO, the total rate of CO disappearance would decrease. This phenomenon would produce a curvature upwards of the type seen in Figure 3. Assuming a total volume of tissue exposed to alveolar gas of only 1,000 ml. (5), since in this instance our interest is limited to tissue that is exposed to alveolar gas and could become saturated with CO, with a total lung gas volume of 4,000 ml. and a partition coefficient for CO of 0.02 (6), at equilibrium the tissue would contain $\frac{1{,}000 \times 0.02}{4{,}000}$ or 0.5 per cent of the CO contained in the alveolar gas. Even if the pulmonary tissue equilibrated with the *initial* alveolar gas concentration and released it at a time when the alveolar CO concentration had decreased to low levels, the increase in the alveolar CO concentration could not be more than 0.5 per cent of the initial value. Inspection of Figure 3 shows that 0.5 per cent of 0.2 per cent CO, or a 0.001 CO change in $\overline{F_{AE}}$ would not substantially change the slope of the curve.

3. *Effect of decreasing lung volume.* During breathholding, the volume of gas in the lung will decrease since CO_2 will be added at a slower rate than oxygen will be removed. This decrease in volume will tend to increase the concentration of CO in the lung gas as time proceeds. However, the total decrease in lung volume during one minute of breathholding is unlikely to exceed 5 to 10 per cent (8), which would produce a proportional increase in CO concentration. It can be seen from Figure 3 that this factor is far too small to produce the curvature observed.

4. *Changes in total alveolar pressure.* The rate of disappearance of CO from the alveolar gases is proportional to its partial pressure, so that an increase in total intrapulmonic pressure produces an increase in the rate of CO loss. If the subjects by thoracic effort produced a higher intrapulmonic pressure early in the period of breathholding than later, the exponential rate of CO disappearance would decrease with time and an upward curvature of log $\overline{F_{AE}}$ would result. According to the results shown in Figure 4, a change of 80 mm. Hg in the intrapulmonic pressure during breathholding did not alter the rate of CO disappearance noticeably. Since the subjects were trying to relax during breathholding under the usual experimental conditions, the intrapulmonic pressure would not have been expected to rise much above 22 mm. Hg (9). It has therefore been concluded that changes in intrapulmonic pressure probably did not cause the curvature seen in Figure 3.

5. *Diffusion of CO into the alveoli from the dead space.* The concentration of CO in the alveolar gas decreases markedly during the period the breath is held. There will consequently be a tendency for CO to diffuse into the alveoli from the dead space. Experiments were performed on two subjects in which several liters of the inspired gas mixture containing CO were followed without interruption by a liter of air so that the dead space was washed free of CO (10). Under these circumstances, the shape of log $\overline{F_{AE}}$ versus time was unchanged. It was therefore concluded that this factor was unimportant in producing the curvature.

6. *Absorption of CO from the inspired gas before it has mixed completely with gases already in the lungs.* Some CO may be absorbed at a higher rate from the inspired gas, rich in CO, before it is completely mixed with gas initially in the lungs. However, it seems likely that mixing of gas within an alveolus is complete within a few thousandths of a second following the completion of inspiration (11). Therefore, this factor cannot be responsible for the curvature after the first second. In addition, there are apparently no sharp discontinuities in the different curves between the 5 second point and the time zero point (Figure 3, for example). Since the latter point is calculated on the assumption of complete mixing of the inspired gas with the residual gas in the lungs before any absorption takes place, it seems reasonable to conclude that this is approximately true.

7. *Presence of significant carboxyhemoglobin in the capillary blood.* When there is COHb in the capillary blood, the alveolar CO concentration will fall to approach a value in equilibrium with it at an oxygen tension and O_2Hb saturation representing an average along the pulmonary capillary. If the presence of the COHb is ig-

nored, an apparent upward curvature of log $\overline{F_{AE}}$ will result. COHb can be present in the pulmonary capillary blood either because it is in the mixed venous blood, or because it is formed in the pulmonary capillary. In either case, the blood COHb can be taken into account by plotting log $(\overline{F_{AE}} - F_c)$ against time instead of log $\overline{F_{AE}}$, where F_c is the concentration of CO in equilibrium with the blood under average pulmonary capillary conditions. This procedure is justified by Equation *17* in the accompanying paper (4).

Considering first the possibility that there was significant COHb in the mixed venous blood, it is interesting to note that it was impossible to choose any value of F_c which would produce a linear plot of log $(\overline{F_{AE}} - F_c)$ for most of the data. Although the highest COHb concentration found in the mixed venous blood of smokers (12) could not explain an upward curvature of the degree seen in Figure 3, estimates of the mean capillary COHb concentration were made before and after a series of experiments in four normal subjects, and values for capillary COHb calculated by interpolation. In three of the subjects, F_c was estimated by the method of Siösteen and Sjöstrand (13), which consisted of a preliminary 4 minute washout of the lungs with 100 per cent O_2 followed by 4 minutes of rebreathing in a 6 liter closed circuit filled with 100 per cent O_2 and including a CO_2 absorber. The final CO concentration was considered F_c at the final O_2 concentration in the closed circuit. This value of F_c was corrected to the actual capillary O_2 tension during the breathholding experiments, by considering F_c proportional to the capillary O_2 tension. In a fourth subject, these estimates of F_c were checked by the blood COHb method of Roughton and Root (14) assuming a Haldane coefficient of 220, which agreed within less than 10 per cent.

Table I presents the data and computations of such a series of experiments in which the alveolar CO concentration was corrected for the presence of the COHb in the capillary blood. The uncorrected data (Col. g) are plotted in Figure 4 as an example of normal uncontrolled intrapulmonary pressure during breathholding. A comparison of $\overline{F_{AE}}$ uncorrected (Col. g) with $\overline{F_{AE}}$ corrected (Col. g) shows that the effect of the blood COHb concentration is small and could not be responsible for the curvature seen in Figure 4. The increase in effective CO gas concentration in equilibrium with the blood (F_c) (Col. (h) 0.0055 per cent CO) during a complete series of experiments is so small as to exert a minimal effect on the curvature of the log $\overline{F_{AE}}$ plot against time. Results were similar in the three other normal subjects. Therefore, it can be concluded that the curvature of the log $\overline{F_{AE}}$ plot is not due mainly to the presence of COHb in the mixed venous blood. However, as a precaution in all series of experiment, the individual experiments were done in the order 60, 10, 50, 20, 40, and 30 seconds to minimize any effect of an increasing mixed venous COHb concentration upon the CO disappearance curve.

There remains the possibility that sufficient COHb forms during a single passage through the alveolus to interfere with the further uptake of CO, either in the lung as a whole, or in certain alveoli. Although the data of Table I indicate that there is no marked rise in the total blood COHb, it must be remembered that large amounts of COHb could have been formed over short periods of time, which when diluted by the total blood volume appear insignificant. It is possible to make a calculation of the ratio of the blood CO tension at the end of the capillary (P_c) to the alveolar CO tension (P_A). A maximal value for the CO diffusing into the capillary equals pulmonary diffusing capacity (D in ml. gas/sec. × mm. Hg) × alveolar CO tension (P_A in mm. Hg). This is maximal because it neglects the presence of CO tension in the blood, either from COHb or from the slowness of the combination of CO with Hb. $\frac{P_A D}{Q}$ where Q is the pulmonary blood flow in ml. per sec., gives the change in COHb concentration in one passage through the lungs in ml. gas per ml. blood. The increase in the capillary CO tension due to this COHb can be computed approximately from the Haldane relation, $P_c = \frac{P_{cO_2}\text{COHb}}{210\, O_2\text{Hb}}$, where P_{cO_2} is the end capillary O_2 tension in mm. Hg and COHb and O_2Hb are the respective concentrations of the compounds in ml. gas per ml. blood. Thus $P_c = \frac{P_A D P_{cO_2}}{Q\ 210\ O_2\text{Hb}}$ and $\frac{P_c}{P_A} = \frac{D P_{cO_2}}{Q\ 210\ O_2\text{Hb}}$.

Under the most severe circumstances, while

breathing 100 per cent O_2, with D certainly no greater than 30 ml. per min. × mm. Hg or 0.5 ml. per sec. × mm. Hg, Q equal to 100 ml. per sec., P_{cO_2} equal to 650 mm. Hg, and O_2Hb equal to 0.19 ml. gas per ml. blood, $\frac{P_c}{P_A} = \frac{0.5 \times 650}{100 \times 210 \times .19} = 0.082$. Therefore, the CO tension at the arterial end of the pulmonary capillary would be 8 per cent of the alveolar CO tension. Even this large an equilibrium tension of CO in the capillary blood could not cause the upward curvature of the plot of log ($\overline{F_{AE}}$) (inspiring 90 per cent O_2) in Figure 3. Actually, 8 per cent is much too high a figure for P_c/P_A because (a) with a high alveolar O_2 concentration the slowness of the reaction of CO with Hb would decrease the effective value of D by at least one-half and (b) the average P_c/P_A along the capillary would be about one-half that at the end of the capillary. Furthermore, regardless of the absolute value of the average tension of CO resulting from an increase in COHb during passage through the alveolus, it would tend to be proportional to the alveolar CO concentration, and would not produce the curvature of Figure 3. Thus it seems possible to rule out an increase in capillary COHb during one passage through the alveoli as a cause of the failure of the mean mixed expired alveolar CO concentration to fall exponentially as regards the lung as a whole. However, there remains a more subtle possibility that in some parts of the lung, the blood flow is so slow, or the diffusing capacity so large, that a significant increase in COHb during a single passage through the alveoli does take place. Under these circumstances the rate of disappearance of alveolar CO would probably be exponential, but would be less than that of the rest of the lung. The true mixed expired alveolar CO concentration being the average of at least two exponential processes under these conditions would show an upward curvature as seen in Figure 3. This possibility cannot be completely ruled out at this time, but can hardly have been occurring in a significant part of the lung, since the measured increase in the total blood COHb is so small.

8. *Chemical action of CO on the alveolar membrane.* At the present time the movement of gases across the pulmonary membrane is considered a passive process. No evidence is available that CO interferes with diffusion by altering the diffusing characteristics of the alveolar membrane.

9. *Variation in the exponential constant during the breathholding period.* Examination of Equation *2* in the accompanying paper (4) shows that the exponential constant is $\left[\frac{-DP_bCt}{V_A}\right]$, where C is a correction factor allowing for the slowness of the rate of combination of CO with Hb. Anything that varied the total value of this expression would vary the rate of decrease of the alveolar CO concentration. The alveolar volume (V_A) was certainly constant during the experiment, as was the barometric pressure (P_b). Because the rate of combination of CO and Hb increases with a decrease in O_2 tension, the tension of CO in the capillary blood resulting from the slowness of this reaction would have decreased with time during breathholding. C would therefore have increased, that is approached unity, as the breathholding continued, which would have had the effect of increasing the rate of fall of alveolar CO concentration, and the plots of Figure 3 should have curved *downwards*. Variation in alveolar O_2 tension cannot therefore have been a cause of the upward curvature found.

There remains the possibility that the pulmonary diffusing capacity (D) decreased during the period of breathholding. It seems unlikely that the specific diffusing characteristics of the tissue composing the alveolar membrane will change greatly in a minute. However, changes in the number, length and distension of the pulmonary capillaries might occur during breathholding and would alter D. For instance, it has been reported (15) that immediately after taking a deep breath the blood flow through the lung first increases greatly, and then gradually decreases as the breath is held. D might be expected to parallel these changes in pulmonary blood flow. This would cause the decay exponent (Equation *2*) to decrease with time during the period of observation and the log $\overline{F_{AE}}$ versus time plot would curve upwards. However, to explain the degree of curvature found D would have to alter sufficiently to decrease the decay exponent by one-third to one-fifth during the 60 seconds of breathholding. Furthermore, the increased blood flow as indicated by Armitage and Arnott's (15) technique subsided after 10 seconds of breathholding while the curvature of the CO

disappearance curves continues out as far as 30 to 40 seconds. One would expect that any change in the diffusing capacity of the lung produced by a maximal inspiration acting through a mechanism such as this would be diminished if smaller and slower breaths were taken. However, in experiments on four normal subjects, inspiration was started from functional residual capacity instead of residual volume, the inspired gas volume was reduced from the usual average of 4 liters to between 600 and 900 ml., and the inspiration was made more slowly than in the case of the larger inspired volumes. The total lung volume during breathholding was the same as in the usual experiments, *i.e.*, approximately maximal. No marked difference was observed between the curves obtained under these conditions and those in which deeper breaths were taken, suggesting that the expansion of the lungs is not an important factor in producing the curvature of the log $\overline{F_{AE}}$ plot.

Several additional experiments were done on one subject to get more clearcut evidence on this point. The subject inspired almost maximally from residual volume, as in the experiments described under Methods, except that he inspired air. He held this volume for 40 seconds and then made a further gentle inspiration of approximately 80 ml. of a mixture of 88 per cent He and 12 per cent CO. This second volume (as well as the first volume) was held for 5 to 15 seconds, and then expired, an alveolar sample being collected as described before. The initial CO concentration was calculated from the He dilution as with the other experiments, the high inspired concentrations of CO giving initial alveolar CO concentrations from 0.1 to 0.2 per cent.[11] If the pulmonary diffusing capacity were decreasing with time during breathholding, or some similar process were going on, the rate of CO disappearance at 40 seconds in this experiment should have been less than the rate of CO disappearance at the start of breathholding, as demonstrated in Figure 3, since we assume that the inspiration with minimal inspiratory effort of 80 ml. into a lung which already contained more than 5 liters would not significantly alter D. However, the actual disappearance rate in these experiments was considerably *greater* than the CO disappearance rate at the start of breathholding breathing air. This is reasonable because of the fall in alveolar O_2 tension which would cause a rise in the CO uptake (7), but is incompatible with the idea that a large decrease in D occurs during the period of breathholding. The possibility that the increase in alveolar CO_2 concentration and/or the decrease in alveolar O_2 concentration with breathholding might produce a gradual decrease in D by a direct chemical effect on the pulmonary vessels, is minimized by the experiment just described. Thus while D might decrease by one-half or one-fifth during the 60-minute period of breathholding, it appears very unlikely.

In summary, up to this point a number of possible explanations for the upward curvature of the plot of log $\overline{F_{AE}}$ against time have been presented and it has been concluded that no one of them is acceptable as a major cause of the effect on the basis of present knowledge. If all the mechanisms enumerated above operated together to produce an upward curvature of the log $\overline{F_{AE}}$ plot, the points would still not fall on a line.

10. *The existence of significantly different diffusing phases in the lung.* As had been previously demonstrated (4) the logarithm of mixed expired alveolar CO concentration ($\overline{F_{AE}}$) plotted against the time will not be a straight line but will curve upwards unless all the exponential constants $\left[\frac{-D_iPbC_i}{V_{Ai}}\right]$ are equal in all phases. $\frac{D_i}{V_{Ai}}$, the ratio between diffusing capacity and volume in an alveolus, is probably related to the number and degree of distension of the pulmonary capillaries. Since the number of capillaries per alveolus varies throughout the lung (17) and the hydrostatic (distending) component of capillary pressure is greater at the base of the lung than at the apex, it would be remarkable if the exponential constants were equal in all parts of the lung. A four- or five-fold variation in $\frac{D}{V_A}$ throughout the lung is not unreasonable and at the present time this is considered the most likely single cause of the curvature of the plot of log $\overline{F_{AE}}$ against time.

[11] Inspired gas appears in the expired alveolar gas after inspirations of as little as 40 ml. (16).

If $\frac{D}{V_A}$ varies throughout the lung, the estimation of the total D of the lung demands precise knowledge of the V_A associated with each D. In other words, the lung would have to be broken up into discrete "diffusing phases." Although the $\overline{F_{AE}}$ curves can be separated into two exponential decay terms, and two "diffusion phases" can be computed, it seems more likely at the present time that there is a statistical distribution of $\frac{D}{V_A}$ throughout the normal lung. This makes the estimation of the total lung diffusing capacity very formidable. A question might even be raised as to the practical value of total D, since it is independent of the ventilation, and a poorly ventilated region of the lung with a large D would not be so effective as would appear from total D alone.

In the preceding paper (4) it was pointed out that if the ratio of diffusing capacity to effective ventilation $\left(\frac{D}{V_T - V_D}\right)$ throughout the lung is not constant, any "steady state" method of estimating the true total diffusing capacity of the lung will be in error. However, $\frac{D}{V_A}$ can vary throughout the lung without $\frac{D}{V_T - V_D}$ of necessity varying.

At present the difficulties of measuring the true total diffusing capacity in the presence of varying ratios of $\frac{D}{V_A}$ and/or $\frac{D}{V_T - V_D}$ are so formidable that methods based on the measurement of an overall or "apparent" total D will have to continue to be used. However, the fact that the "apparent" total D so measured is influenced by factors other than the diffusing capacity of the lung, must be remembered when differences in this measurement are interpreted in terms of pathological change.

The uptake of chemically inert gases from the lungs has been recently discussed by Kety (18). The relationships presented by him can be expressed in terms of different perfusion phases, that is phases with different blood flow per unit alveolar volume, or per unit alveolar ventilation. It may be found necessary to use this approach to reconcile experiment and theory.

SUMMARY

1. The disappearance of CO from the alveolar gas of the lung during breathholding has been investigated in seven normal subjects.

2. The alveolar CO concentration did not fall exponentially with time as had been assumed by previous workers.

3. The most likely single explanation of this phenomenon is that the diffusing capacity per unit gas volume varies throughout the lung.

4. This finding is relevant to the consideration of the validity of present methods of measuring the diffusing capacity of the lungs.

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REFERENCES

1. Krogh, M., Diffusion of gases through the lungs of man. J. Physiol., 1915, **49**, 271.
2. Fowler, W. S., Lung function studies. III. Uneven pulmonary ventilation in normal subjects and in patients with pulmonary disease. J. Applied Physiol., 1949, **2**, 283.
3. Roughton, F. J. W., The average time spent by the blood in the human lung capillary and its relation to the rates of CO uptake and elimination in man. Am. J. Physiol., 1945, **143**, 621.
4. 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.
5. Jackson, C. M., Ed., Morris' Human Anatomy. 9th ed., Philadelphia, Pa., The Blakiston Company, 1933, 1481 p.
6. Lawrence, J. H., Loomis, W. F., Tobias, C. A., and Turpin, F. H., Preliminary observations on the narcotic effect of xenon with a review of values for solubilities of gases in water and oils. J. Physiol., 1946, **105**, 197.
7. Roughton, F. J. W., The kinetics of the reaction $CO + O_2Hb = O_2 + COHb$ in human blood at body temperature. Am. J. Physiol., 1945, **143**, 609.
8. Otis, A. B., Rahn, H., and Fenn, W. O., Alveolar gas changes during breath holding. Am. J. Physiol., 1948, **152**, 674.
9. Rahn, H., Otis, A. B., Chadwick, L. E., and Fenn, W. O., The pressure-volume diagram of the thorax and lung. Am. J. Physiol., 1946, **146**, 161.

10. Fowler, W. S., Lung function studies. II. The respiratory dead space. Am. J. Physiol., 1948, 154, 405.
11. Rauwerda, P. E., Unequal ventilation of different parts of the lung and the determination. Gröningen University, 1946.
12. Forbes, W. H., Sargent, F., and Roughton, F. J. W., The rate of carbon monoxide uptake by normal men. Am. J. Physiol., 1945, 143, 594.
13. Siösteen, S. M., and Sjöstrand, T., A method for the determination of low concentrations of CO in the blood and the relation between the CO concentrations in the blood and that in the alveolar air. Acta physiol. Skandinav., 1951, 22, 129.
14. Roughton, F. J. W., and Root, W. S., The estimation of small amounts of carbon monoxide in blood. J. Biol. Chem., 1945, 160, 123.
15. Armitage, G. H., and Arnott, W. M., Effect of voluntary hyperpnoea on pulmonary blood flow. J. Physiol., 1949, 109, 64.
16. Briscoe, W. A., Forster, R. E., and Comroe, J. H., Jr., Alveolar ventilation with very small tidal volumes. To be published.
17. Miller, W. S., The Lung. Springfield, Ill., Charles C Thomas, 1937, 209 p.
18. Kety, S. S., The theory and applications of the exchange of inert gas at the lungs and tissues. Pharmacol. Rev., 1951, 3, 1.