J. Physiol. (1960), **152**, *pp.* 167–175
With 2 text-figures
Printed in Great Britain

NITROGEN ELIMINATION FROM THE TISSUES DURING OXYGEN BREATHING AND ITS RELATIONSHIP TO THE FAT:MUSCLE RATIO AND THE LOCALIZATION OF BENDS

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(*Received* 8 *February* 1960)

In a previous paper (Lundin, 1953) it has been shown that the nitrogen eliminated from the tissues during oxygen breathing seems to be released in an exponential way in three stages. First, there is a rapid phase with an elimination half-time of about 1·5 min, then a slower phase with a half-time of 12–13 min and lastly a slow phase with a half-time of about 110–200 min. On theoretical grounds it is probable that the first phase corresponds to the nitrogen from the highly vascular tissues, such as liver, brain, heart, intestines, etc., the middle phase to nitrogen mainly from the muscles, and the slowest to nitrogen mainly from fat. Probably the free intestinal nitrogen escapes with the third fraction.

The values obtained above for the rapid and slow phases were very much the same as those obtained by Lawrence, Jones, Berg, Henry & Ivy (1948). However, the muscular phase had a higher elimination rate than that found in their experiments. Jones (1951) claims that the values of Lawrence *et al.* accord with the theory that the muscles give rise to the N_2 bubbles appearing during decompression, which are the cause of the group of symptoms called decompression sickness. In the work of Lundin (1953) the tissue N_2 elimination was determined from the accumulation of N_2 during O_2 breathing in a closed spirometer system. Because of technical difficulties involved in the collection and measurement of the small amount of N_2 given off from the tissues during O_2 breathing in a closed spirometer system, the importance of a further examination of this problem with another technique became evident. In the present study we have followed the elimination of N_2 during O_2 breathing by continuously measuring the changes in N_2 concentration of end-tidal air.

METHODS

The N_2 concentration in end-tidal air during O_2 breathing was measured with a Lundin–Åkesson nitrogen-meter. An improved model of the instrument was used which was some five times as sensitive as the previous version (Lundin & Åkesson, 1954). There was no loss

of stability and measurement of changes in N_2 percentages as low as 0·002 % became possible. The experiments were carried out with the subject in a low-pressure chamber in order to achieve a further increase in the output of the instrument. With an unchanged ventilatory minute volume the relative percentage of N_2 in the expired gas is increased at low barometric pressure.

In preliminary experiments the pressure was lowered in two stages, with a period of 90 min at 380 mm Hg followed by 150 min at 190 mm Hg. In this way the N_2 sensitivity would be increased during the later period of slow N_2 elimination at 190 mm Hg, after the rapidly eliminated N_2 had been washed out at 380 mm Hg. Although five experiments on three subjects were carried out in this manner, calculation of the results proved difficult because of the necessity for correction for the slight N_2 contamination, 0·3 %, of the O_2 inhaled, and its increased concentration because of the O_2 used by the body. However, it became evident that the output and stability of the N_2 meter was sufficient to permit the use of a barometric pressure of about 285 mm Hg (or a simulated altitude of 7500 m) which gave a relative increase in the concentration of expired N_2 of

$$\frac{\text{Barometric pressure}}{285}.$$

In order to achieve a further increase in the sensitivity of the N_2 meter the CO_2 and water vapour were absorbed from the expired gas before it reached the N_2 meter. In this way the following increase in sensitivity was obtained,

$$\frac{\text{Barometric pressure} - pCO_2 - pH_2O}{285 - pCO_2 - pH_2O}:$$

so that a given number of N_2 molecules occupied about 3·5 times the volume at ground level. The ventilatory minute volume being the same, this therefore means a 3·5 times as big a N_2 percentage in the CO_2-, H_2O-free end-tidal air. With the more sensitive meter the total increase in sensitivity was about 17·5 times that previously available and it became possible to read changes in the analysed N_2 percentages with an accuracy of about $\pm$0·0006 %. The altitude of 7500 m was chosen because there is very little likelihood of the occurrence of decompression sickness in a resting subject at that altitude.

Procedure

The subject reclined in a comfortable chair and breathed room air for a resting period of 20–30 min, during which the experimental arrangements were tested, the subject being connected to an ordinary air-craft pilot's respiratory demand valve through which he breathed pure O_2. The pressure within the chamber was then lowered with the subject breathing normally. No attempt was made to wash out the lung N_2 by over-ventilation. After 5–6 min the observer outside read the N_2 percentages in the CO_2-, H_2O-free end-tidal air, which was sampled with a modified Otis–Rahn pump (Rahn & Otis, 1947). With this system the air was taken through a container filled with granulated soda-lime to the pumping bladder. A needle valve was placed between the soda-lime container and the pump through which the air to be analysed was sucked to the N_2 meter. This needle valve could be closely controlled from outside the chamber with the help of a servo mechanism. When the chamber pressure had fallen to 285 mm the needle valve was adjusted until the pressure in the ionization chamber of the N_2 meter became 2 mm.

Before the sampled gas entered the ionization chamber it traversed a cooling drier consisting of a Dewar bottle filled with 96 % ethanol and solid CO_2. When the gas passed through a glass tube immersed in this solution its H_2O pressure fell to practically zero.

The N_2 content of the end-tidal air was followed for 240 min. During the first 20 min a reading was taken every 60 sec, and after that time every fifth minute. In a few experiments, with subjects where the fall in nitrogen content of end-tidal air was very uniform,

readings were taken every 10 min after an initial period of 100 min. After 4 hr the experiment was terminated and the subject restored to normal barometric pressure.

In order to avoid leakage of N_2 into the breathing system the subject sat with head and shoulders in a tent of plastic film through which O_2 was streaming. The exhaled air, after passing through an ice-filled copper cooler, went out into the top of this tent. The demand valve and its connexion, which were inside the tent, were surrounded by a small polyethylene bag through which O_2 was streaming from a container outside the low-pressure chamber. After having passed through the bag the O_2 flowed out into the first-mentioned tent. In this way the head of the subject, after a few minutes, was surrounded by an almost pure O_2 atmosphere. This minimized the danger of leakage which, in our experience with the

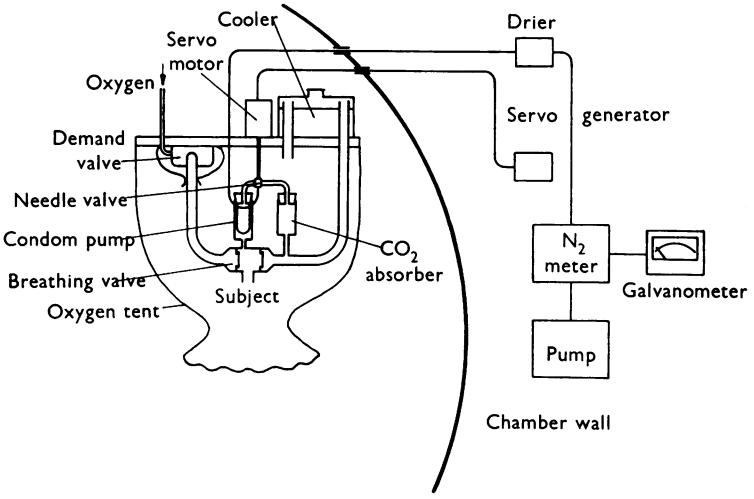

Fig. 1. Semi-schematical drawing of the experimental arrangements.

system, was most likely to occur in the demand valve system, or somewhere along the contact of the soft rubber face mask with the face of the subject. The softness and the seating properties of this mask were arranged to depend upon the gas pressure in a tube which formed its rim, and the subject could control this pressure by means of a rubber balloon. This pump bladder was connected to the mask through a tube which passed through an opening in the wooden disk which made up the roof of the tent. The subject was accustomed to the control of the rim pressure by several blank experiments in the low-pressure chamber. However, even with such precautions some leakage might occur, particularly after some hours, but this was detected on the recording meter and the subject advised to adjust the face mask. A small leakage did not disturb the shape of the elimination curve. After 1–2 min the deviation from the curve disappeared and representative readings could be obtained as before.

Figure 1 is a semi-schematic drawing of the experimental arrangements. The inspired O_2 was analysed for N_2 content with the help of the N_2 meter, and the readings obtained were corrected for the influence of increase in concentration at 7500 m brought about by the O_2 consumption. This correction was made in the following way:

$$N_2 \text{ meter readings} \times \frac{\text{Pressure at 7500 m}}{\text{Pressure at 7500 m} - 38/0{\cdot}8}, \qquad (1)$$

where 38 is the average end-tidal CO_2 pressure previously measured in our subjects, and 0·8 is the assumed R.Q. The value obtained was subtracted from the registered values without transformation into percentages since there was no reason for performing such a

transformation when the alveolar ventilation was not measured. The scale on the N_2 meter was linear in the range of measurements and the values obtained were thus proportional to the percentage values. The corrected values were plotted against time, with time in minutes as the linear abscissa and the N_2 meter readings as the logarithmic ordinate. If the ventilation for each subject was the same throughout an experiment, then this curve should give a picture of the N_2 elimination rate during O_2 breathing for each individual.

The curves obtained were treated in the same way as the N_2 elimination curves in previous work (Lundin, 1953). This means that the last part of the curve, representing the slowest eliminated N_2 fraction and forming a straight line, is projected to the left, and this straight line is used to separate out other N_2 fractions representing tissues with more rapid elimination rates.

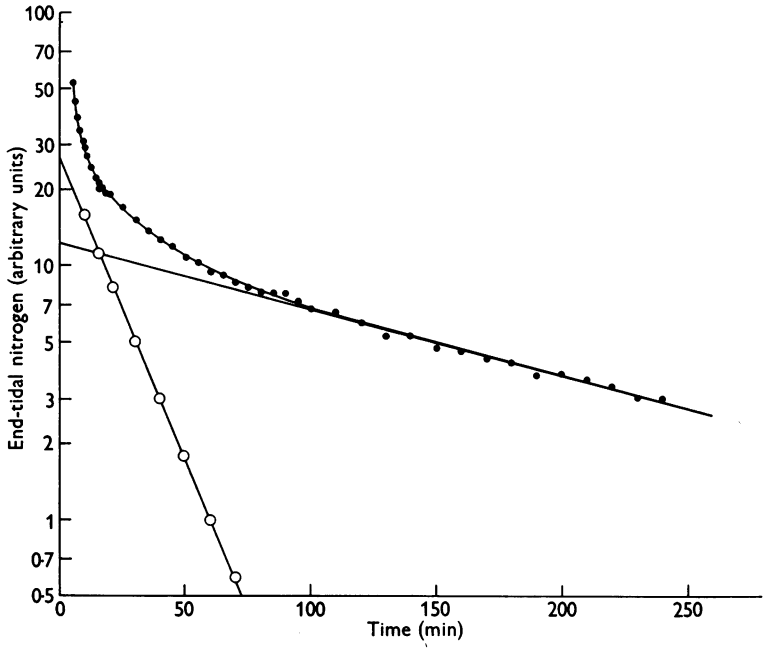

Fig. 2. Nitrogen elimination curve. Semi-log. scale.

The nitrogen value R_t at any point of the nitrogen elimination curve can be expressed:

$$R_t = R'_0 e^{-k_1 t} + R''_0 e^{-k_2 t}, \tag{2}$$

where R'_0 and R''_0 are the values for N_2 at time zero for the component curves, t is time in minutes, k_1, k_2 are time constants, or fraction of N_2 eliminated per minute. The relative amount of N_2 under each curve component is given by

$$N = \frac{R_0}{k}. \tag{3}$$

RESULTS

Thirty experiments were made on six subjects, the number of experiments on each subject varying between 2 and 11. The treatment of the semi-logarithmic curves described above permits the isolation of two com-

ponents. The rapid component reported in our earlier work (representing highly vascular tissues) could not be obtained because the N_2 elimination was not measured during the first 6–8 min. The average values for each subject are summarized in Table 1.

From the table it can be seen that the more rapid phase has mean k values between 0·046 and 0·061 and the slow phase k values between 0·0047 and 0·0079, indicating that the first phase represents tissues which eliminate between 4·6 and 6·1 % of their N_2 content per minute, and the

TABLE 1

Subject	Age	Weight (kg)	Height (cm)	No. of experiments	R_{0_M}	k_M	N_2 eliminated (arbit. units)	Desaturation half-time (min)
				Fraction M				
L.Å.	33	79	182	5	27·5	0·055	500	13·0
B.E.	28	59	165	5	28·0	0·061	460	12·0
G.L.	45	80	183	11	30·0	0·051	588	13·5
I.L.	16	60	163	4	11·7	0·050	234	13·5
S.A.	16	53	163	3	24·0	0·046	520	15·0
L.D.	22	60	176	2	25·0	0·051	490	13·5
				Fraction F		k_F		
L.Å.	33	79	182	5	12·1	0·0059	2050	120
B.E.	28	59	165	5	12·8	0·0048	2670	145
G.L.	45	80	183	11	13·5	0·0047	2870	150
I.L.	16	60	163	4	8·0	0·0051	1570	135
S.A.	16	53	163	3	7·1	0·0079	900	90
L.D.	22	60	176	2	7·6	0·0059	1290	120

Fraction M: the phase of more rapid elimination of N_2 during O_2 breathing, presumably from muscle. Fraction F: the phase of slowest elimination, presumably from fat. N_2 eliminated given by $\frac{R_{0_M}}{k_M}$ and $\frac{R_{0_F}}{k_F}$, respectively. k: N_2 elimination time constant of equation (2). R_0: nitrogen elimination rate at time zero of equation (2), in arbitrary units.

second phase tissues with a N_2 elimination rate of between 0·47 and 0·79 %/min. The elimination half-times vary between 12 and 15 min for the faster and between 90 and 150 min for the slower phase.

There is no relationship between age and elimination rate for the slow phase: but the subjects with the relatively largest N_2 volumes from this phase, namely G. L., B. E. and I. L., have the slowest elimination rates and the subjects with the smallest relative amount of N_2 the most rapid elimination rate. The rate of N_2 elimination from the 'muscle' phase is not obviously related to age. The youngest subject had a somewhat slower elimination rate than the other subjects but was the only one who occasionally slept during the experiment and it is probable that his metabolism was more basal than that of the others. Compared with our previous

results these experiments give the same values for the N_2 elimination rates from the two slowest components of the elimination curve. There is a 10:1 relationship between relative elimination rates for the two fractions labelled 'muscle' and 'fat' nitrogen.

DISCUSSION

Earlier unpublished investigations with the closed-circuit technique by W. M. Boothby & G. Lundin have shown that the elimination rate of N_2 during O_2 breathing is the same at ground level in Lund, and at a simulated height of 7500 m in a low-pressure chamber. These findings indicate that the cardiac output and vascularity of the body tissues remain unchanged. From earlier work (Lundin, 1953) we have seen that the ventilatory minute volume and O_2 consumption remained fairly constant during 4 hr of O_2 breathing. In the present series of open-circuit measurements the subject was quite as comfortable as before and therefore we have assumed that the ventilation and O_2 consumption have been constant during the whole experiment. The N_2 percentage in the end-tidal air should then reflect the amount of N_2 and the blood flow through the body tissues. In our earlier series of experiments (Lundin, 1953) the amount of N_2 from the two phases showed a good relationship to probable amount of muscles and fat of the subjects. In this series of experiments a direct calculation of probable amount of fat and muscles was not possible because the values were given in relative figures. However, an indirect calculation of fat and muscle of the subjects can be made if we assume that the two curves represent nearly all N_2 from the muscle and fat tissues. The area under each component of the curve should be proportional to the amount of N_2 given off.

From anatomical observations we know that about 50 % of the weight of the lean body mass (= total body minus fat) consists of muscle tissue (Vierordt, 1906). From the investigations of Campbell & Hill (1933), among others, we know that the solubility of N_2 per unit weight in fat tissue is about six times that in tissues such as muscle and blood, with high content of water and negligible fat. We can then construct the following equations for the determination of the absolute amount of fat and muscle tissue.

$$(W-y)\times 0{\cdot}50 = X. \tag{4}$$

Where W is total weight, X weight of muscles and y weight of fat, in kg, and $W-y$ thus equals the lean body mass. X is thus the muscle weight.

But

$$\frac{X}{y} = \frac{N_M}{\frac{1}{6}N_F}, \tag{5}$$

when N_M is the surface area of the curve representing the rapid 'muscle'

fraction of the N_2 elimination and N_F is the surface area of the 'fat' curve determined from equation (3).

The amounts of muscle M and fat tissue F in the subjects, calculated from the expression (4) and (5) are given in Table 2.

The values accord fairly well with what could be expected from the habitus of the subjects, and from other observations (Vierordt, 1906; Keys & Brozek, 1953). On subjects L. Å., B. E. and G. L. specific weight

TABLE 2

	Muscle (kg)*	Fat (kg)*
L.Å.	29·5	20·1
B.E.	20·0	19·2
G.L.	28·5	23·1
I.L.	19·3	21·5
S.A.	23·2	6·7
L.D.	24·6	10·8

* Weights are calculated from equations (4) and (5).

determinations were made by Dr W. von Döbeln by weighing the subject in air and in water. The weight of fat, F, in these subjects was calculated from the equation:

$$F = 4{\cdot}5 \times V - 4{\cdot}09\,W, \qquad (6)$$

where V is the volume of the subject corrected for the lung volume and W is the weight of the subject. The weight of fat was for L. Å. = 20·8, B. E. = 18·7 and G. L. = 22·8 kg. These values, together with the fact that the k values in this series of experiments are very much the same as in our former experiments, strongly indicate the reliability of the measurements and probability of the assumptions that the two components represent mainly nitrogen from muscle and fat tissue.

It has been shown by Jones (1951) and by Roughton (1952) among others that the circulation rate through the tissues is proportional to the measured N_2 elimination rate (expressed in terms of the factor k) and also proportional to the solubility of N_2 in tissue when compared with the solubility in blood. Thus from the present experiments the blood flow through muscle tissue should be between 46 and 61 ml./min/l. muscle tissue, and for fat between 24 and 40 ml./min/l. fat tissue.

Do these N_2 elimination values provide any information concerning the origin of the N_2 bubbles which have long been known to give rise to decompression sickness? A final answer to this question probably cannot be obtained from experiments of this nature; but the rapid elimination rate of N_2 from the muscles does not fit in with Jones's theory of the muscles as the place of formation of the N_2 bubbles. Also, the similarity in elimination rate of the muscle phase for younger and older subjects does not

agree with the observations that older subjects are more prone to develop 'bends' and other symptoms at altitude, and that a much longer period of pre-oxygenation is required in older subjects than in younger to avoid the bends.

One observation made in this series of experiments accords well with the assumption that fat tissue, or tissue with the same N_2 elimination rate as fat, is the place where, at least in resting subjects, the bubbles causing bends originate. Two preliminary experiments, performed in two stages, on subject G. L. (with 60 min at 5500 m) had to be stopped because of the symptoms of bends at a pressure of 190 mm. Subject G. L. (who has an experience of several hundred low-pressure chamber runs to different altitudes) is prone to develop bends at 30,000 ft. (9140 m) when the reduction of pressure is rapid. If it is assumed that the bubbles causing the bends are formed on the venous side of the circulation (Behnke, 1951), then the gas pressure in the venous blood at ground level is about 707 mm Hg (573 mm N_2, 47 mm CO_2, 47 mm H_2O and 40 mm O_2). At 30,000 ft. the barometric pressure is 226 mm, a 3·1:1 ratio between the gas pressure at ground level and at this altitude. In the present experiments, with the values obtained for N_2 elimination rate from the 'fat' of subject G. L. it is found that 60 min pre-oxygenation gives a 24 % decrease of N_2 content in the 'fat' tissue. The subject should then have a reduction of N_2 pressure in the venous blood from this tissue of $573 \times 0{\cdot}24 = 137$ mm. The pressure relationship between gases in the venous blood and barometric pressure 190 mm should then be

$$\frac{707-137}{190} \quad \text{or} \quad 3:1$$

which is very much the same as the relationship between ground level and 30,000 ft. with zero pre-oxygenation time. Bends would thus be expected. There is also an old observation that bends in resting subjects are rare at heights below 30,000 ft.

A prolongation of pre-oxygenation to 90 min would give a reduction of 'fat' N_2 of 34 %, or a gas tension in venous blood of

$$707-(573 \times 0{\cdot}34) = 512 \text{ mm},$$

a ratio to final pressure 190 mm, of 2·7:1. This would be equivalent to a rapid reduction of pressure up to 26,000 ft. (7925 m) without pre-oxygenation. No symptoms of decompression sickness were observed in any of the five experiments made under this schedule, nor in any of the other twenty-five experiments where this pressure ratio at the beginning of the stay at altitude was about 707:285 = 2·5.

From these theoretical considerations it seems reasonable to assume a close relationship in desaturation time between the tissues producing the

N_2 bubbles of bends and the fat tissue. The well known fact that fat subjects are more apt to develop decompression sickness strongly suggests that the bends tissue is fat or a tissue which changes its rate of elimination of N_2 in proportion to the amount of body fat.

SUMMARY

1. Nitrogen content of end-tidal air during oxygen breathing has been followed by means of a sensitive nitrogen meter.

2. The nitrogen desaturation rate of the body could be measured.

3. The desaturation curve could be separated into exponential fractions.

4. These fractions can be used to calculate the amount of fat and muscle tissue in the subjects.

5. The blood flow through these tissues can be calculated.

6. A relationship between nitrogen desaturation rate of fat tissue and decompression sickness seems to be probable.

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