STUDIES ON THE INTRAPULMONARY MIXTURE OF GASES. II. ANALYSIS OF THE REBREATHING METHOD (CLOSED CIRCUIT) FOR MEASURING RESIDUAL AIR

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The closed rebreathing circuit has been widely used for residual air measurement (1, 2, 3, 4, 5, and others). The essential features of the apparatus and procedure were the same in all. A spirometer filled with high oxygen (or with a hydrogen-containing) gas mixture was attached to the test subject. The carbon dioxide was removed with soda lime or other alkali in the circuit and the subject breathed for a period of time until the nitrogen (or hydrogen) was redistributed in the circuit. The residual lung gas volume was calculated from the amount of redistributed gas.

In most of the work it was assumed that at the end of five or more minutes of quiet breathing the gas in lungs and spirometer was uniform in composition, except for a slight excess concentration of inert gases in the lungs. This excess was assumed to be the same as that occurring during the breathing of room air. On the basis of these assumptions, the net change in spirometer gas concentration was considered the same as that in the lungs during the rebreathing.

By actual measurement of the gas concentration in the various parts of the spirometer system and the alveolar gas, Lassen, Cournand, and Richards (6) concluded in the study of normal subjects that these assumptions were not valid. Rather than an excess, they found a lower concentration of nitrogen in the lungs than in the spirometer at the end of a breathing period. They explained these findings as due to the constantly increasing nitrogen concentration which resulted from the steadily diminishing spirometer volume. This effect they called the "oxygen storage" effect. To correct for it, they introduced alveolar air concentrations into the calculation and found that this correction made a difference of several hundred cubic centimeters in the residual air value in some subjects.

Other workers have found variable results in testing the need for this correction. Anthony (7), using a larger spirometer and a powerful motor blower, failed to find any need for alveolar measurements. The discrepancy from Lassen, Cournand, and Richards' finding seemed to be explained by the difference in apparatus. Kaltreider, Fray, and Hyde (8) believed from the study of six normal subjects that the correction due to the "oxygen storage" effect was negligibly small. Herrald and McMichael (9) confirmed the presence of a significant "oxygen storage" effect and proposed a modified procedure in which the spirometer volume was kept constant by added oxygen.

In severe cases of pulmonary emphysema, Cournand, Lassen, and Richards (10) found no such predictable "oxygen storage" effect as in normal subjects. Moreover, they found extreme variability of results in these subjects on successive measurements. Some figures obtained seemed improbably high, considering the external chest measurements of the subjects. From this they suggested that in these subjects there was an unequal distribution of respiratory gases within the lungs. If this were true, the alveolar air as measured was not a true index of average lung gases. Previously, Sonne and his coworkers (11, 12, 13) had shown this lack of uniformity of the alveolar air not only in emphysema but also in some normal subjects. By fractional analyses of alveolar air after breathing hydrogen, Roelsen (14, 15) has shown an even greater factor of unequal distribution than was evident in analyses of the normal respiratory gases. In the residual air measurements, it is possible that this factor may be still more magnified in its effect, since here the entire gaseous

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lung contents are presumably measured and used in the calculation, contrasted with Sonne's studies of the variations in that part of the lung gases which can be expired.

The purpose of the present investigation is to detect and quantitate the effect of imperfect distribution on closed circuit residual air measurements, not only in order to test the reliability of the method, but also to detect poor mixing as one of the factors disturbing normal lung function. The approach is necessarily indirect. It is proposed to determine the residual air values by several modifications of the method, in which poor mixing should give opposite effects. Conclusions will be based on a comparison of the different values so obtained.

Discussion of effect of poor mixing on standard method

Let us first consider the probable direction of the error due to this factor of unequal distribution by noting the variables in the formula from which the functional residual air [2] is calculated in the standard method. This formula in its simplest form is

$$\text{F. R. A.} = \frac{(\text{N}_2 \text{ in spirometer system at end}) - (\text{N}_2 \text{ in spirometer system at start})}{\text{alv. } \bar{a}\ \text{N}_2 - \text{alv. } \bar{p}\ \text{N}_2} - (\text{N}_2 \text{ excretion factor}),$$

where alv. $\bar{a}$ N_2 is the alveolar nitrogen concentration at the start, alv. $\bar{p}$ N_2 that at the end of the breathing period.

It will be seen that the numerator of the fraction is the difference of two definitely measurable volumes, involving no assumptions. In the denominator, however, one or both of the values alv. $\bar{a}$ and alv. $\bar{p}$ may be in error if there is imperfect distribution of gases within the lungs. Under these conditions, each alveolar specimen will more likely represent the gas in the relatively well aerated portions of the lung.

In the instance of the alv. $\bar{a}$ specimen taken on room air breathing, the gas in the poorly ventilated areas of the lung will show a relative nitrogen concentration, as long as these areas are perfused with blood from the pulmonary arteries. This arises from the fact that the R_t Q. is normally less than 1. Thus probably alv. $\bar{a}$ nitrogen as measured is lower than the average lung gas concentration.

The likely error in alv. $\bar{p}$ is not so simple. At the start of the experiment the lungs are high in nitrogen, the spirometer system low. The first few inspired breaths are low in nitrogen, then rise as nitrogen from the lungs enters the spirometer. Furthermore, with the reduction in volume of the spirometer gas due to oxygen used, the successive inspiratory samples increase in nitrogen concentration. If there is unequal distribution, the alv. $\bar{p}$ specimen as measured will be more influenced by recent inspiratory gas and less by more distant breathing mixtures which may be trapped in the poorly aerated regions. One can enumerate three periods of previous breathing which may influence the gaseous content of the deeper regions whose contents are not measured: (*1*) the high nitrogen of the original room air breathing, (*2*) the very low nitrogen of the first few breaths of the experiment, and (*3*) the somewhat lower nitrogen of the immediately preceding spirometer samples, due to decreasing volume during the period of alleged equilibrium. If the volume is kept constant as in McMichael's work (9) and in some of ours, the third factor is eliminated. The first factor will make alv. $\bar{p}$ as measured too low, the second and third, too high. Thus the error depends on the balance of these factors. Regardless of the direction of the resultant error, it will probably be less negative than that in alv. $\bar{a}$, which is due to factor (*1*) alone. Thus the difference between alv. $\bar{p}$, a value either high or slightly low, and alv. $\bar{a}$, which is definitely too low, will also be too low. Substituting this in the calculation, the F. R. A. value will be too high. Since the difference, alv. $\bar{a}$ − alv. $\bar{p}$ is usually less than 0.3 atmosphere, this error will be magnified by the form of the calculation. It should be emphasized again that this conclusion is based on a series of assumptions of probability. There may be other sources of error which have

[2] Functional residual air is the volume of air in the lungs and air passages at the end of a normal quiet expiration.

not been considered, or there may be more cancellation of errors than allowed for. Realizing this, we may still take as a probability the premise that the factor of imperfect lung gas mixture causes too high a value for residual air by the Christie procedure.

A reversed technique, its purpose, and the probable influence of poor mixing[8]

If the above arguments are valid as the explanation of errors in residual air determination by the Christie method, then it should be possible, by *reversing* the shift of nitrogen during rebreathing, to reverse also the direction of sign of the error. Specifically, if the lungs are first filled with pure oxygen, then a rebreathing of nitrogen (or room air) is carried out, the resultant F. R. A. figure in subjects with imperfect distribution should be too *low*. Such a technique has been devised: the subject is allowed to breathe oxygen for a prolonged period, then connected to the spirometer filled with room air for the rebreathing period. In such a procedure, the net nitrogen shift is from spirometer to lung, in contradistinction to the Christie method where the nitrogen shift is from lung to spirometer. For brevity in future references, we shall call the original method, that of *decreasing* lung nitrogen, Method I; and the new modification, that of *increasing* lung nitrogen, Method II.

The calculation formula of Method II is analogous to that of Method I:

$$\text{F. R. A.} = \frac{(\text{N}_2 \text{ in spirometer system at start}) - (\text{N}_2 \text{ in spirometer system at end})}{\text{alv. } \bar{p}\,\text{N}_2 - \text{alv. } \bar{a}\,\text{N}_2} - (\text{N}_2 \text{ absorption factor}).$$

As before, all values are definitely measurable without assumptions, except those in the denominator of the formula. If these alveolar measurements represent true average lung gas samples, the F. R. A. value should be an accurate volume measurement. However, if there is imperfect gas mixture in the lungs, either alv. $\bar{a}$ or alv. $\bar{p}$ or both will be in error. Under such circumstances, alv. $\bar{a}$ taken after pure oxygen breathing may be too low in nitrogen, since it will not measure the nitrogen still trapped in poorly ventilated areas of the lung. As in Method I, several factors may influence the accuracy of alv. $\bar{p}$: (*1*) too high in nitrogen due to trapped oxygen from the previous oxygen breathing; (*2*) too low due to very high nitrogen in the first few breaths of the rebreathing period; (*3*) too high due to lower nitrogen in spirometer gas during the course of rebreathing. The net result of these three factors is a probable positive error in alv. $\bar{p}$. Thus the difference between alv. $\bar{a}$ (too low) and alv. $\bar{p}$ (too high) will show a probable positive error. From this, in the calculation F. R. A. will be smaller than the true volume if the factor of unequal distribution is important. It should be noted from this discussion that a cancellation of errors is less likely in this method than in the standard method.

As noted in the analysis of both methods, the factor of diminishing volume in the system is one important feature contributing to the possible errors due to poor intrapulmonary gas mixture. Herrald and McMichael (9) have removed this factor by continual replacement of the oxygen used during the rebreathing period. A similar procedure has been carried out in this work, not only in the usual Method I, but also in Method II, so that in subjects fully studied, the results of four separate procedures are presented. In those instances where the volume is allowed to diminish, the methods are designated as Ia and IIa; where the volume is kept constant by oxygen replacement, as Ib and IIb.

A comparison of figures obtained by all four methods or by any pair of figures should give some measure of the factor of imperfect gas distribution within the lungs. If all agree in any subject, the reliability of the method may be assumed, but poor distribution will not be entirely ruled out, since there may be a cancellation of errors. However, a difference between the results by the different methods will be of positive significance as an index of slow gas mixture within the lungs. This type of data, on the other

[8] A preliminary report of this investigation was published in the Proceedings of the American Society for Clinical Investigation, 1938 (J. Clin. Invest., 1938, **17**, 536).

hand, gives no information as to the true value for the residual lung volume.

METHODS

Method Ia (decreasing lung nitrogen, diminishing volume)

This is the Christie method as modified by Lassen, Cournand, and Richards (6) to include alveolar air measurements before and at the end of the rebreathing period. The apparatus shown in Figure 1 is fundamentally that of a Benedict-Roth basal metabolism apparatus, with a few additions. The soda lime carbon dioxide absorber (*S.L.*) and the flutter valves (F_1, F_2) are inserted outside the spirometer. The chief addition is a valve (V_1) adjacent to the mouthpiece (*M*), which can be turned either into the spirometer circuit or into a side circuit fitted with similar flutter valves (F_3, F_4). In the expiratory side of this circuit are inserted the alveolar sampling tubes. The dead space from mouthpiece to these tubes is approximately 100 cc. A valve (V_2) is inserted to close the inspiratory gas flow during alveolar sampling.

In preparing the apparatus for Method Ia, the dead space was washed repeatedly with room air by raising and lowering the spirometer bell. The water level in the spirometer was kept at a measured level. At this level the dead space of the spirometer system had been previously determined (Christie). After washing, the valve (V_1) was turned to the side circuit, thus closing the spirometer circuit. Oxygen was then admitted through *R.I.*, usually about 4000 cc. The actual amount was measured on the graphic tracing after constant temperature had been reached. In practice, the amount of oxygen added was varied, depending on the size of the residual air and the oxygen consumption expected. Ideally, it should be such an amount that the final spirometer gas after rebreathing should be slightly under 50 per cent oxygen. The apparatus was tested for leaks by registering a short period on the drum with a weight placed on the spirometer bell. A straight horizontal line should be drawn.

The subject, preferably under basal conditions, then inserted the mouthpiece and applied the nose clip. When breathing quietly through the side circuit, he was instructed to exhale fully for an alveolar specimen which was taken at the end of three to five seconds of forced expiration. Quiet breathing was then resumed and continued one to two minutes, or until the effect of the exertion of alveolar sampling had passed. With the drum moving,

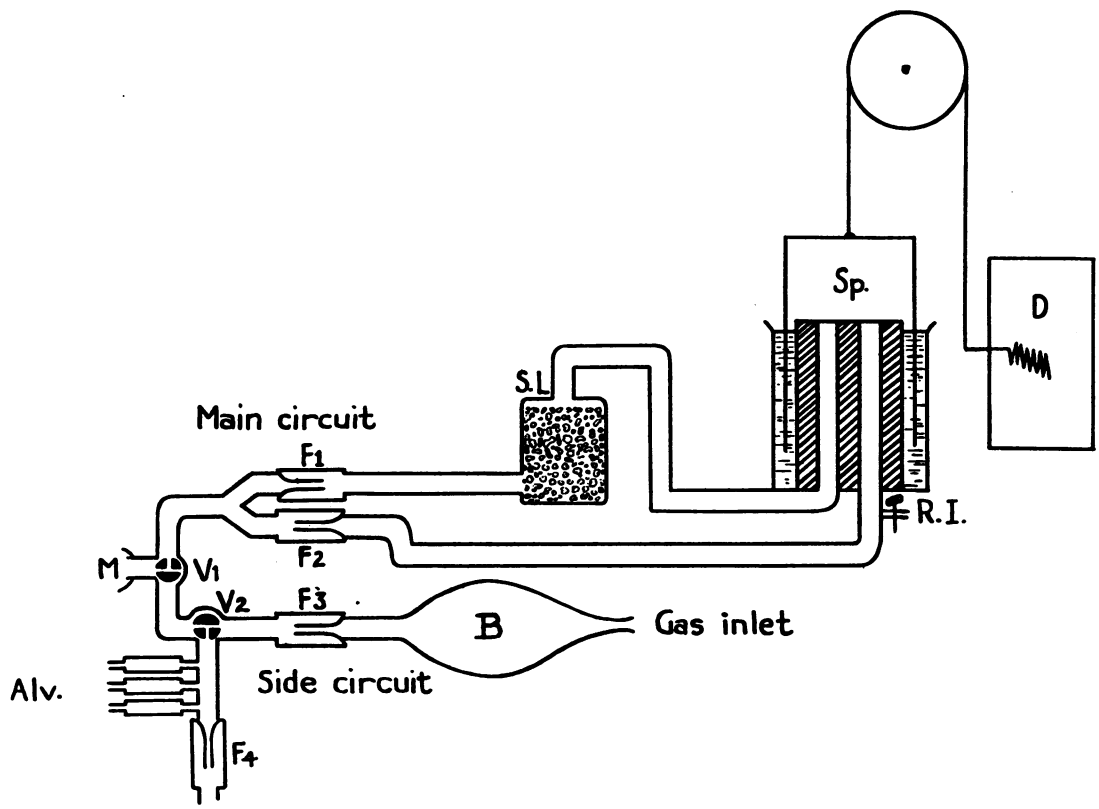

FIG. 1. DIAGRAM OF CLOSED CIRCUIT APPARATUS FOR ANALYSIS OF INTRAPULMONARY MIXTURE OF GASES

M, mouthpiece. *Alv.*, set of three evacuated gas sampling tubes. V_1, V_2, three-way respiratory valves. F_1, F_2, F_3, F_4, one-way rubber flutter valves. *B*, rubber anesthesia bag. *S.L.*, soda lime absorber for CO_2. *Sp.*, spirometer. *R.I.*, side valve for sampling spirometer gases. *D*, recording drum.

the valve (V_1) was then turned to the spirometer circuit as nearly as possible at the end of a quiet expiration. Quiet breathing was recorded for the standard time of seven minutes, or a longer period, as desired in some data to be reported. Near the end of this period, the spirometer temperature was read. At the end of this time, the valve (V_1) was turned to the side circuit and simultaneously the subject expired fully for alveolar sampling. It was not necessary that this valve shift occur at the end of a normal expiration. In fact, it was found better to turn it immediately after the start of the expiration. The spirometer gas sample was then taken by placing a weight on the spirometer bell, opening *R.I.*, allowing approximately half the gas to escape, and then taking the sample.

There were three gases to be analyzed, the spirometer and two alveolar specimens, which we shall call alv. $\bar{a}$ and alv. $\bar{p}$. For calculation, only the nitrogen percentage needed to be measured as the difference between the total and the combined oxygen and carbon dioxide. Actually, frequent analyses were made for carbon dioxide separately: on the spirometer to check the efficiency of the soda lime, and on the alveolar specimens to be sure that specimens were not significantly diluted with dead space gas. Analyses were made with a Haldane apparatus in which the burette was calibrated for up to 50 per cent absorbable gases.

All volume measurements were made from the tracings, together with the previously measured dead space of the apparatus. The volume of added oxygen in the spirometer was recorded at the time it was run in; the oxygen consumption during the experiment was measured from the slope of the tracing. The initial volume minus this consumption equals the final spirometer volume to which the dead space volume was added to obtain the final volume of spirometer system.

The formula for the F.R.A. calculation is obtained from a mathematical statement that the nitrogen in the lungs and spirometer at beginning and end is equal (with a correction for nitrogen excretion).

Let $V\bar{a}$ = vol. N_2 containing gas in spirometer system at start (in this case = D.S., dead space).

$V\bar{p}$ = final spir. vol. + D.S.

Spir. $\bar{p}$ = analysis of spir. gas for N_2 expressed as part of an atmosphere.

Alv. $\bar{a}$ / Alv. $\bar{p}$ = alveolar N_2 analyses in same units.

F.R.A. = functional residual air in cc.

Then:

$$\text{F.R.A.(alv. } \bar{a}) + V\bar{a}(0.791) = \text{F.R.A.(alv. } \bar{p}) + V\bar{p}(\text{Spir. } \bar{p}) - N_2 \text{ excretion.}$$

Solving:

$$\text{F.R.A.} = \frac{V\bar{p}\ \text{Spir. } \bar{p} - V\bar{a}(0.791) - N_2 \text{ excretion}}{\text{alv. } \bar{a} - \text{alv. } \bar{p}}.$$

From Paper I of this series (16):

$$N_2 \text{ excretion} = \frac{\text{alv. } \bar{a} - \text{alv. } \bar{p}}{0.80} \times 220.$$

(For seven minutes' breathing time; 10 cc. added to 220 cc. for each minute after seven, if longer period.)

Substituting:

$$\text{F.R.A.} = \frac{V\bar{p}\ \text{Spir. } \bar{p} - V\bar{a}(0.791)}{\text{alv. } \bar{a} - \text{alv } \bar{p}} - 275.$$

All analyses with Haldane apparatus give proportions of dry gas, so $V\bar{p}$ and $V\bar{a}$ were corrected to dry gas at the temperature of the experiment before substituting in the formula. The F.R.A. value then obtained may need a slight correction if the rebreathing period did not begin at the exact end of a normal expiration. Such a correction was determined from examination of the tracing. After this correction the F.R.A. volume was corrected to 37° C. and saturation with water vapor.

Method Ib (decreasing lung nitrogen, constant volume)

This procedure differed only in a few details from Ia. Immediately after the start of the rebreathing period, a steady flow of oxygen was begun through *R.I.* (Figure 1), equal to the resting oxygen consumption. This figure was estimated from a previous tracing. For regulating the flow, a Forregger flow measuring valve was used with a fine and coarse water manometer gauge. This flow of oxygen was turned off fifteen seconds before the end of the rebreathing period.

The formula for calculation is unchanged. $V\bar{a}$ is still the D.S. (dead space). A base line, drawn on the tracing as in Ia, will be approximately horizontal. $V\bar{p}$ will be D.S. + O_2 volume added at start ± correction for any deviation of tracing from horizontal.

Method IIa (increasing lung nitrogen, diminishing volume)

This method required the same apparatus with only one addition. On the inlet valve of the side circuit a rubber anesthesia bag (*B*) was attached. This in turn was connected with an oxygen tank fitted with reduction and flow measuring valves.

In preparing the apparatus, the spirometer and dead space were washed with room air as before, then partly filled with room air and tested for leaks. The volume of room air admitted to spirometer was usually 2000 to 3000 cc., but was conveniently made larger with subjects of large F.R.A. and smaller with small subjects.

With V_1 open to the side circuit and the oxygen flow in that circuit maintained at 7 to 8 liters per minute, the subject was then attached to mouthpiece and allowed to breathe quietly for ten minutes. Oxygen flow was adjusted to keep the anesthesia bag partly full. The ten-minute period was chosen because preliminary experiments measuring alveolar nitrogen values at frequent intervals during oxygen breathing showed that in normals a low plateau level for alveolar nitrogen was reached after three to four minutes; in severe emphysema a slightly higher plateau level was reached in ten to twelve minutes.

At the end of ten minutes of oxygen breathing, an alveolar specimen was taken with the inlet valve (V_2) closed for the few seconds of the procedure. Oxygen breathing was continued for two additional minutes to reach a resting level; then at the end of a normal expiration,

valve V_1 was turned to the spirometer system. For seven minutes or longer, spirometer breathing was recorded on the drum and an alveolar specimen taken at the end, as in Method I. It was found advisable to partly wash out the tubing of the side circuit with room air before taking the alv. $\bar{p}$ specimen, in order that the high oxygen previously there would not too greatly dilute the nitrogen of the alveolar air.

Using the same symbols as in Method I, the calculation formula is similarly derived:

$$\text{F.R.A.(alv. } \bar{a}) + V\bar{a}(0.791) = \text{F.R.A.(alv. } \bar{p}) + V\bar{p}(\text{Spir. } \bar{p}) + \text{N}_2 \text{ absorbed.}$$

Solving:

$$\text{F.R.A.} = \frac{V\bar{a}(0.791) - V\bar{p}(\text{Spir. } \bar{p}) - \text{N}_2 \text{ absorbed}}{\text{alv. } \bar{p} - \text{alv. } \bar{a}}.$$

Substituting formula for N_2 absorption:

$$\text{F.R.A.} = \frac{V\bar{a}(0.791) - V\bar{p}(\text{Spir. } \bar{p})}{\text{alv. } \bar{p} - \text{alv. } \bar{a}} - 275.$$

$V\bar{a}$ in this case is the dead space plus the room air added to system. $V\bar{p}$ is this value minus the oxygen absorbed during the rebreathing period. Corrections are made as in Method I for inexact starting point, temperature and for water vapor.

Method IIb (increasing lung nitrogen, constant volume)

This modification of Method II is exactly analogous to the "b" modification of Method I. The volume was kept nearly constant by a steady flow of oxygen into *R.I.* during the rebreathing period, up to fifteen seconds before the end.

The calculation formula is likewise unchanged. $V\bar{p}$ in this case equals $V\bar{a}\pm$ any necessary correction for deviation from horizontal in the slope of the tracing.

Actually the Method IIb was used more frequently than IIa in those cases in which only a comparison of results by Methods I and II was desired. One reason for this was that in the "b" modification there is no chance of obtaining a final breathing mixture of less than 21 per cent oxygen, as is possible in IIa.

RESULTS

The subjects for this work consisted of six normal persons and ten patients with severe pulmonary emphysema. The normal subjects included physicians and ambulant hospital patients with non-pulmonary diseases. In the patients with emphysema, the diagnosis was established by the physical signs, the x-ray and the spirographic tracings. All suffered from severe dyspnea; six out of ten showed a reduced arterial oxygen saturation. Only one subject (Ant. C.) seemed to be suffering from some degree of cardiac failure, and in this case the pulmonary disturbance seemed predominant. Two subjects who have since died were examined at autopsy and the diagnosis of emphysema confirmed. They also showed some degree of bronchiectasis and it is probable that some of the living subjects also showed this common complication of advanced obstructive emphysema. The group of abnormal subjects is therefore a picked group, chosen not as average cases but as extreme and advanced cases.

Table I shows the results on the entire series by Methods Ia and IIb. In addition, calculated figures for the Ia experiments are listed, using

TABLE I

Functional residual air determinations by closed system

Subject	Length of test	Decreasing lung N_2 method—Ia: Number of determinations	Christie calculated average	Lassen *et al.* calculated average	Increasing lung N_2 method—IIb: Number of determinations	Average
NORMAL SUBJECTS						
	minutes					
J. D.		3	1225	1115	3	1065
J. L.	7	12	1420	1320	12	1300
	12				3	1210
A. C.	7	2	1895	1745	3	1770
	10				1	1735
T. R.	7	4	5500	4375	3	4460
	10				1	4300
D. W. R.	7	3	4040	3540	2	2930
	10	1		3470	1	3310
R. C. D.	7	5	3990	3250	6	2330
	10	2		3810	2	2340
Mean of group			3010	2560		2310
S.D.			1740	1480		1370
S.E.$_m$			710	602		503
t*			0.5		0.3	
SUBJECTS WITH PULMONARY EMPHYSEMA						
M. K.		4	4100	3320	4	2390
J. O.		5	5180	4380	4	2530
M. H.		15	6060	5360	4	3845
J. F.	7	5	4760	4240	4	2280
	12	1		5040	2	2085
M. A.		7	7490	5465	9	3320
Ant. C.		2	3490	3240	2	2850
J. C.	7	4	7210	6570	4	5430
	12	2		6285	2	5470
D. H.		2	3365	3140	2	2530
H. K.	7	3	3790	3835	3	2250
	12	2		3765	2	1940
F. H.		1	3160	3020	1	1945
Mean of group			4860	4260		2960
S.D.			1680	1270		1120
S.E.$_m$			531	401		354
t*			0.9		2.4	

* $t = \frac{\Delta_m}{\text{S.E. Difference of Means}}$.

the original Christie calculation and neglecting the alveolar measurements. The average value of a series of determinations is given for each of the three calculations. Results of Methods Ib and IIa are omitted from this table, but will be presented in a later detailed listing of results on four subjects, together with an analysis of the variations by each single method.

A comparison of columns 1 and 2 gives confirmation to the findings of Lassen, Cournand, and Richards (6) on the influence of alveolar measurements in the calculation. Of the six normal subjects, the Christie calculation gave larger results in every instance, the difference varying from 100 to 1125 cc. Of the ten emphysematous subjects, nine showed higher results by the Christie calculation, the difference varying from 140 cc. to almost 2000 cc. In one (H. K.) the Christie calculation gave practically identical results.

A comparison of columns 2 and 3 gives the evidence by which the factor of unequal distribution in the lungs may be discovered and roughly quantitated.

Normal subjects

Of the six normal subjects, the two methods give practically identical results in four. Of these four, three were subjects with functional residual air of less than 2000 cc. In the case of the other two normal subjects, both with rather large functional residual air, there was a difference of 600 cc. and 900 cc., respectively, in the average values by the two methods. It should be mentioned that no abnormalities could be found clinically or by x-ray of the chest in these two subjects. Thus it would seem that in these two normal subjects there was indirect evidence that the alveolar air as measured does not indicate the gas concentration throughout the lungs, under the conditions of the experiment.

Theoretically, this situation might be corrected by increasing the rebreathing time beyond the usual seven minutes. Such an experiment was carried out on these two subjects. In a single pair of ten-minute tests on one of them, there appeared to be better agreement than in the seven-minute tests. In the case of the other subject, however, no closer agreement could be reached.

Subjects with emphysema

In the experiments on the emphysematous subjects, it will be seen from Table I that there was in every instance a significant difference between average results by Methods Ia and IIb. This difference ranged from 400 cc. in the case of Ant. C. to 2000 cc. in the case of J. F. In seven out of ten cases, the difference was greater than 1000 cc. It would seem that these findings have similar significance to those in the case of the last two normals, except that the degree of change is more marked in the pathological cases. Evidently the alveolar air samples as obtained were in all cases a poor measurement of the average lung gases, even though the alveolar carbon dioxide level in the gases analyzed was regularly above 5 per cent. The comparable findings in some normal subjects and all emphysematous subjects would seem to follow closely Sonne's findings of variations in different parts of the measurable alveolar air in the same groups of subjects.

As in the case of the normal subjects, an attempt was made to repeat the experiments, using longer breathing periods. Such experiments are technically somewhat more difficult. In three cases, results of a twelve-minute breathing period are presented in the same table (Table I). It will be seen that in no case was agreement obtained between the two values. Furthermore, there is no definite trend toward decreasing the difference between them. In two there is actually an increase in the difference; in the third, a slight decrease, probably not significant.

Table I also shows a statistical analysis of the seven-minute figures by the various methods. Such an analysis of small groups of subjects, however, introduces a large variable in the size of the chest within the group, so that the tests of significance between the different methods are no measure of significant differences due to the method alone. In spite of this unfavorable type of comparison, it will be seen that in the group of abnormal subjects there is a statistically significant difference between the means of results by Methods Ia and IIb.

To obtain a comparison of the groups in which only the factor of difference of method is involved, a second table is presented (Table II), expressing only the relative values by each

TABLE II

Relative mean values of functional residual air by closed circuit methods

Subject	Decreasing lung N_2 method—Ia		Increasing lung N_2 method—IIb
	Christie calculation	Lassen *et al.* calculation	
NORMAL SUBJECTS			
J. D.	1.10	1.00	0.96
J. L.	1.07	1.00	0.98
A. C.	1.09	1.00	1.02
T. R.	1.26	1.00	1.02
D. W. R.	1.14	1.00	0.83
R. C. D.	1.23	1.00	0.72
Mean	1.15	1.00	0.92
S.D.	0.087		0.133
S.E.$_m$	0.034		0.054
t	4.4		1.5
SUBJECTS WITH PULMONARY EMPHYSEMA			
M. K.	1.24	1.00	0.72
J. O.	1.18	1.00	0.59
M. H.	1.13	1.00	0.72
J. F.	1.12	1.00	0.54
M. A.	1.37	1.00	0.64
Ant. C.	1.08	1.00	0.88
D. H.	1.21	1.00	0.81
J. C.	1.10	1.00	0.82
H. K.	0.99	1.00	0.59
F. H.	1.05	1.00	0.64
Mean	1.15	1.00	0.70
S.D.	0.115		0.121
S.E.$_m$	0.036		0.038
t	4.2		7.9

method, arbitrarily setting the value by Method Ia as unity in each subject. In this comparison it will be seen that the Christie calculation gives significantly higher values in both groups. Considered as a group, Methods Ia and IIb are not significantly different among the normals. In the abnormal group, the striking difference of the two methods is clearly shown.

Table III presents the more complete data on four subjects studied thoroughly: one normal subject with small residual air, one normal subject with large chest who showed discrepancies between results by the contrasting methods, and two subjects with pulmonary emphysema. Such a comparison is more valuable than analysis of groups since the subjects were picked as strikingly abnormal cases and, also, the evaluation of any method may depend on its applicability to the extreme and unusual case.

In the case of J. L., a small normal subject, it is evident that the results by all the methods using alveolar measurements are not significantly different. Even here, however, the Christie calculation is significantly higher than that using alveolar measurements. It should also be noted, in this small subject, that there is much less variability of results by the same method than in the other three subjects.

The tabulation on R. C. D. merely repeats in statistical form the results on this subject

TABLE III

Functional residual air values by closed circuit methods, statistical analyses on individual subjects

	J. L. (Normal)				R. C. D. (Normal)				M. H. (Emphysema)				M. A. (Emphysema)			
	Number of determinations	Mean	S.D.	S.E.$_m$	Number of determinations	Mean	S.D.	S.E.$_m$	Number of determinations	Mean	S.D.	S.E.$_m$	Number of determinations	Mean	S.D.	S.E.$_m$
Method Ia																
Christie Calculation...	12	1420	105	30	5	3990	442	198	15	6060	821	212	7	7490	1740	660
Lassen *et al.* Calculation	12	1320	108	31	5	3250	359	160	15	5360	748	193	7	5465	553	209
Method Ib	6	1290	48	35					5	4630	105	47	5	4560	373	167
Method IIa	8	1230	82	29					8	3865	368	130	10	3475	170	54
Method IIb	15	1270	79	20	6	2330	221	90	4	3845	316	158	9	3320	196	65
Values compared	t				t				t				t			
Christie Calculation vs. Lassen *et al.* Calculation	2.3				2.9				2.4				2.9			
Ia vs. Ib	0.6								3.7				3.4			
IIa vs. IIb	1.1								0.1				1.8			
Ia vs. IIb	1.3				5.0				6.1				10.2			
Ib vs. IIb	0.5								4.9				6.3			

in Table I. It will be seen that the comparison to show the effect of "oxygen storage" and also the comparison to show the possible effect of poor mixing both give statistically significant differences.

The figures on M. A. and M. H. are not only an analysis of those in Table I, but also include figures by Method Ib (which is essentially the modification of McMichael), and by Method IIa. The results by Methods Ia and IIb are widely divergent, as is evident from inspection. A comparison of the values by Ia against Ib, and by IIa against IIb will show how far the maintenance of constant volume corrects the error due to imperfect intrapulmonary gas mixture. In these two subjects the values by Ib were significantly lower than by Ia, yet there is even more difference between either of these and Method II. Furthermore, in the case of "a" and "b" modifications of Method II, there is no significant difference.

The findings in these two subjects are characteristic of several others so studied. In no case did the maintenance of constant volume remove the evidence for error due to malmixture within the lungs.

DISCUSSION

These data not only give us some information on the limitations of the rebreathing methods for measuring residual air, especially in severe cases of emphysema, but they also furnish an indirect measure of imperfect gas mixture in the lungs. The reversed Christie procedure, Method II, was devised in such a way that, if uniform mixture within the lungs occurred, the two methods should give identical results. Furthermore, the probable direction of the error in case of imperfect mixture was predicted in each method, assuming that there was gas trapped in the lungs from previous breathing which did not contribute to the alveolar air measured.

The uniform evidence of unequal distribution in the emphysematous subjects is not surprising. It has long been evident that such might be the case from the anatomical changes in the lungs of such subjects. Analysis of gas obtained by direct puncture from large emphysematous bullae has shown very high nitrogen values (17). Sonne's fractional analyses of an alveolar sample showed directly a marked variability. Roelsen repeated the fractional alveolar analyses following a single breath of hydrogen and deduced from the results that the mixture was even more imperfect than was evident from the carbon dioxide and oxygen analyses alone. This latter finding with the use of hydrogen would seem to give the clue to the reason why the factor of maldistribution has been so rarely appreciated. The fractional analyses of alveolar air for carbon dioxide and oxygen give evidence of unequal distribution only if the poorly aerated lung regions are relatively well perfused with blood. If in the course of disease the blood circulation in those areas diminishes parallel to the drop in aeration, then the alveolar samples may be uniform and the alveolar carbon dioxide tension may correspond well to that in the arterial blood. This correspondence between alveolar and arterial blood carbon dioxide tensions has been an important piece of evidence and has been interpreted to signify uniformity of gas mixture within the lungs.

In spite of the previous evidence that gas mixture is slow in emphysematous lungs, it has been assumed that the seven-minute period was adequate to overcome difficulties arising from this slow mixture. It seems likely from our data that the factor of poor mixture in the lungs is an important source of error not only after seven minutes' breathing, but also after ten to twelve minutes. It seems doubtful whether increasing the time further would remove the difficulty. Furthermore, the magnitude of the discrepancies due to poor mixture in our data seems to indicate that this factor is a major one, not only for the measurement of residual air, but probably also for the general problem of the pulmonary disturbance.

The finding of a factor of imperfect distribution in certain normal subjects was somewhat more surprising. Sonne's work showed that it existed, but not that it was as large as the data here indicate. Possibly our method is sensitive to detect small degrees of maldistribution.

It is evident that our data give no conclusive evidence as to the true residual air value in the cases showing different results by the various methods. The residual air volume has been considered an important index in emphysema of the degree of disability, usually expressed in relationship to the vital capacity or the total capacity. It is possible in these cases that the residual air value, as measured by the Christie method, is not a true volume measurement, but represents

the true volume plus an added value due to the factor of poor gas mixture. In order to prove this, it would be necessary to have another method for comparison which minimizes or eliminates the effects of mixing.

A detailed consideration of the rebreathing method has shown why, in cases of poor mixing, the chances of true equilibrium between lungs and spirometer are not good. Even keeping the volume constant as in McMichael's work or our Method Ib, there is a constantly changing breathing mixture for the first few minutes of the rebreathing. If the volume is diminishing, the changes are more complicated and the inspiratory gas mixture is always changing. Furthermore, the size of the usual spirometer is such that the change in alveolar nitrogen is usually 0.2 to 0.3 atmosphere. In the course of calculation, such a small change leads to a three- to five-fold magnification of error in the residual air value.

Thus, a method which would allow a uniform inspiratory gas mixture and would utilize a maximum alveolar change should offer greater likelihood of reaching a true measure of residual air. Such a method will be presented in Paper III of this series (18).

SUMMARY AND CONCLUSIONS

1. Modifications of the Christie method of residual air measurement are presented which (*1*) keep the spirometer volume constant, (*2*) reverse the shift in nitrogen and therefore reverse the direction of error due to unequal distribution within the lungs.

2. Results are presented using the different modifications and the original method on six normal and ten subjects with pulmonary emphysema.

3. These results give agreement by all methods in four normal subjects only, three of whom had small residual air volume.

4. In the remainder of the normal subjects and all the subjects with emphysema, there are wide discrepancies between the results by the different methods. Keeping the spirometer volume constant does not correct the discrepancies. These data give positive evidence of unequal gas distribution within the lungs.

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