STUDIES ON THE INTRAPULMONARY MIXTURE OF GASES. I. NITROGEN ELIMINATION FROM BLOOD AND BODY TISSUES DURING HIGH OXYGEN BREATHING

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If a normal subject under resting conditions is changed from breathing atmospheric air to breathing 100 per cent oxygen, his lungs will then be progressively washed free of their normal content (about 80 per cent by volume) of the inert gas nitrogen, the latter being replaced by oxygen.

In addition, the nitrogen gas in physical solution in body tissues (in amount about 1000 cc.) will be gradually eliminated, carried from the tissues to the lungs by the blood stream, and will finally diffuse into the alveolar air spaces, which have been depleted of their nitrogen.

This procedure involves several physiological phenomena of interest in connection with pulmonary ventilatory function. The investigation of these will form the subject matter of this and the following papers. The phenomena concerned may be described as follows:

(*a*) The *rate of emptying* of nitrogen from the lung during pure oxygen breathing will depend upon a number of factors: the volume of residual air in the lungs, the volume of tidal air, rate of respiration, and the adequacy of distribution of each tidal breath to deeper pulmonary spaces (1). This rate of emptying is also an effective means of measuring *efficiency* of the ventilatory process; that is, the emptying of nitrogen from a pulmonary air space, with a given breath, gives an index of the effectiveness of this breath in removing carbon dioxide from, or adding oxygen to, this same air space.

The great defects that may exist in this function, in pathological lungs, can be illustrated by example. Figure 1 describes an experiment in which the nitrogen concentration of alveolar air has been measured during the course of pure oxygen breathing (*1*) in a normal subject, and (*2*) in a patient with advanced pulmonary emphysema. The delay in emptying the lungs of nitrogen, in the latter case, is striking. In the third paper of this series (2), we will return to a further consideration of this type of procedure.

(*b*) The nitrogen in the lungs has been found to serve as a convenient means of measuring lung volume. In the simplest and most widely used of these methods, that of Christie (3), the subject rebreathes for seven minutes in a closed circuit containing a spirometer filled with oxygen. The concentration of nitrogen in the lungs at the start of the procedure, the concentration in lungs and spirometer at the end, and the volume of the gases in the spirometer are known. From these values the volume of gas in the lungs can be calculated. The calculation assumes a nearly even mixture of nitrogen through the lung-spirometer closed circuit at the end of the rebreathing period. In this case the lungs are emptied of a known fraction of the contained nitrogen, rather than of all nitrogen. In the second paper of this series, we attempt to study the adequacy of this assumption of even intrapulmonary mixture, in normal and pathological subjects, in the Christie type of lung volume determination.

(*c*) In any method which attempts to measure intrapulmonary nitrogen by washing a part or all of this gas out of the lungs, it is obvious that a correction will be necessary due to the nitrogen excreted from the body into the lungs, whenever the normal alveolar nitrogen concentration is lowered. The present paper is concerned with the determination of an adequate correction factor due to nitrogen excretion from the body, and its application in the measurement of residual lung volumes.

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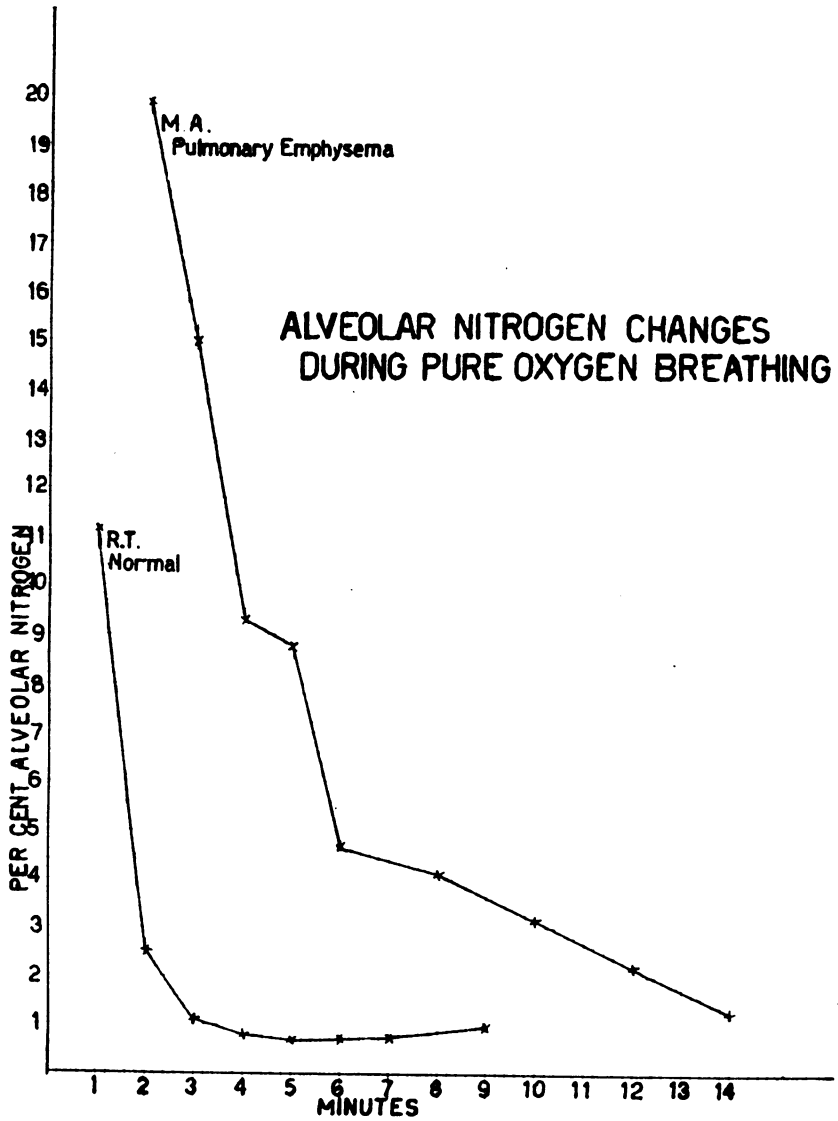

FIG. 1. RATE OF EMPTYING OF NITROGEN FROM THE LUNGS DURING PURE OXYGEN BREATHING

Ordinates, per cent of nitrogen in alveolar air sample.
Abscissae, time (in minutes) after starting pure oxygen breathing.

The factors involved in the excretion of nitrogen (from the body into the lungs) may be listed as follows:

1. Gradient of partial pressure of nitrogen between pulmonary blood capillaries and alveolar air spaces. In normal subjects, as shown in Figure 1, alveolar nitrogen falls to a low figure within two minutes of oxygen breathing. During this time the partial pressure of nitrogen in the tissues is still high, whereas the nitrogen in the lungs has been rapidly decreased by successive normal respirations. The rate of excretion of nitrogen from the body will therefore be highest during the early minutes of oxygen breathing (after the first few breaths have established a blood-alveolar gradient), and will decrease as the tissue nitrogen is depleted.

2. Since the alveoli are exposed to only a part of the circulating blood at any one time, the second important factor is the rate of blood circulation. This principle has been used for estimations of cardiac output (4, 5), but has now

been discarded because of technical difficulties. Thus the nitrogen excretion should be lowest under basal conditions; *i.e.*, when the cardiac output is at a minimum. It is useful to visualize the cardiac output as limiting the nitrogen excretion during any given period of time.

3. Time factor. This is simply the converse of the first two considerations, and its importance is sufficiently indicated above.

4. Total amount of nitrogen in the body. The size of the individual is obviously a significant factor. It was also shown many years ago by Vernon (6), later by Campbell and Hill (7), that a disproportionate part of body nitrogen exists in fatty tissues, in which the gas is five times as soluble as in water or blood.

A careful study of nitrogen excretion from the body during oxygen breathing, as well as its reabsorption during nitrogen breathing, has been carried out by Shaw and others (8), using dogs. They studied the process of nitrogen excretion only during the period *after* the residual lung volume had been washed nearly free of nitrogen; that is, after the first seven minutes of quiet breathing. Their findings were: (*a*) the curves of nitrogen excretion during oxygen breathing, and of nitrogen resaturation during air breathing after oxygen breathing, were similar; (*b*) nitrogen excretion was proportional to the nitrogen pressure gradient between blood and lungs; both facts predictable if the nitrogen is a simple dissolved gas.

For the calculation of a correction factor in lung volume determinations, it is, of course, the nitrogen excreted into the lungs during the first few minutes of oxygen breathing that is important. Several investigations of this aspect of nitrogen excretion have been made. In general, the procedure has been to measure the total nitrogen eliminated from the lungs during a given period of oxygen breathing, then to subtract from this the excess of nitrogen in the lungs at the start of oxygen breathing over that still in the lungs at the end of the oxygen breathing period. It might seem that no satisfactory solution can be reached if the residual air must be known before nitrogen excretion can be calculated, and vice versa. Actually, the lungs can be nearly freed of nitrogen (down to 4 per cent) by a few quick deep breaths; in this way any *error* in residual air figure will involve an error only 1/25 (4 per cent) as large in the final nitrogen excretion figure.

Using a small rebreathing circuit, Bornstein (4) found about 90 cc. of nitrogen excreted during the three minutes following a period of seventy seconds of overbreathing with oxygen to wash out the alveolar nitrogen.

Campbell and Hill (7) measured it for a similar initial three-minute period and for subsequent two-minute periods up to fourteen minutes. Assuming but not measuring 60 cc. excreted during the first minute, they found values of 185 to 217 cc. during the first five minutes of pure oxygen breathing and 10 to 15 cc. per minute for later minutes. Their values during exercise were considerably higher.

Behnke, Thomson, and Shaw (9) have measured nitrogen excretion in three normal men, using a technique similar to that of Shaw, Behnke, *et al.* in their dog experiments. The nitrogen excretion during the first five minutes of oxygen breathing was not included. These investigators found that the curve of nitrogen excretion could be expressed by two equations, the first describing the early phase (first twenty-five minutes) of nitrogen excretion, the second the later phase, when nitrogen stores in body fat depots were being depleted. It is of interest that the time for complete unsaturation in man is longer than in dogs, and that the two times are inversely proportional to the cardiac output per unit of body weight in the two species. Using the equation for the first part of their measured period, they have extrapolated to determine the curve for the first five minutes. From their data we have calculated the nitrogen excretion during the first seven minutes and found the figures to average 241, 181, and 212 cc. in the three normal subjects they studied.

In our experiments we have measured the nitrogen excretion only during the first seven minutes of oxygen breathing, since this is the period of interest in connection with lung volume measurement. In order to measure it during as much as possible of the first minute, we have made the preliminary period for washing out the alveolar nitrogen only twenty seconds. This seems to be adequate for the normal subjects we

are studying, in that the alveolar nitrogen concentrations at the end of this washing-out period were low.

Our apparatus (shown in Figure 2) consists of two open breathing circuits fitted with appropriate flutter valves (F_1, F_2, F_3, F_4) to control the direction of flow. We use the term open circuit to contrast with that of closed or rebreathing circuit. In the former case the expirations do not mix with the inspiratory supply, but are collected separately. A valve (V_1) adjacent to the mouthpiece is used to shift the subject into either of the two circuits. The inlet side of each circuit is fitted with a rubber anesthesia bag (B_1 and B_2) which is connected in turn to an oxygen tank with appropriate pressure reduction and flow regulating valves. An additional valve (V_2) is inserted in the side circuit to close the inflow of oxygen during alveolar sampling. On the outlet tubing of the side circuit a series of three evacuated gas sampling tubes for alveolar sampling is inserted close to the mouthpiece. The dead space from the mouthpiece to these tubes was kept less than 100 cc. The outlet tubing of the main circuit led to a Tissot gasometer (T) of 100-liter capacity.

Before each experiment all the tubing and the gasometer were thoroughly flushed out with oxygen. It was found by repeated analyses that six successive washings of 15 to 20 liters each were sufficient to reduce the nitrogen content of the gasometer dead space to that of the oxygen supply.

After the preliminary washing a steady flow of oxygen of 2 to 3 liters per minute was maintained through the tubing of both circuits. The subject, who had rested for thirty minutes, then inserted the mouthpiece and attached a nose clip. The valve was then turned to the side circuit and he was instructed to take four breaths of maximum depth, each followed by full expiration. The total time of this was adjusted to approximately twenty seconds. During this time the oxygen flow was adjusted to keep the anesthesia bag full. At the start of the fourth expiration, the valve V_2 was closed; at the end of this expiration a gas sample was taken at "alv." This furnished an estimate of the alveolar gas at that time and is designated "alveolar $\bar{a}$" sample.

Immediately thereafter the center valve V_1 was shifted to the main circuit and the subject instructed to breathe naturally. The oxygen flow was adjusted now on this cir-

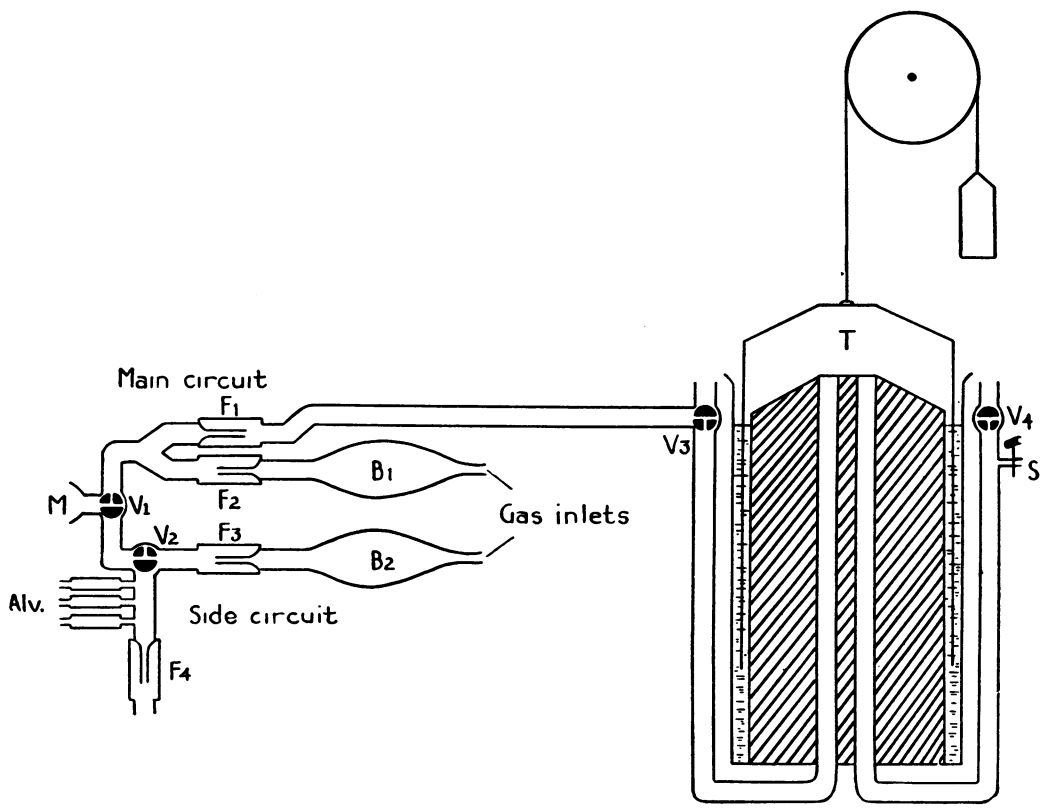

FIG. 2. APPARATUS FOR MEASUREMENT OF NITROGEN EXCRETION

M, mouthpiece. *Alv.*, group of three evacuated gas sampling tubes. V_1, V_2, V_3, V_4, three-way respiratory valves. F_1, F_2, F_3, F_4, one-way rubber flutter valves. B_1, B_2, small rubber anesthesia bags. *T*, one hundred liter (Tissot) gasometer. *S*, valve and attachment for obtaining gasometer samples. For further explanation, see text.

cuit so as to keep the anesthesia bag just full. At the end of seven minutes, the center valve was turned back to the side circuit. At the same time the subject was instructed to expire fully for an alveolar sample which was taken at "alv." This sample is designated "alveolar $\bar{p}$."

Before reading the volume in the gasometer, the tubing in the main circuit was flushed with 8 to 10 liters of oxygen. Then the gasometer inlet valve (V_3) was closed, the volume read, and a sample taken, which we shall designate as "Tissot" sample.

Thus there were three gas samples to be analyzed in each experiment: two "alveolar" and one "Tissot." Gas from the oxygen tank was analyzed whenever a new tank was opened. The dead space under the bell of the gasometer was determined by measurement and geometrical calculation (1100 cc. in our apparatus). This is the total dead space needed in the calculation since all the tubing has been washed out with tank oxygen at the start and end of the experiment.

For the gas analyses, either the Van Slyke-Neill manometric apparatus or the Haldane apparatus could be used. We have used the latter throughout.

Accurate analyses of the gas samples with a Haldane apparatus offered a problem since they all contained 5 per cent or less of nitrogen. Using an apparatus with a burette graduated from 5.0 to 10.0, we have tried several methods of diluting the sample with nitrogen so that the final volume could be read on the scale. The following procedure was found to be the most accurate, chiefly because it utilized a full 10 cc. sample of the unknown gas, whereas the other methods of dilution utilized only a 4 or 5 cc. sample.

A preliminary sample of room air is freed of oxygen and carbon dioxide in the machine, leaving 5 to 7 cc. of nitrogen in the burette. This is read accurately and the reading may be designated as R_1. This gas is then driven over into the oxygen-absorbing chamber. To do this, the upper level of the water riding above the mercury in the burette is raised just to the lower level of the burette stopcock. The burette stopcock is then turned to the inlet for admitting the sample, and the center stopcock is turned from the oxygen absorber to the carbon dioxide absorber. An approximate 10 cc. sample of gas to be analyzed is next admitted to the burette as usual, and the burette stopcock is turned to connect with the carbon dioxide absorber. The level is adjusted and the volume read accurately. This reading may be designated as R_2. The carbon dioxide can be absorbed immediately but usually we are interested only in the nitrogen measured as the residual after absorption of both oxygen and carbon dioxide in alkaline pyrogallol. For this the center stopcock is turned to connect the burette and oxygen absorber. This connects the burette containing 10 cc. of gas with the oxygen absorber containing the previously measured 5 to 7 cc. of nitrogen. This total volume at the start is larger than customarily used in the machine, so the levels of pyrogallol and of alkali in the trap must previously be adjusted in order that no gas is driven through the U-tube in the oxygen absorber. With this precaution the gases are absorbed as usual until a constant volume of residual nitrogen is obtained. This reading may be designated as R_3.

For the calculation one must have previously measured the volume of the burette stopcock bore, and the volume of acidified water in the burette.

Then

(1) Volume of unknown gas sample $= R_2 -$ vol. H_2O

(2) Volume of N_2 used for diluting $= R_1 -$ vol. H_2O $-$ stopcock vol.

(3) Final gas volume $= R_3 -$ vol. $H_2O = N_2$ used for diluting $+ N_2$ in unknown

(4) Substituting (2) and (3) and transposing:

Volume N_2 in unknown $= R_3 -$ vol. $H_2O - (R_1 -$ vol. $H_2O -$ stopcock vol.)

(5) Volume N_2 in unknown $= R_3 - R_1 +$ stopcock vol.

(6) Per cent N_2 in unknown

$$= \frac{R_3 - R_1 + \text{stopcock vol.}}{R_2 - \text{vol. } H_2O} \times 100.$$

There is one theoretical inaccuracy in this method. Because of the slight positive pressure necessary to drive the diluting gas into the oxygen absorber, the nitrogen in the capillary tubing is slightly compressed. Thus the reading R_2 is somewhat too high, actually not more than 0.01 cc. In the calculation such an error does not change the nitrogen percentage significantly when that percentage is less than 10.

By this method duplicate analyses regularly agree within 0.03 per cent and analyses of gas from oxygen tanks are in the range specified by the makers; *i.e.*, less than 0.4 per cent nitrogen.

With these measurements we can calculate the nitrogen excretion.

(1) Total N_2 in gasometer and effective dead space

$$= \frac{(\text{Tissot vol.} + 1100)(\%N_2 \text{ Tissot gas})}{100}$$

(2) N_2 originally in this gas

$$= \frac{(\text{Tissot vol.} + 1100)(\%N_2 \text{ in } O_2 \text{ tank})}{100}$$

(3) N_2 added to gases during 7′ rebreathing

$$= \frac{(\text{Tissot vol.} + 1100)\ (\text{Tissot } N_2\% - O_2 \text{ tank } N_2\%)}{100}$$

Of this gas part comes from lowering the nitrogen content of the lungs, part from excretion.

(4) N_2 from lung spaces

$$= \frac{(\text{Resid. air vol.})\ (\text{alv. } \bar{a}\ N_2\% - \text{alv. } \bar{p}\ N_2\%)}{100}.$$

The figure for residual air volume in this instance need be only an approximation since the expression (alv. $\bar{a}$ − alv. $\bar{p}$) is usually less than 5 per cent (as already discussed above). Actually the subjects used in this investigation had residual air measurements by several methods to be described in succeeding papers, and in all we obtained consistent reproducible values by more than one method.

(5) $\therefore$ N_2 excretion

$$= \text{increase in } N_2 \text{ in gases breathed} - N_2 \text{ from lung spaces}$$

$$= \frac{(\text{Vol. Tissot} + 1100)\ (\text{Tissot } N_2\% - \text{tank } N_2\%)}{100} - \frac{(\text{Resid. air})\ (\text{alv. } \bar{a}\ N_2\% - \text{alv. } \bar{p}\ N_2\%)}{100}.$$

In this way we have repeatedly measured the nitrogen excretion under both basal and non-basal resting conditions in four normal subjects. The results are shown in Table I. There is a very considerable variation in values, even in the same subject, which was to be anticipated in view of the large volumes of air involved, the

TABLE I

Normal subjects

Subject		Basal		Non-basal	
		Date	N_2 Excretion	Date	N_2 Excretion
J. L.		January 21	129	February 28	316
Weight	*kgm.* 48	January 21	89	March 4	210
Height	*cm.* 140	January 21	122	March 21	186
Body surface	*sq.m.* 1.41				
Residual air	*cc.* 800				
Average			115		240
A. C.		October 15	228	September 29	274
Weight	*kgm.* 79	October 29	195	October 3	395
Height	*cm.* 178	November 19	185	October 29	286
Body surface	*sq.m.* 1.97			November 19	423
Residual air	*cc.* 1200				
Average			200		345
D. R.		October 18	184	September 27	399
Weight	*kgm.* 80	October 27	177	October 11	229
Height	*cm.* 183	November 17	273	October 27	359
Body surface	*sq.m.* 2.02	December 7	259	November 17	263
Residual air	*cc.* 1500			December 7	246
Average			225		300
R. D.		October 22	154	September 26	335
Weight	*kgm.* 93	October 27	205	October 3	336
Height	*cm.* 182	November 19	282	October 27	249
Body surface	*sq.m.* 2.15	December 3	298	November 19	302
Residual air	*cc.* 1800			December 3	362
Average			235		315
Average of 4 normals			195		300
D. H. (Emphysema)		November 7	581		
Weight	*kgm.* 53	November 8	663		
Height	*cm.* 165	November 17	521		
Body surface	*sq.m.* 1.57				
Residual air	*cc.* 3100				
Average			555		

small percentages of nitrogen in the expired air, and the fact that the final result is a difference of two factors.

The subjects are listed in this table in order of increasing body surface. It will be seen that the average results for nitrogen excretion are lowest in the smallest person and highest in the heaviest, as would be expected.

There is also apparently a significant increase in the non-basal figures over those under strict basal conditions.

Our average results under basal conditions in normal subjects agree well with those previously determined in the literature. The average figure is 195 cc., as compared with 300 cc. under non-basal resting conditions. To these figures there should be added a small correction due to the amount of nitrogen excreted during the initial twenty seconds of hyperventilation, adding approximately 20 cc. to the average figure.

The average of Campbell and Hill's figures (7) is 220 cc. for seven minutes of oxygen breathing; that of Behnke, Thomson, and Shaw (9) under similar conditions, 210 cc.

In subsequent studies of residual lung volumes we have used the figure 220 cc. for the average nitrogen excretion in seven minutes, for an alveolar nitrogen change of 0.8 atmosphere. Usually, in residual air volume calculations, the alveolar nitrogen change is less than this, and the correction factor is then proportionally reduced. In the few experiments where the breathing period is longer than seven minutes, we have added 10 cc. to this 220 cc. for each minute after the seventh. This is an approximate figure obtained from the data of Campbell and Hill (7).

Furthermore, in experiments to be presented it has been necessary to apply a correction for nitrogen reabsorbed into the blood during the breathing of room air, following a period of oxygen breathing. In these cases we have used the same figure for nitrogen absorption as for excretion during a like period of time. The work of Shaw and others on dogs would seem to justify this assumption.

The use of a single correction standard in all subjects may seem too gross an assumption. But, in the calculation of residual air, any error in this figure (220 cc.) will be magnified only by a factor of 1.25 (the reciprocal of 0.80) in the final residual air value, an insignificant error in this larger volume.

In Table I are also listed the results on one patient with emphysema, which are typical of a small group so studied. The figures for nitrogen excretion are much higher than in any normals; in fact impossibly high if we consider that the cardiac output definitely limits the amount of nitrogen excretion in a given time. Let us consider the figure of 660 cc. obtained in patient D. H. on November 8, and let us assume that the conditions most favorable for nitrogen excretion were present; namely, that the nitrogen tension of the blood has not started to fall in seven minutes (obviously too favorable) and that the blood has lost all its nitrogen in going through the lungs. The solubility of nitrogen in blood at atmospheric pressure and 37° C. is slightly less than 1 per cent. Then the figure of 660 cc. would require at least 66 liters of blood going through the lungs in seven minutes, or a mini-

mum cardiac output of over 9 liters per minute. Such an increase over normal circulation is unlikely.

The cause of this unsatisfactory result in emphysema patients is apparent from the figures obtained for the alveolar nitrogen after four deep breaths of oxygen, which in D. H. were over 30 per cent, as compared with 3 to 8 per cent in normal subjects. In other words, he could not rapidly wash the nitrogen out of his lung spaces. It seems probable that the figure of 30 per cent was much lower than the actual nitrogen content of the deeper lung spaces. This nitrogen, which was not washed out in the preliminary four deep breaths, appears slowly in the later oxygen breathing and is calculated as excreted nitrogen. It is possible that a similar situation exists to a less degree in normal subjects with large chests. In that case our results on the larger normal subjects are possibly too high.

There appears to be no theoretical reason for greatly increased bodily nitrogen or increased nitrogen excretion in emphysema. A possible exception exists only in the rare instances of high blood nitrogen content reported by Leiner (10). Since we have been unable to devise any satisfactory method of measuring nitrogen excretion in such cases, we have used our results on normal subjects for the correction factor in residual air determinations on all cases.

CONCLUSIONS

1. Nitrogen excreted in seven minutes of pure oxygen breathing varied from 115 cc. to 235 cc. in a series of four normal subjects tested under basal conditions. These figures are in general agreement with similar data in the literature.

2. Under non-basal conditions, nitrogen excretion during oxygen breathing is significantly greater than under basal conditions.

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