CONCERNING THE AMOUNT OF NITROGEN GAS IN THE TISSUES AND ITS REMOVAL BY BREATHING ALMOST PURE OXYGEN.

By J. ARGYLL CAMPBELL AND LEONARD HILL.

(*From the National Institute for Medical Research, Hampstead.*)

UNDER normal conditions, that is, at one atmosphere pressure, the total nitrogen content of the human body is usually estimated as being about 1000 c.c., nearly half of this being present in the fatty tissues. Recently Burns [1921] gave the following figures for nitrogen content of a man of 70 kg.: blood, 30 c.c.; fat, 530 c.c.; bone, 0 c.c.; residue, 435 c.c.; with a total of 995 c.c.

So far as we know, there are no direct observations upon nitrogen content of whole tissues. Many results have been published for body fluids and, in general, these contain much the same amount of nitrogen as water under similar temperatures and pressures. It has been assumed that tissues, with the exception of fat, contain nitrogen in quantities to be expected in solution in the water content of these tissues. Vernon [1907] showed that olive oil, cod-liver oil and lard contain about 5 c.c. of nitrogen per 100 c.c. of substance both at 15° and at 37° C. From this he concluded that fat of mammals dissolves at least five times as much nitrogen as do water, blood and blood plasma. He compared the solidifying point, melting point, specific gravity and iodine value of human fat with those of olive oil, cod-liver oil and lard and, since all three latter contain about 5 c.c. of nitrogen per 100 c.c. of substance, he considered he was fully justified in assuming that human fat would behave similarly as regards gaseous content. In Part I of our present paper we confirm these conclusions by direct observations with a mammalian fatty tissue, *i.e.* yellow bone-marrow.

In connection with problems of decompression of divers it seemed of value to estimate the amount of nitrogen which could be removed from the body by breathing almost pure oxygen. This we have attempted in Part II of our paper.

PART I. ESTIMATION OF NITROGEN CONTENT OF
BONE-MARROW AND BRAIN.

Method. Vernon used Geissler's mercury gas pump to remove the gases from the fluid fats mentioned above, and he then analysed the gases in Haldane's apparatus. We chose bone-marrow and brain, because these tissues could be easily macerated. This was done in a closed graduated cylinder. The macerated tissue was well shaken with water and a measured quantity of almost pure oxygen in a water bath at 37–45° C. After 25–60 minutes' shaking the gas was drawn off and analysed, often in duplicate, in the large Haldane apparatus, and the nitrogen content of the tissue was calculated from the increase in nitrogen content of the gas in the cylinder.

The main object was to devise a simple apparatus (Fig. 1) for macerating the tissue and extracting the gas, without admission of outside air. Air was excluded by placing all the apparatus in a water bath and then carrying out all the manipulations with the apparatus completely covered by water.

The marrow was placed in the graduated cylinder (CY) containing a known quantity of water, the rise in the level of the water giving the volume of tissue added. A disc of paper was then placed over the marrow to prevent the marrow rising to the top of the water. Then the whole cylinder was filled with water at 15° C. and the stopper (E) put in

Fig. 1.

firmly. This stopper was pierced by three openings, a central opening for the shaft (S) of the macerator and two side openings for glass tubes. The macerator (M) consisted of a number of narrow brass prongs (P) radiating from the central shaft and carrying on their under surface a perforated circular plate of zinc (Z), the perforations being 1·5 mm. in diameter. A similar zinc disc covered the top of a small block of solid rubber (R) at the bottom of the cylinder. The tissue was macerated between these two zinc plates by up-and-down and rotatory movements of the shaft (S), which was manipulated by hand.

Before the tissue was macerated a measured quantity, usually about 30 c.c. of oxygen, was run into the cylinder from the rubber bladder (O_2) by way of tubes A, B and C, whilst a similar quantity of water was run out of the cylinder by way of tube D.
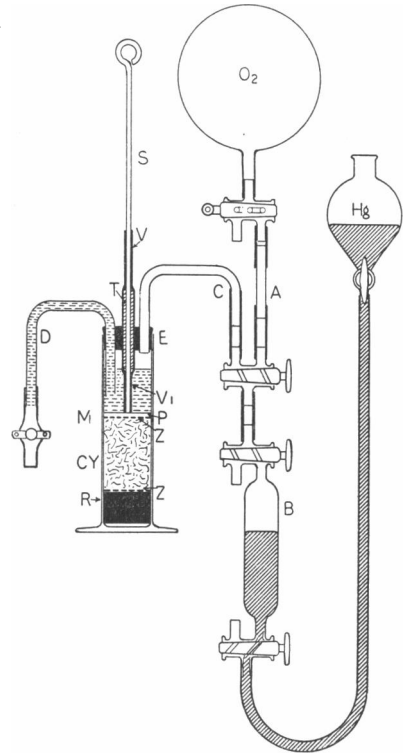
The shaft (*S*) of the macerator was free to move through a metal tube (*T*) in the stopper (*E*). It was tightly clasped by rubber tubings (*V* and V_1) above and below the stopper (*E*), so that although the shaft (*S*) could be moved fairly easily up and down through *T*, only a slight leakage of grease occurred under *V* and V_1, which were made to adhere closely to the shaft (*S*) by means of wire strands fixed fairly tightly around their outer surfaces. The shaft was well lubricated with grease and tube *T* was filled with water to exclude air before the shaft (*S*) was inserted through *V* and V_1; the macerator (*M*) could be screwed off its shaft to allow of this insertion.

All tubes were filled with water at 15° C. and the apparatus submerged before finally assembling for admission of oxygen and maceration of tissue.

Before the tissue was macerated the cylinder was connected by way of *C* with a water manometer so that pressure changes during maceration could be observed, and finally the pressure in the cylinder was equalized with that of the outside air.

After maceration the tissue—still under water at a temperature high enough to melt the fat—was well shaken with the oxygen and water in the cylinder for about 30 minutes or more. Then the level of gas in the cylinder was read off and the gas withdrawn by way of tubes *C* and *B* for analysis. Only a trace of combustible gas was present.

For control exactly similar observations were made with water, but without the tissue. In such experiments, after making allowance for any nitrogen in the water and in the oxygen, there was always an excess of nitrogen, usually about 0·53 c.c. This excess had come probably in part from the rubber fittings and, although great care had been taken to exclude all air, some very small bubbles may have escaped notice. Twenty blank experiments of this nature were performed, details of some of them being given in Table I. The average excess nitrogen

TABLE I. Blank experiments.

Date	Volume of water taken (c.c.)	N_2 c.c. unaccounted for at 37° C. and 750 mm. Hg
16. vi. 30	150	0·46
20. vi. 30	142	0·46
24. vi. 30	144	0·59
24. vi. 30	143	0·56
30. vi. 30	108	0·56
28. vii. 30	92	0·56
29. vii. 30	86	0·65
31. vii. 30	88	0·51
1. viii. 30	96	0·50
	Average	0·53

worked out at about 0·53 c.c., and this amount has been deducted in each experiment with tissue. Examples of calculations used in a blank experiment and in a bone-marrow experiment are given below.

Blank experiment. 24. vi. 30. 144 c.c. tap water at 15° C. placed in cylinder with 30 c.c. gas ($O_2 = 98\cdot34$ p.c., $N_2 = 1\cdot66$ p.c.), and shaken up in water bath at 37° C., for 25 minutes; $P = 747$ mm. Hg. Nitrogen content of gas after shaking = 9·6 p.c. of 30 c.c. = 2·88 c.c.

Nitrogen content of gas at start $=0.5$ c.c.: N_2 content of water at start $=1.94$ c.c.; at end at $37°$ C. water still contained about 0.15 c.c. N_2 $\left(\dfrac{9.6 \text{ p.c.}}{80 \text{ p.c.}} \text{ of } 1.4 \text{ c.c.}\right)$ so that N_2 from water $=$ 1.79 c.c. Total accounted for in gas at end $=2.29$ c.c. (0.5 c.c. $+ 1.79$ c.c.). Therefore nitrogen unaccounted for $=2.88 - 2.29 = 0.59$ c.c.

Experiment with bone-marrow. 30. vii. 30.   22 c.c. sheep's bone-marrow placed in cylinder with 60 c.c. water at $15°$ C. and 33 c.c. gas ($O_2 = 98.79$ p.c., $N_2 = 1.21$ p.c.) at $37°$ C. and $P = 749$ mm. Hg. After maceration and shaking N_2 content of gas $=8.72$ p.c. or 2.87 c.c. The volume of gas present after shaking was much the same as before shaking because the amount of nitrogen and carbon dioxide freed from the tissue and water nearly equalled the amount of oxygen which passed into these. N_2 from water and gas $=0.74$ c.c. and 0.4 c.c. $= 1.14$ c.c.   Difference $= 2.87 - 1.14 = 1.73$ c.c., from which 0.53 must be deducted for blank experiment, which leaves 1.2 c.c. N_2 from 22 c.c. bone-marrow, which still contains $\dfrac{8.72 \text{ p.c.}}{80 \text{ p.c.}}$ of its total; this total therefore equals about 1.3 c.c., so that 100 c.c. bone-marrow would contain 5.9 c.c. at $37°$ C. and 749 mm. Hg or 4.8 c.c. at N.T.P. If bone-marrow contains 96 p.c. fat, then pure bone-marrow fat would contain about 5 c.c. N_2 at N.T.P.

Some observations were carried out with olive oil, using the above method, and the results (Table II) obtained agree fairly closely with

TABLE II. N_2 c.c. content of olive oil.

Date	Volume of oil taken (c.c.)	N_2 c.c. N.T.P. per 100 c.c. oil
8. v. 30	250	5.1
9. v. 30	250	5.2
20. vi. 30	141	5.5
24. ix. 30	114	4.8
	Average	5.1

those given by V e r n o n for this oil, so that the present method may be regarded as sufficiently accurate.

Our main results with pure marrow fat are recorded in Table III; we have taken the fat as forming 96 p.c. of the whole marrow as given by H a l l i b u r t o n [1898 a]. Most of our experiments gave about 5 c.c. of nitrogen at N.T.P. per 100 c.c. of marrow fat; this is the nitrogen content after exposure of the animal or fat to atmospheric air. Some of our lower figures, e.g. 4.2, 4.4, were probably due to the presence of more fibrous tissue than usual; this we observed by the naked eye after maceration in the case of some of the ox-marrow. It may be concluded from all our observations with marrow fat from the horse, ox and sheep, that mammalian fat resembles olive oil, cod-liver oil and lard as regards its nitrogen content, so that V e r n o n's assumption is proved.

Some similar observations were made with brain tissue at room temperature, $16°$ C. The method seemed to give accurate results (Table IV)

TABLE III. Bone-marrow fat, N_2 content.

Date	Animal	Volume of marrow used (c.c.)	N_2 c.c. at N.T.P. per 100 c.c. bone-marrow fat
17. vi. 30	Horse	49	5·3
18. vi. 30	,,	37	5·3
19. vi. 30	,,	34	5·2
19. vi. 30	,,	31	5·4
23. vi. 30	,,	39	5·4
23. vi. 30	,,	36	4·8
26. vi. 30	,,	29	4·9
27. vi. 30	,,	30	5·0
30. vi. 30	,,	30	6·0
22. ix. 30	,,	32	5·4
		Average	5·3
2. vii. 30	Ox	50	4·2
3. vii. 30	,,	50	4·3
3. vii. 30	,,	50	5·0
		Average	4·5
30. vii. 30	Sheep	22	5·0
7. viii. 30	,,	24	5·6
14. viii. 30	,,	26	4·4
		Average	5·0

TABLE IV. N_2 c.c. in brain tissue.

Date	Animal	Volume of brain (c.c.)	N_2 c.c. N.T.P. per 100 c.c. brain
15. v. 30	Calf	125	1·23
16. v. 30	,,	125	1·02
4. vi. 30	Guinea-pig	24	1·10
6. vi. 30	Calf	16	0·64
10. vi. 30	,,	27	0·65
12. vi. 30	,,	30	1·19
13. vi. 30	,,	50	1·08
13. vi. 30	,,	50	1·13

if large volumes of tissue were used, *e.g.* 125 c.c. Then the results were much as expected; brain is stated [Halliburton, 1898 *b*] to contain about 72–82 p.c. of water and only about 5–8 p.c. of fat [Thudichum, 1901], so that 100 c.c. of brain tissue would in all contain only about 1 c.c. nitrogen. Therefore if only small volumes of tissue, *e.g.* 20 c.c., are used, the amount of nitrogen to be detected is small and errors will be much increased. Accurate results were obtained with 50 c.c. of brain. We wished to use brain because it could be removed almost entire and placed under water in the cylinder with exposure of only its surface to the outside conditions; thus, in experiments with animals exposed to compressed air, it might be possible to estimate roughly the nitrogen content under the new pressures. Some such results are given in Table V

TABLE V. N_2 c.c. in brain tissue of guinea-pig after exposure to
+ 9 atmospheres for 30 minutes.

Date	Volume of brain taken (c.c.)	N_2 c.c. N.T.P. per 100 c.c. brain
2. vi. 30	16	3·96
3. vi. 30	16	3·44
5. vi. 30	16	5·40
	Average	4·30

for four guinea-pigs compressed for 30 minutes at + 9 atmospheres.
They were very rapidly decompressed and their brains removed entire
and placed at once below water in the cylinder. Some bubbles were
lost, but in the third experiment the manipulations were carried out
more rapidly than in the other experiments, and 100 c.c. of brain tissue
contained about 5 c.c. of nitrogen; we used 16 c.c. of brain tissue and
got about 1 c.c. of nitrogen from this. At one atmosphere we have seen
that 100 c.c. brain contains about 1 c.c. nitrogen, so that at + 9 atmo-
spheres (*i.e.* 10 total) it should contain 10 c.c. when fully saturated; but
if it be only half-saturated, as it probably is after only 30 minutes'
exposure to the increased pressure and after partial desaturation during
the rapid decompression, we should expect to find only about 5 c.c.
nitrogen. This is near enough to our third result.

An attempt was made to estimate the rate of saturation of bone-
marrow with nitrogen during compression, the animal being killed before
decompression; these results will be published separately.

PART II. NITROGEN REMOVED FROM THE BODY BY BREATHING ALMOST PURE OXYGEN.

Pflüger [1868] showed many years ago that nitrogen was removed
from the blood of a dog when the animal breathed gas without nitrogen.
Leonard Hill [1912] showed that by breathing oxygen the nitrogen
dissolved in urine could be markedly lowered. Bornstein [1913], in a
study of stroke-volume of the heart, found that about 98 c.c. of nitrogen
could be removed from the body in 3 minutes by re-breathing about
3 litres of oxygen from a small bag. He had previously washed out the
nitrogen from the lungs by re-breathing very deeply for 70 seconds from
a bag containing 8–10 litres of oxygen; he calculated the residual air of
the lungs from the amount of nitrogen removed during this 70 seconds.
The amount of nitrogen removed from the body during the first few
minutes was thus about 30 c.c. per minute. This is near the figure to be
expected, since the total blood of the body holds about 30 c.c. of nitrogen

and takes about 1 minute to pass through the lungs. Henderson and Haggard [1925] objected to Bornstein's results for use in estimation of circulation rate, because he estimated the nitrogen indirectly in a Zuntz-Geppert gas analyser. This objection does not appear to be of great significance for our purpose because, apart from oxygen and carbon dioxide, only very small amounts of gases other than nitrogen are expired from the lungs. Parsons [1930] recently found only small amounts of combustible gases, hydrogen and methane, in expired air.

Technique. We have used a spirometer method and chiefly a modification of Bornstein's bag method.

The arrangement of the apparatus in the spirometer method is shown diagrammatically in Fig. 2. About 7 litres of oxygen were placed in the spirometer (*S*) and all the tubes,
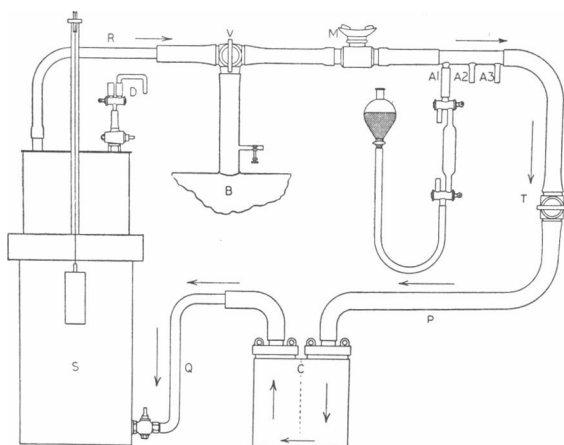

Fig. 2.

etc., were well flushed out with the same oxygen to remove all the air. The subject, sitting at rest, inserted the mouthpiece (*M*) fitted with Roslin valves, and took four very deep breaths of oxygen from the bag (*B*) and expired each breath to the air through tap *T*. At the end of the fourth deep expiration a sample of alveolar air was taken by mercury suction at *A* 1, the subject holding his breath and leakage from bag *B* being prevented. The tap *T* was then turned to shut the passage to the air and to open the passage to the soda-lime canister (*C*); also the bag (*B*) was shut out by tap *V* so that now the mouthpiece communicated with the spirometer (*S*) through tube *R*. The subject re-breathed, with deep inspirations and expirations, the gas in the spirometer after it had circulated by way of tubes *T*, *P*, *Q* and *R*. At the end of 3 minutes the subject made a very deep expiration and a sample of alveolar air was taken at *A* 2 and a sample also was taken from the spirometer (*S*) by way of tube *D* and the volume of gas in the spirometer read off. Then the subject re-breathed the same gas again from the spirometer for another 3 minutes and a sample of alveolar air was taken at *A* 3, also a sample from the spirometer, the volume of gas therein being again obtained. The nitrogen removed from the body was calculated by difference between the amounts of nitrogen present in the tubes, canister, spirometer and

lungs at the start and at the end of each period of 3 minutes' re-breathing. The residual air of each subject was assumed to be similar to that obtained for the same subject by the bag method. The results for nitrogen removed from the body agreed fairly closely with those obtained with the bag method, which will be described in more detail.

In the bag method there were five bags, No. 1 containing about 7–10 litres oxygen, and Nos. 2, 3, 4 and 5 about 5 litres or less of oxygen (see Fig. 3). The subject breathed quietly through a mouthpiece (*M*) without valves to outside air by way of tap *T*, the other outlet of the mouthpiece being blocked by a stopper (*S*). At a signal he made as deep an expiration as possible, and then tap *T* was turned to shut the passage to the outside air and to open the passage to a soda-lime canister (*C*) and to bag No. 1, in and out of which the subject breathed oxygen very deeply for about 70 seconds; then, after a very deep expiration into this bag, the taps were turned to shut the passage into bag No. 1 and
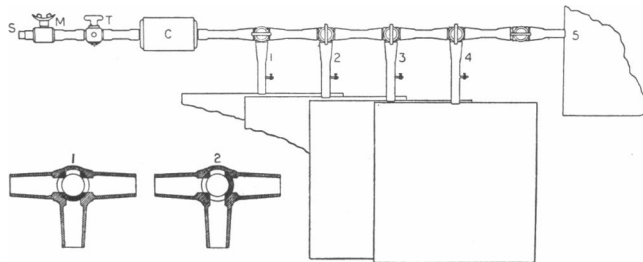

Fig. 3.

to open the passage into bag No. 2. The positions of the different taps at this stage are shown in the figure. The subject breathed deeply in and out of bag No. 2 for 2 minutes, at the end of which time he made a very deep expiration into this bag, and then the taps were turned to shut off bag No. 2 and to open the passage to bag No. 3. He re-breathed the gas in this bag for 2 minutes and later that in bags Nos. 4 and 5. From the nitrogen content of bag No. 1 it was possible to estimate the residual air; and from the nitrogen content of bags Nos. 2, 3, 4 and 5 the nitrogen removed from the body during the next 8 minutes was determined.

The whole apparatus had been well tested under water for leakage before use, all rubber junctions being double wired. The tubes, bags, etc., were well flushed out with oxygen just before the experiment commenced. Then the bags were filled with the required amount of oxygen, and control samples were withdrawn from each bag for analysis just before the subject inserted the mouthpiece. At the end of the experiment samples were withdrawn from each bag and the volume of gas in each bag accurately measured by collection over water in a graduated 10-litre flask. The analyses were carried out in a large

Haldane apparatus, about 5 c.c. of the gas being mixed with a known volume of nitrogen. The same routine for analyses of control and experimental samples was followed, so that any small errors were constant and would cancel out. The control samples for the different bags agreed closely for nitrogen content, and the average was used in the calculations. The capacities of all the tubes, taps, etc., were determined. Control experiments proved that there was no leakage of nitrogen into the apparatus or any great absorption of nitrogen during the time required for the manipulations. Ordinary small Douglas bags (30 litres) of thick corded material were employed. Sample tubes were not evacuated for withdrawal of samples, these being obtained by mercury suction only, to reduce possibility of entrance of any small quantity of outside air.

Example for estimation of residual air after a very deep expiration to the outside air. About 8 litres of nearly pure oxygen were re-breathed for 70 seconds from bag No. 1. At the start the nitrogen in the tubes, bag, etc., was 200 c.c.; at the end of the 70 seconds' re-breathing the nitrogen was 800 c.c. Let $X =$ residual nitrogen in the lung at start; then residual air in lung at start $= 5/4\ X$, assuming that nitrogen forms about 80 p.c. by volume of the air. Then N_2 in tubes, bag, etc., at start (200 c.c.) $+ N_2$ in residual air $(X) = N_2$ in tubes, bag, etc., at end (800 c.c.) $+ N_2$ in residual air at end (10 p.c. of $5/4\ X$; 10 p.c. estimated from alveolar air; the alveolar air is regarded as being of the same composition as that in the bag at the end of the re-breathing); from this $X = 685$ c.c. and residual air $= 5/4\ X \times 685$ c.c. $= 855$ c.c. at 17° C. and 750 mm. Hg or 778 at N.T.P. We have assumed that about 60 c.c. N_2 come, not from the air in the lungs, but from the blood and tissues during the 70 seconds' hyperpnœa, so that there were really 860 c.c. at the end in the tubes, bag, etc.; 60 c.c. have been deducted from this, giving 800 c.c. The chief facts regarding our results for residual air are given in Table VI.

TABLE VI. Residual air c.c. at N.T.P.

Subject	Age	Weight (kg.)	Height (cm.)	No. of observations	Residual air		
					Average	Highest	Lowest
L. H.	64	81·5	183·0	6	1428	1479	1365
J. A. C.	46	60·0	170·0	3	1373	1481	1274
R. S.	35	65·4	177·8	3	1327	1401	1233
C. P.	36	56·0	162·7	11	800	982	658
J. R.	20	64·2	170·7	8	844	1028	710

The figures given in the literature for residual air vary greatly, *e.g.* 600 to 2000 c.c., and each subject must be dealt with separately. Various factors influence the result, particularly rigidity of the chest wall. Figures given in recent editions of text-books are 600 to 1200 c.c. [Evans, 1930], and 1600 c.c. [Halliburton, 1924]; Bornstein obtained about 900 c.c. We have included the dead space of the trachea, pharynx, etc. in the residual air.

Example of experiment with bags. 21. iii. 30. Subject R. S., resting. Residual air estimated from bag No. 1 = 1401 c.c. At start bag No. 2, canister and tubes contained 161 c.c. N_2; at end of 2 minutes' re-breathing these contained 354 c.c. N_2; gain of $N_2 = 193$ c.c., but 113 c.c. of these had come from the lung air as estimated from alveolar and residual air, so that 70 c.c. only had come from blood and tissues. Similarly in 2 minutes' re-breathing from bag No. 3, 109 c.c. N_2 was removed from the body, whereas with bag No. 4 only about 10 c.c. N_2 were removed from the body in 2 minutes, and with bag No. 5 only 29 c.c. N_2 were removed. Assuming that 60 c.c. N_2 were removed in the first 70 seconds' deep re-breathing from bag No. 1 we have

N_2 removed during 70 seconds   = 60 c.c.
     ,,   second and third minutes = 70 c.c. or 35 c.c. per min.
     ,,   fourth and fifth   ,,   = 109 c.c. or 54 c.c. per min.
     ,,   sixth and seventh ,,   = 10 c.c.
     ,,   eighth and ninth   ,,   = 29 c.c.

Total in 9 minutes = 278 c.c. at 15° C. and 746 P
or 254 c.c. at N.T.P.

We are not able to conclude that the above figures are very accurate for separation into each period of 2 minutes without assuming that the lungs were equally well emptied at the end of each period. The error, we think, was not very great, and certainly the total cannot be appreciably affected.

Results. Some of the experiments were carried out during rest, and others during exercise under normal atmospheric pressure. Exercise consisted sometimes of quick stepping movements with the apparatus suspended on the back of the subject, and sometimes the subject worked at 12, 150 kg. metres per hour on a bicycle ergometer for periods up to 30 minutes before the re-breathing commenced. The exercise was expected to help in removal of nitrogen on account of the increase in circulation rate. In other experiments the subjects, sitting at rest, were exposed to increased atmospheric pressure (+ 1 or + 2 atmospheres) in a compression chamber at Messrs Siebe Gorman's premises. The averages of the results are given in Table VII.

TABLE VII. Nitrogen c.c. at N.T.P. removed from the body in 5 min., when breathing nearly pure oxygen (average figures).

Subject	Resting	Exercise	Resting + 1 atmosphere	Resting + 2 atmospheres
C. P.	200 (5)*	214 (6)	420 (2)	—
J. R.	205 (4)	268 (3)	—	—
L. H.	185 (2)	279 (4)	279 (1)	—
J. A. C.	189 (1)	205 (2)	295 (1)	—
R. S.	217 (1)	—	—	730 (1)

* Figures in brackets give number of experiments.

It will be noted that, during rest, about 30–40 c.c. nitrogen were removed per minute during the first 5 minutes of breathing oxygen; 40 c.c. may seem a little high, but the circulation rate was probably increased owing to the hyperpnœa which was always carried out by the resting subjects. With exercise there was an increase in nitrogen removed in all subjects over that removed during rest; but this increase was definite in only two of the subjects, namely J. R. and L. H.

After being under + 1 atmosphere pressure for 30 minutes C. P.

showed a marked increase, about 100 p.c., in nitrogen removed when breathing oxygen, over the normal figures for rest. He used the bag method. Subjects L. H. and J. A. C. used the spirometer method, and although showing definite increases under + 1 atmosphere, the increase was not so well marked as in C. P. The breathing was probably altered in raising and lowering the spirometer by each breath, and this method is not so efficient as the bag method in keeping the nitrogen in the air breathed at a low percentage.

Under + 2 atmospheres about 730 c.c. of nitrogen were removed from subject R. S., the figure for normal conditions being 217 c.c. He had been exposed for 40 minutes to + 2 atmospheres of ordinary air, but it is not possible to determine exactly how much extra nitrogen his body would contain after this time. The difficulty in making any calculation is that different parts of the body become saturated with nitrogen at different rates owing to local variations in circulation. Rates of decompression have been worked out from experience gained in experiments and in the practice of diving and of caisson work. In these rates, allowance has been made for parts of the body which half-saturate only in about $1\frac{1}{4}$ hours [Haldane, 1922]; of course some parts of the body may become saturated in 5 minutes. The parts that saturate very slowly will require long periods of time to desaturate.

Our observations prove that when breathing almost pure oxygen during rest, the more easily removable nitrogen, about 200 c.c., is rapidly removed, that is to say, in about 5 minutes. Then there appears to be some decrease in the rate of removal which seems to be due to the nitrogen not diffusing fast enough into the blood from the more remote areas of the tissues, *i.e.* areas poorly supplied with blood vessels. Sometimes, as during exercise, the nitrogen may be removed at the rate of 30 c.c. a minute up to the eighth or ninth minute. Results illustrating these points are given in Table VIII. No great accuracy is claimed for the figures when they fall below 20 c.c. per minute. It might have been more profitable to use a more accurate method for estimating nitrogen. Such a method would hardly be convenient, and in any case the conclusions will not be greatly altered.

We have seen above that about one-third to one-fourth of the nitrogen—taking the normal total as about 1000 c.c.—may be removed from the body in about 5 minutes by breathing oxygen; then the rate of removal is much decreased, owing to the rest of the nitrogen in the tissues diffusing slowly into the blood. There can be no doubt that any nitrogen reaching the circulation will be removed from the blood in the

TABLE VIII. Nitrogen c.c. per min. at N.T.P., removed by breathing almost
pure oxygen at normal atmospheric pressure.

Subject	Condition	1st min.	2nd min.	3rd min.	4th min.	5th min.	6th min.	7th min.
C. P.	Resting	54	45	45	17	17	9	9
	,,	—	—	—	—	—	19	19
	Exercise	54	53	53	23	23	29	29
	,,	—	—	—	—	—	30	12
J. R.	Resting	54	40	40	41	41	9	9
	Exercise	54	77	77	51	51	30	30
	,,	—	—	—	—	—	27	13

Subject	Condition	8th min.	9th min.	10th min.	11th min.	12th min.	13th min.	14th min.
C. P.	Resting	12	12	—	—	—	—	—
	,,	15	15	14	14	—	—	—
	Exercise	23	23	—	—	—	—	—
	,,	12	9	9	13	13	9	9
J. R.	Resting	14	14	—	—	—	—	—
	Exercise	13	13	—	—	—	—	—
	,,	13	15	15	14	14	14	14

lungs if the pressure of nitrogen in the alveoli is kept low enough by
breathing nearly pure oxygen. We have tried to estimate the alveolar
nitrogen percentages at the different stages; these are given in Table IX.

TABLE IX. C. P., resting; deep breathing of almost pure oxygen
under normal atmospheric pressure.

Period	N_2 removed c.c. per min. at N.T.P.	N_2 p.c. in gas breathed	N_2 p.c. in alveolar air (or arterial blood)
1st min.	54	2·25	8·30
2nd and 3rd min.	45	2·25	5·17
4th and 5th min.	13	2·25	3·39
6th and 7th min.	13	2·25	2·62
8th and 9th min.	14	2·25	2·95

The figures show clearly that after the fifth minute the head of nitrogen
pressure falls very low, the percentage of nitrogen in the alveolar air—
or arterial blood—being much the same as in the air breathed. Obviously
the nitrogen in the tissues is not coming out so rapidly into the blood
after the fifth minute.

TABLE X. R. S., resting; deep breathing of almost pure oxygen at +2 atmospheres,
after 40 min. exposure to this pressure of air.

Period	N_2 removed c.c. per min. at N.T.P.	N_2 p.c. in gas breathed	N_2 p.c. in alveolar air (or arterial blood)
1st min.	162	3·80	19·77
2nd and 3rd min.	232	3·80	10·44
4th and 5th min.	52	3·80	6·56
6th and 7th min.	20	3·80	4·81
8th and 9th min.	28	3·80	4·15

Results for an experiment with subject R. S. under + 2 atmospheres pressure illustrate the same point (see Table X).

It seems possible that after the more easily removable nitrogen has been removed from the more vascular tissues, *e.g.* grey matter of brain, and at the same time the oxygen tension therein greatly increased, some antagonistic action might occur, such as constriction of blood vessels, to keep out the oxygen. Indeed Tinel [1927] claims to have observed constriction of vessels of the brain during breathing of oxygen. To test this Dr Duke-Elder kindly examined the blood vessels of the eye with the ophthalmoscope after our subject had been breathing pure oxygen for over 5 minutes; Dr Duke-Elder did not detect any narrowing of vessels. One of us [J. A. C., 1930] has detected an increase in carbon dioxide tension in the tissues as an effect of breathing oxygen at high pressure, and this might be interpreted as due to constriction of blood vessels. Leonard Hill and MacLeod [1912] also proved that the carbon dioxide output is decreased whilst breathing oxygen at high pressure. This might have been due in part to constriction of vessels. These workers, however, observed a fall of body temperature at the same time indicating a fall in metabolism.

SUMMARY.

The gaseous nitrogen content of bone-marrow fat of an animal (ox, horse, sheep) breathing air is about 5 c.c. per 100 c.c. fat; the results agree with Vernon's figures for cod-liver oil, olive oil and lard. Brain tissue contains about 1 c.c. nitrogen per 100 c.c. tissue.

The more easily removable nitrogen (200–300 c.c. under normal atmospheric pressure) in the human body is removed in a few minutes by breathing oxygen. After exposure to + 1 and + 2 atmospheres of ordinary air, the amount removed in the same time is about doubled and trebled respectively.

REFERENCES.

Burns, D. (1921). *An Introduction to Biophysics*, p. 270.

Bornstein, A. (1913). *Z. exp. Path. Ther.* **14**, 135.

Campbell, J. A. (1930). *J. Physiol.* **68**, *Proc.* p. vii.

Evans, C. L. (1930). Starling's *Principles of Physiology*, 5th ed. p. 847.

Haldane, J. S. (1922). *Respiration*, p. 346.

Halliburton, W. D. (1898 *a*). Schäfer's *Text Book of Physiology*, **1**, 17.

Halliburton, W. D. (1898 *b*). Schäfer's *Text Book of Physiology*, **1**, 115.

Halliburton, W. D. (1924). *Handbook of Physiology*, 17th ed. p. 351.

Henderson, Y. and Haggard, H. W. (1925). *Amer. J. Physiol.* **73**, 193.

Hill, L. E. (1912). *Caisson Sickness*, p. 188.

Hill, L. E. and MacLeod (1912). *Caisson Sickness*, p. 138.

Parsons, T. R. (1930). *J. Biochem.* **24**, 585.

Pflüger, E. (1868). *Pfluegers Arch.* **1**, 251.

Thudichum, J. L. (1901). *Die Chemische Konstitution des Gehirns der Menschen und der Tiere*, p. 292.

Tinel (1927). *C.R. Soc. Biol. Paris*, **96**, 665.

Vernon, H. M. (1907). *Proc. Roy. Soc.* B, **79**, 366.