J. Physiol. (1959) 146, 572–582

THE EFFECT OF AGE, BODY SIZE AND LUNG VOLUME CHANGE ON ALVEOLAR-CAPILLARY PERMEABILITY AND DIFFUSING CAPACITY IN MAN

BY MARGARET W. McGRATH AND M. L. THOMSON

From the Department of Applied Physiology, London School of Hygiene and Tropical Medicine, London, W.C. 1

(*Received* 8 *January* 1959)

The published figures for diffusing capacity for carbon monoxide (D_{CO}) in normal human lungs show a wide scatter even for the same method. Minimum, maximum and mean values for six or more normal, resting, seated subjects by the single-breath D_{CO} method have been recently reported as follows: 11·0, 37·5, 24·0 (Ogilvie, Forster, Blakemore & Morton, 1957); 12·0, 22·0, 16·8 (Bates & Pearce, 1956); 18·3, 41·9, 27·9 (Lewis, Lin, Noe & Komisaruk, 1958); 14·5, 30·1, 23·9 (Curtis, Bauer, Loomans & Rasmussen, 1958); 21·5, 37·3, 30·2 (Marks, Cugell, Cadigan & Gaensler, 1957); 14·4, 33·6, 24·8 (Shephard, 1958); 18·0, 39·0, 30·0 (Forster, Roughton, Cander, Briscoe & Kreuzer, 1957). This variation is to some extent due to errors of method and minor differences in technique. Since, however, D_{CO} estimates the capacity of the alveolar-capillary membrane of the lung as a whole to transfer CO, differences in area of membrane between persons may account for a large proportion of the total variation.

Few authors have made any attempt to allow for body size; some have corrected by expressing D_{CO} values per unit surface area (e.g. Marks *et al.* 1957). The true graph, however, is almost certainly curvilinear over the full range of surface area and therefore greater accuracy can be obtained by applying a regression equation. This has been done by Ogilvie *et al.* (1957) whose equation, discussed below, is supported by published values which have been reviewed by Forster (1957).

The effect of age on D_{CO} has also been reviewed by Forster (1957) who found the evidence inconclusive, although Cohn, Carroll, Armstrong, Shephard & Riley (1954) had reported a significant relationship between age, height and maximal D_{O_2}.

Evidence is presented in this paper that D_{CO} depends on age as well as on body size, but that the permeability, '*k*' of Krogh (1915), which also depends on age, appears to be independent of body size. On this account, and because it is more reproducible and more easily measured, it appears to be a superior index to D_{CO}.

In addition to the wide variation for the same method, most authors (Bates & Pearce, 1956; Marks *et al.* 1957; Forster *et al.* 1957; Shephard, 1958), have reported higher values, ranging from 12 to 62 % more for breath-holding than for steady-state D_{CO}, for the same subjects. With the aim, among others of explaining this disagreement, the effect was investigated of changes in lung volume on k and on D_{CO}.

METHODS

The permeability and diffusing capacity were measured in thirty-nine normal male persons by the single-breath method of Krogh (1915) using a portable box-bag apparatus (Thomson, 1958). The accuracy of this apparatus appears to be at least as great as for non-portable forms.

The single-breath D_{co} method and the instruments used for gas analysis have been described in full by Ogilvie *et al.* (1957). Briefly, the subject exhales fully into the box and then inspires the test-gas mixture (He 14; CO 0·28; O_2 20; N_2 65·72 %) from the inspirate bag. The breath is then held for 10 sec and a rapid deep expiration is made, of which the first 750 ml. is discarded into the box and the remainder, the alveolar sample, is collected in the expirate bag. Analysis of expirate and inspirate is then made using a katharometer for He and an infra-red analyser for CO.

The katharometer is sensitive to CO_2, which must therefore be absorbed before He determination. The CO_2-free gas then passes through the infra-red analyser. No correction is therefore required when calculating k since the correction factor appears in numerator and denominator (equation (1) below). In calculating D_{co} a correction factor of 5 % has been allowed for oxygen consumption after correcting the inspired volume to S.T.P.D. It should also be noted that calibration of the instruments is not critical when, as in these calculations, ratios and not absolute values are required. In all but three subjects the tests were made in duplicate and repeated if duplicates differed by more than 10 %. The subjects were invariably seated in this and the following trials.

In eight persons we ascertained the effect on k and D_{co} of altering alveolar volume (V_A) over the widest range possible, i.e. from total lung capacity to residual volume (RV) + 1200 ml., comprising 750 ml. required to wash out dead space and 450 ml. for analysis. Determinations were carried out in duplicate on successive days, as in the first study above, but according to a prearranged plan where V_A was randomized with time. On the rare occasions when more than two determinations were made on one day an interval of several hours separated the additional measurements, to allow blood carboxyhaemoglobin to return to within the normal range.

Krogh's permeability (k) is the time constant of the exponential decay of CO concentration; during breath-holding.

$$F_{E_{CO}} = F_{IN_{CO}} . e^{-kt}$$

from which

$$k = \frac{1}{t} . \ln \frac{F_{IN_{CO}}}{F_{E_{CO}}} = \frac{1}{t} . \ln \frac{F_{E_{He}} . F_{I_{CO}}}{F_{I_{He}} . F_{E_{CO}}} \text{ min}^{-1}:$$

where $F_{IN_{CO}}$ = initial concentration of CO in the lungs after dilution but before absorption ; $F_{I_{He}}$, $H_{E_{He}}$, $F_{I_{CO}}$, $F_{E_{CO}}$ are respectively concentrations of He and CO in inspirate and expirate; t = time in minutes from beginning of inspiration to time at which expired gas sample was collected. The diffusing capacity was then found from

$$D'_{co} = \frac{V'_A . k}{B.P. - 47} \text{ ml./min} \times \text{mm Hg}: \quad (2)$$

where $B.P.$ = barometric pressure in mm Hg; V'_A = lung volume in millilitres derived from a single-breath He dilution technique, which estimates the volume reached by CO during the diffusion process ($V'_A = V . F_{I_{He}}/F_{E_{He}}$); V is the inspired volume corrected to S.T.P.D. and for O_2 consumption.

It has been recommended (Forster, Cohn, Briscoe, Blakemore & Riley, 1955) that alveolar volume (V_A) used in calculating D_{co} should be taken as $V + RV$ where RV is the residual volume determined by a multibreath method. (Strictly, the anatomical dead space should be subtracted from $V + RV$ to give V_A).

Although in six subjects RV has been determined by a closed-circuit helium-dilution method (Bates & Christie, 1950), the single-breath values have been used throughout this paper and have, therefore, been denoted V'_A, RV' and D'_{co}.

RESULTS

In Table 1 the physical characteristics of thirty-nine normal male subjects are listed. The values given for V'_A, RV', k and D'_{co} are the mean of two or more measurements in all but three subjects. In six subjects (numbered as in Table 1) the following values (litres) for RV were found by the multibreath method, nos. (3) 1·40; (15) 1·62, 1·34, (mean 1·48); (16) 1·54; (20) 1·72; (25) 2·95, 2·40, (mean 2·68); (35) 2·07. As expected there is close agreement (within 8%) between V_A and V'_A (Table 1) in these normal subjects. There is no reason to believe that any conclusion reached in this paper would have been altered by using multibreath values.

Multiple regression analysis of the relationship between D'_{co} and age and body surface area (SA) gave a highly significant result (variance ratio = 34·89, degrees of freedom 2 and 36)

$$D'_{co} = -0{\cdot}289 \times \text{Age} + 24{\cdot}246 \times \text{SA} - 3{\cdot}405 \quad (3)$$

The coefficients of both age ($t = 6{\cdot}342$, $P < 0{\cdot}001$) and SA ($t = 4{\cdot}307$, $P < 0{\cdot}001$) terms contributed to the significance of the results. In Fig. 1 values of D'_{co} have been plotted against age, together with the regression lines corresponding to equal increments of SA, in an attempt to illustrate the inter-relationship between the three quantities.

The effect of age and of V'_A on k was simultaneously examined and was also found to be highly significant (variance ratio = 24·00, degrees of freedom 2 and 36) $k = -0{\cdot}038 \times \text{Age} - 0{\cdot}035 \times V'_A + 5{\cdot}986$. However, this was entirely due to the k-versus-age correlation ($t = 6{\cdot}709$, $P < 0{\cdot}001$); the coefficient of V'_A did not differ significantly from zero ($t = 0{\cdot}272$, $P > 0{\cdot}8$). Thus k was independent of V'_A where V'_A (as in this method) approximated to total lung capacity. When k was corrected for age and then plotted against SA, there was no evidence of any relationship. The regression of k on age then becomes

$$k = -0{\cdot}038 \times \text{Age} + 5{\cdot}780. \quad (4)$$

In Fig. 2 values of k have been plotted against age and the regression line given by equation (4) drawn through the points. (Distinction is made in this figure and in Fig. 1 between smokers and non-smokers. There is no obvious effect of smoking.) In normal adults k may therefore be predicted from equation (4) or D'_{co} from equation (3). We have calculated the residual variation about the regression in both equations and found the coefficients of variation to be 18·3% for D'_{co} and 14·9% for k. Thus the predictive accuracy is greater for k than for D'_{co}.

TABLE 1. Lung function measurements and physical characteristics of thirty-nine male subjects

Subject	Age (yr)	Weight (kg)	Height (cm)	SA (m^2)	Vital capacity (ml.)	V'_A (ml.)	RV' (ml.)	Mean k (min^{-1})	Mean D'_{CO} (ml./min × mm Hg)
1	15	44·5	155	1·40	2750	3800	1080	5·14	24·90
2	15	57·0	158	1·58	3250	3950	780	4·31	21·70
3	17	75·0	180	1·84	4280	5520	1360	4·25	30·35
4	17	86·5	174	1·98	4400	5360	1060	5·40	37·20
5	17	75·5	170	1·88	4800	5310	640	5·57	41·50
6	17	65·0	168	1·74	4600	5430	980	4·96	37·80
7	18	79·0	186	2·03	4900	6040	780	5·50	42·80
8	18	69·5	178	1·87	4800	5620	1260	5·89	42·40
9	22	66·0	178	1·83	4200	5010	900	6·34	40·70
10	22	64·5	177	1·80	4900	6330	1530	5·20	42·40
11	23	75·5	186	2·00	5070	6680	1650	5·56	47·00
12	24	78·0	174	1·93	4950	5700	1480	4·66	34·30
13	27	66·0	177	1·82	4250	4820	840	4·63	28·60
14	28	72·5	177	1·88	3900	5050	1220	4·78	31·10
15	30	79·0	177	1·96	4180	5460	1430	3·75	26·80
16	32	72·5	160	1·76	3450	4600	1170	4·44	26·40
17	32	68·0	175	1·83	4100	5270	1320	4·96	33·45
18	35	70·5	175	1·86	3750	4660	1340	4·05	26·40
19	36	—	—	1·70	4050*	5820*	1770*	4·11*	26·80*
20	38	93·0	170	2·04	3500	4730	1450	4·52	27·86
21	38	71·5	188	1·98	4700	6840	2290	5·24	45·90
22	40	59·0	177	1·74	3550	5830	2400	3·20	23·70
23	43	66·0	177	1·82	3650	5260	1680	3·27	22·00
24	44	79·0	178	1·98	4300	5630	1480	4·25	33·61
25	49	71·0	184	1·94	4950	6880	2230	3·39	32·50
26	51	58·5	165	1·65	3650	4780	1200	3·66	22·40
27	53	72·0	170	1·83	4300	5840	1840	4·26	32·33
28	57	68·0	164	1·74	2800	3880	1270	4·38	22·20
29	58	49·0	173	1·58	3150	4270	1370	3·47	18·90
30	60	52·5	172	1·62	3600	4840	1700	2·70	17·25
31	62	70·0	174	1·86	2450*	3960*	1510*	4·15*	20·80
32	63	88·5	183	2·07	2800*	4540*	1760*	3·20*	18·83*
33	63	77·0	170	1·88	3800	6260	2580	2·89	24·35
34	65	82·0	184	2·05	3150	4540	2120	3·65	23·68
35	65	64·5	170	1·75	3350	5260	1970	4·15	28·25
36	65	54·5	158	1·54	2400	3740	1820	4·25	20·77
37	66	46·0	165	1·48	3530	5210	1850	2·26	15·10
38	72	54·0	173	1·64	2550	4440	1920	3·07	17·34
39	75	74·5	165	1·83	2500	4210	1700	3·20	16·90
Mean	40·3			1·81	3827	5161	1506	4·27	28·90

The forms V'_A (mean alveolar volume) RV' (mean residual volume) and D'_{CO} (diffusing capacity) are used to indicate that these values are based on the method of single-breath He dilution.

* Single observations.

The effect on k and D'_{co} of changing V'_A

The above results have been obtained for different subjects, measured at maximum V'_A. Figure 3 shows the effect on k and D'_{co} of deliberately changing

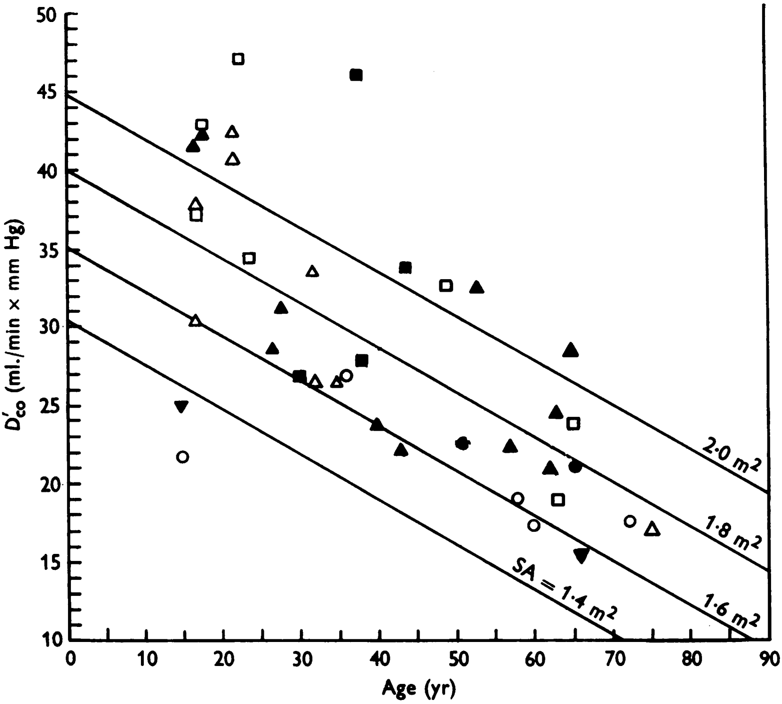

Fig. 1. Relationship between diffusing capacity, age and body surface area for thirty-nine male subjects. SA, 2·1–1·9 m², □; 1·9—1·7 m², △; 1·7–1·5 m², ○; 1·5–1·3 m², ▽. Solid signs indicate smokers.

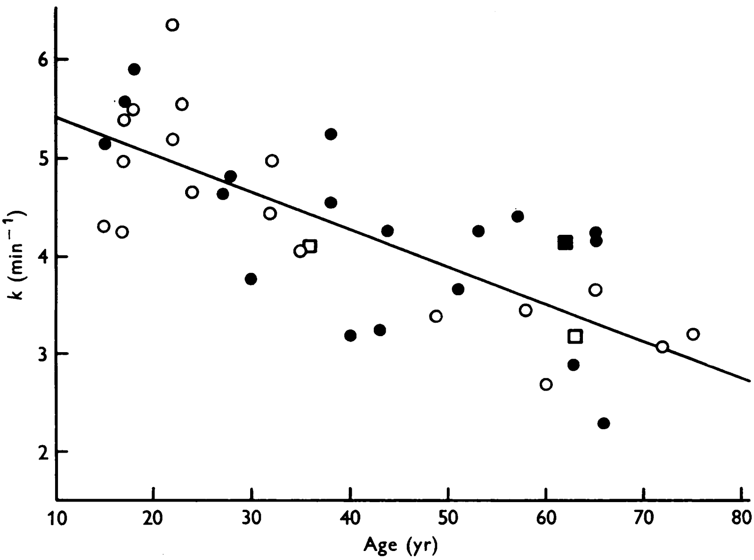

Fig. 2. Relationship between permeability (k) and age for thirty-nine male subjects. Single observations, □; mean of two or more observations, ○. Solid signs indicate smokers.

V'_A over the widest possible range for each of eight normal subjects. In most cases the graphs appear to be divisible into two portions at a point v in the neighbourhood of 5 l. > V'_A > 4 l. Krogh (1915), Bøje (1933) and Wilson, Evans, Johnson & Dempsey (1954) postulated a biphasic graph of this kind,

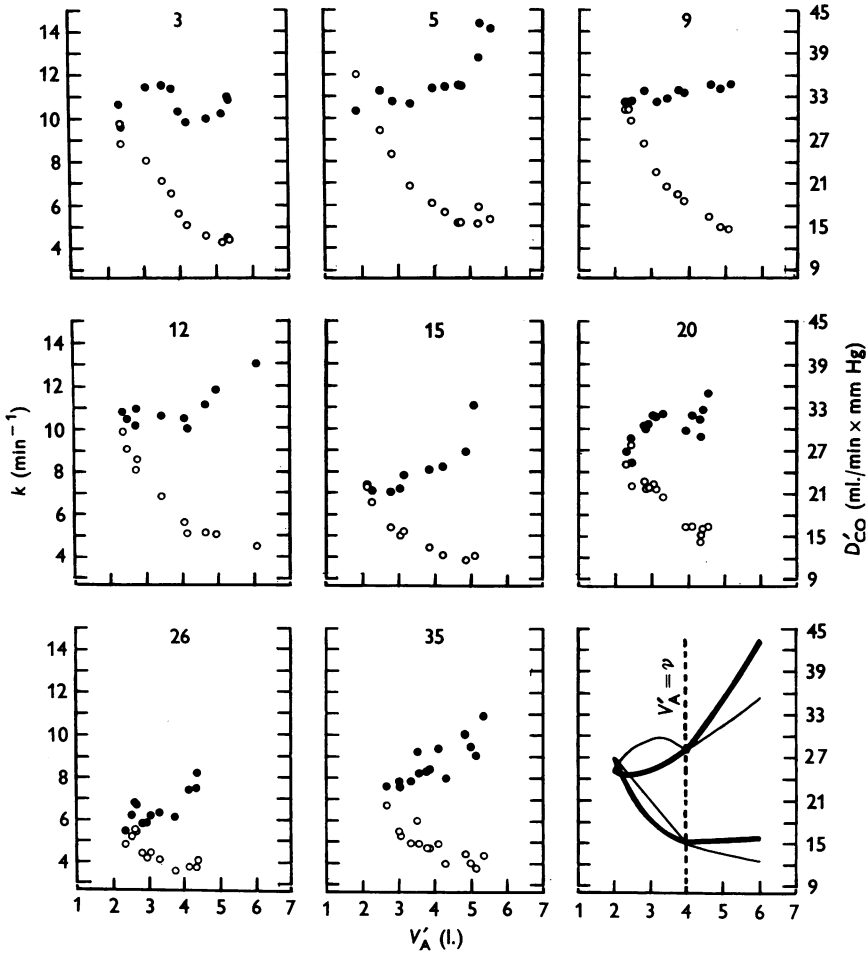

Fig. 3. The effect of changing lung volume on k (open circles) and D'_{co} (solid circles) in eight subjects (numbered as in Table 1). Lower right, interpretatory diagram.

and although we have not been able to show that the two parts differ significantly from each other, their work, together with the apparent recurrence of this feature in six out of the eight individuals in this group, strongly suggests that they do.

At present the basic form, or forms, of these graphs cannot be decided with certainty. Two graphs which may represent the true (k, V'_A) relationship have been drawn in Fig. 3 (lower right) together with the corresponding (D'_{co}, V'_A), graphs $(D'_{co} = k\ V'_A/713)$. There is theoretical justification, discussed below, for the heavy-line graph which, to the left of v, has the reciprocal form $k-3 = 6/(V'_A - 1)$, and to the right of v, the form $k-3 = 1{\cdot}38\ (V'_A - 1)^{\frac{1}{2}}$.

The linear graph $k = -1{\cdot}5V'_A + 12$, in the first phase, produces the somewhat unexpected inverted U-shaped D'_{co} graph, similar to that found in nos. 3 and 20 of Fig. 3. The reciprocal form has been prolonged to the right of v: this appears to fit best the graphs of subject no. 9 in this range.

From a practical point of view measurements of k will give best reproducibility at maximum V'_A; a slight departure from maximum should not introduce serious error.

The *form* of the (D'_{co}, V'_A) graphs is more variable than that of the (k, V'_A) graphs because the conversion involves the direct factor V'_A which is always greater than unity. The curve to the left of v generally rises with V'_A; that to the right of v rises more rapidly. By contrast with k, therefore, dependence of D_{co} measurements on the achievement of a maximum inspiration is critical. The general form is in agreement with Shephard's (1958) results in two subjects.

The scatter about the mean value of points within 1 l. of the maximum V'_A attained for each subject has been calculated for k and D'_{co} so as to compare the effect on the accuracy of measurement of these indices of failing to secure maximum inspiration. Mean coefficients of variation for eight subjects were 4·6% and 8·6% for k and D'_{co} respectively. Although the number of points was small these were, without exception, higher for D'_{co} than for k and the difference was, therefore, highly significant.

The eight subjects have been arranged in Fig. 3 in order of increasing age from no. 3 to no. 35. Effects which appear to be attributable to age are, diminution of volume range, a decline in the reproducibility of the results and, as has already been shown in the group of thirty-nine subjects, a fall in k at maximum V'_A.

Marshall (1958) showed in one subject that D_{co} by single-breath method at V_A = total lung capacity was higher than at V'_A = functional residual capacity and concluded that the difference between steady-state and single-breath D_{co} was due to the different lung volumes at which the tests were carried out. The mean D'_{co} of our eight subjects at total lung capacity was 34·5 and at estimated functional residual capacity was 26·3 ml./min × mm Hg. The mean increase (31%) is close enough to that of the values reported above in the introduction (39%) to confirm this explanation.

DISCUSSION

There is fairly good agreement between the rise of D_{CO} with surface area given here and that given by Ogilvie *et al.* (1957), where they predict D_{CO} from surface area only

$$D_{CO} = 18{\cdot}85 \times SA - 6{\cdot}8.$$

The regression of D_{CO} on SA from our results becomes

$$D_{CO} = 27{\cdot}12 \times SA - 20{\cdot}26.$$

The agreement is better if the results of three children (aged 8, 10 and 10) in the values of Ogilvie *et al.* (1957) are excluded.

Cohn *et al.* (1954) found a significant effect of age and height on maximal (exercise) diffusing capacity for oxygen,

$$D_{O_2} = 0{\cdot}67 \times \text{Height} - 0{\cdot}55 \times \text{Age} - 40{\cdot}9. \quad (5)$$

Since the membrane offers only about half the total resistance, as has been shown by Roughton (1945) and Roughton & Forster (1957), the factor $D_{O_2} = 1{\cdot}23\ D_{CO}$ should not be used, and on this and other grounds the coefficients in equation (5) would not be expected to agree closely with those reported in this study in equation (3).

Estimating diffusing capacity of the diseased lung

On one point all authors seem to agree, that multibreath and not single-breath V_A should be used in calculating breath-holding D_{CO}. The rationale for this is not at first sight clear. Alveolar volume by multibreath method will exceed that obtained by single-breath He dilution by a quantity which will depend on the uniformity of ventilation. For simplicity this may be visualized as a separate volume X. The membrane of the extra volume X is available for diffusion if ventilation and perfusion could be improved and is probably of some respiratory value, even with the existing ventilatory defect, under the more natural, steady-state conditions. The existing practice would make allowance for this membrane as though its permeability were equal to that of the well-ventilated space, i.e. the measured k. (Incidentally the volume of the anatomical dead space has been similarly credited in the modified method (Forster *et al.* 1955; Ogilvie *et al.* 1957)). Whether this is so will depend on the nature of the abnormality. Thus D_{CO} based on $V'_A + X$ is likely to be a maximum and on V'_A a minimum.

In the same circumstances k would estimate a mean permeability per unit area of the well-ventilated space V'_A. This might well be a more useful index than D_{CO} in the abnormal lung. If, however, the total capacity to transfer gas is required, the clinician may use, for calculating D_{CO}, V'_A together with that fraction of X which is most applicable having regard to the nature of the disease. In bronchospastic disease $V'_A + X$ would yield the best estimate of D_{CO}; in emphysema this would not necessarily be true.

Cause of the fall in k with age

A change in specific permeability of the membrane is only one of the factors which may be causally related to the fall in k with age. The cause may be in the blood component of resistance; it may be a reduction in area of blood-gas membrane per unit volume due to a fall in the number or diameter of functioning capillaries, or coalescence of a number of alveoli into one chamber with loss of diffusing surface. Increase in membrane thickness would have the same effect.

Changes in k within individuals at different lung volumes

The reciprocal phase. To the left of v (Fig. 3) the relationship between k and V'_A appears to be reciprocal in the majority of cases, as suggested above. This will be so if PA/H is constant. The specific permeability of the membrane (P) is unlikely to alter over the test period and, since the order of tests was randomized, any such effect would have been eliminated. The consensus of opinion (Krogh, 1915; Macklin & Macklin, 1944; Wilson *et al.* 1954) is that over this range of V'_A the alveoli are collapsing with their sides falling together, and, if this is true, A/H (area/thickness) would be constant. Again, A/H would be constant if the intercapillary areas shrank leaving the area of blood-alveolar membrane unchanged.

Since the (k, V'_A) curves appear to be asymptotic at positive k and V'_A values, the reciprocal equation would then have the following general form

$$k - C_2 = \frac{PA}{H(V_A - C_1)}$$

The thick line in Fig. 3 (lower right) to the left of v is a specific case of this equation. C_1 is an irreducible lung volume which seems in most cases to be about one litre; C_2 is a portion of k which is constant and associated with the volume C_1. The occurrence of these minima could be explained if, in addition to the expansible portion of RV, there was a relatively inexpansible respiratory part of volume C_1 of which the k was constant and equal to C_2. The diffusing capacity $(k - C_2)(V_A - C_1)$ of the expansible lung would then be constant and equal to PA/H over the reciprocal phase, provided such variables as exercise and posture were controlled.

The second phase. Krogh (1915) suggested that k remained constant above mid capacity. The results presented here confirm this but suggest the commencement of a second phase at a value of V'_A between 4 and 5 l., a volume considerably above mid capacity. The present results indicate the dependence, in this second phase, of k on A and H such as would be the case when the alveolar walls were stretching, as opposed to folding, which was characteristic of the first phase. It can be shown that when a spherical alveolus expands in such a way that the volume of the investing membrane remains constant, the reduction in thickness more than compensates for the decrease in k associated with decrease in area/unit volume. The net result is that k increases approximately with the radius (r), or the cube root of the volume, where membrane thickness is small compared with the radius. Thus the (k, V'_A) relationship for lung volumes greater than v has been represented in Fig. 3 by one of the family of curves

$$k - C_2 = C_3(V_A - C_1)^{\frac{1}{3}} \qquad (6)$$

since $r \propto (V_A - C_1)^{\frac{1}{3}}$. Here C_3 is a constant equal to $PA/H\ (v - C_1)^{\frac{2}{3}}$, found by substituting in (6) the k, V_A values common to both curve phases, i.e. at $V_A = v$.

It should be pointed out that k, like D_{CO}, depends on gas uptake in the blood as well as on transfer across the membrane. The above discussion is limited to membrane effects. In general the investigation supports the theory that in any individual there is a specific lung volume (about 4–5 l. for male adults) below which the alveolar membrane collapses, its physical condition remaining unaltered, and above which the membrane stretches.

SUMMARY

1. In thirty-nine normal males aged 15–75 there was a highly significant fall in alveolar-capillary permeability with age ($k_{CO} = -0{\cdot}038$ Age $+ 5{\cdot}8$). A highly significant multiple regression was also obtained between age, surface area and D_{CO}, based on single-breath helium-dilution lung capacity determination ($D_{CO} = -0{\cdot}29$ Age $+ 24$ SA $- 3{\cdot}4$). Even when corrected for surface area the predictive accuracy of D_{CO} was less than that of k.

2. In eight subjects at lung volumes (V_A) up to 1 l. from maximum the (k_{CO}, V_A) relationship appears to be reciprocal, consistent with constant area, thickness and specific permeability of the membrane.

3. Within 1 l. of maximum k_{CO} was relatively independent of V_A, as would occur if the membrane were stretching: by contrast, D_{CO} was critically dependent on V_A over this range. This adversely affects the precision of D_{CO} determination. In calculating D_{CO}, V_A appears as a direct multiplier and therefore the (D_{CO}, V_A) relationship shows a more variable form than does (k_{CO}, V_A).

4. In addition to being independent of body size and relatively reproducible at maximum V_A, the use of k has the advantage that it avoids the measurement of lung volume with the laborious corrections for temperature, pressure and oxygen consumption. It is concluded that k is a better index than D_{CO}.

We are very grateful to Professor G. P. Crowden and to Dr P. Armitage for advice, to the staff of the School who acted as subjects, and to the University of London Central Research Fund for a grant for apparatus.

REFERENCES

BATES, D. V. & CHRISTIE, R. V. (1950). Intrapulmonary mixing of helium in health and in emphysema. *Clin. Sci.* **9**, 17–27.

BATES, D. V. & PEARCE, J. F. (1956). The pulmonary diffusing capacity; a comparison of methods of measurement and a study of the effect of body position. *J. Physiol.* **132**, 232–238.

BØJE, O. (1933). Über die Grosse der Lungen diffusion des Menschen während Ruhe und körperliche Arbeit. *Arbeitsphysiologie*, **7**, 157–166.

COHN, J. E., CARROLL, D. G., ARMSTRONG, B. W., SHEPARD, R. H. & RILEY, R. L. (1954). Maximal diffusing capacity of the lung in normal male subjects of different ages. *J. appl. Physiol.* **6**, 588–597.

CURTIS, J. K., BAUER, H., LOOMANS, S. & RASMUSSEN, H. K. (1958). Pulmonary diffusing capacity studies 2. Clinical results using a modified carbon monoxide breathholding technique. *Amer. J. med. Sci.* **236**, 57–64.

FORSTER, R. E. (1957). Exchange of gases between alveolar air and pulmonary capillary blood: pulmonary diffusing capacity. *Physiol. Rev.* **37**, 391–452.

FORSTER, R. E., COHN, J. E., BRISCOE, W. A., BLAKEMORE, W. S. & RILEY, R. L. (1955). A modification of the Krogh carbon monoxide breath holding technique for estimating the diffusing capacity of the lung; a comparison with three other methods. *J. clin. Invest.* **34**, 1417–1426.

FORSTER, R. E., ROUGHTON, F. J. W., CANDER, L., BRISCOE, W. A. & KREUZER, F. (1957). Apparent pulmonary diffusing capacity for CO at varying alveolar O_2 tensions. *J. appl. Physiol.* **11**, 277–289.

KROGH, M. (1915). The diffusion of gases through the lungs of man. *J. Physiol.* **49**, 271–300.

LEWIS, B. M., LIN, T., NOE, F. E. & KOMISARUK, R. (1958). The measurement of pulmonary capillary blood volume and pulmonary membrane diffusing capacity in normal subjects; the effects of exercise and position. *J. clin. Invest.* **37**, 1061–1070.

MACKLIN, W. H. & MACKLIN, C. C. (1944). The size of human lung alveoli. *Trans. Roy. Soc. Can.* **38**, 63–82.

MARKS, A., CUGELL, D. W., CADIGAN, J. B. & GAENSLER, E. A. (1957). Clinical determination of the diffusion capacity of the lungs. *Amer. J. Med.* **22**, 51–73.

MARSHALL, R. (1958). Methods of measuring pulmonary diffusing capacity and their significance. *Proc. R. Soc. Med.* **51**, 101–104.

OGILVIE, C. M., FORSTER, R. E., BLAKEMORE, W. S. & MORTON, J. W. (1957). A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J. clin. Invest.* **36**, 1–17.

ROUGHTON, F. J. W. (1945). The kinetics of the reaction $CO + O_2Hb \rightleftarrows O_2 + COHb$ in human blood at body temperature. *Amer. J. Physiol.* **143**, 609–620.

ROUGHTON, F. J. W. & FORSTER, R. E. (1957). Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung. *J. appl. Physiol.* **11**, 290–302.

SHEPHARD, R. J. (1958). 'Breath-holding' measurement of carbon monoxide diffusing capacity. Comparison of a field test with steady-state and other methods of measurement. *J. Physiol.* **141**, 408–419.

THOMSON, M. L. (1958). A portable apparatus for spirometry and for measuring timed vital capacity and diffusing capacity of the human lung. *J. Physiol.* **142**, 17–19*P*.

WILSON, R. H., EVANS, R. L., JOHNSON, R. S. & DEMPSEY, M. E. (1954). An estimation of the effective alveolar respiratory surface and other pulmonary properties in normal persons. *Amer. Rev. Tuberc.* **70**, 296–303.