XXVIII. A GAS ANALYSIS APPARATUS ACCURATE TO 0.001 %. MAINLY DESIGNED FOR RESPIRATORY EXCHANGE WORK.

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THE safest and by far the simplest method of determining the total respiratory exchange of large animals and of man is that introduced by Jaquet, according to which a respiration chamber is ventilated by a current of pure air which is maintained and measured by a gas meter working as a pump. From the current of outgoing air an average sample is collected and analysed, and from the analytical data combined with the ventilation the gas exchange can be calculated. As the determination of the ventilation can easily be made more than accurate enough for the purpose the total accuracy depends on the sampling and the gas analysis.

In gas analysis by current methods [Haldane, 1912; Petterson-Sondén, described by Sondén and Tigerstedt, 1895], the carbon dioxide can be determined with any desired accuracy (to 0.0005 %), but to obtain accurate oxygen determinations is more difficult and even for the best instruments it is not claimed that they can accomplish more than 0.005 % while in actual practice errors of 0.01 % are common enough.

As the oxygen deficit in the air leaving a respiration chamber cannot be increased beyond 2 % and ought not for several reasons to be increased beyond 1 % the accuracy obtainable is scarcely satisfactory, though it must be admitted that, even as it stands, the accuracy of the Jaquet method is certainly not inferior but on the whole superior to that of the Regnault method, the more so as the respiratory quotients obtained with large Regnault apparatus are rather unreliable as shown by the researches of Carpenter [1915].

A great deal would be gained if the accuracy of the gas analysis could be increased sufficiently to allow an oxygen deficit of 0.5–1 % to be analysed without causing errors on the gas exchange exceeding 0.2 %, and this is what I have endeavoured to attain.

The sources of error which prevent the oxygen analyses from being as accurate as the CO₂ determinations are intimately connected with the presence of water and dirt in the gas burette. Water must be present to insure the saturation of the gas with vapour, and dirt will accumulate rather rapidly from the contact of the mercury with the rubber tubing and with oxygen.

In analyses of atmospheric air or the outgoing air from respiration chambers the volume of the burette between the 0 mark at the lower end and the reading after the absorption of oxygen is always about 21 %, and this volume is usually partly expanded into a bulb, as a cylindrical stem with divisions capable of being read to 0.001 % would become inconveniently long. The amount of water and dirt present in this bulb cannot be constant, and this is the chief reason why individual analyses differ. If the burette is kept scrupulously clean and is moistened with water in a constant manner before each analysis, as done by Benedict [1912], the variation of the results can be kept well below ± 0.01 %, and the results will remain comparable over long periods, but when this precaution is not taken the variations become larger, and two analyses of the same air made with an interval during which 5–10 other analyses are carried out may differ considerably. This is due, as pointed out before by A. and M. Krogh [1913], chiefly to the amount of water in the burette which does not remain constant, but decreases steadily by distillation into the absorption pipettes which contain strong solutions of alkali, possessing low vapour tensions¹. I attempted to obviate the difficulties by measuring the air in a state of complete dryness, but this involved so many difficulties that I had to abandon the plan.

DESCRIPTION OF APPARATUS.

I have now attained the end desired by two improvements on the usual arrangements.

The most important of these is to use three separate gas burettes (Fig. 1 (1, 2 and 3)) of which one (1) is employed exclusively for moving the air to and from the absorption pipettes, while the second (2) is of a suitable size to measure the air before and after the absorption of CO_2 and the third (3) to measure it after the absorption of O_2 . The water vapour necessary for saturating the sample of air, when it has become partially dried in the absorption pipettes, will be supplied by the first burette, and the variations in the amount of water present in this burette will have no influence upon the accuracy of the measurements. The two other burettes (2 and 3) contain just enough water to insure that the sample remains saturated. This water cannot interfere with the readings, because it does not enter the divided stems of the burettes, and its amount will remain constant over a long series of analyses, because the air is always saturated before being brought in contact with it.

The second improvement is that in my apparatus the mercury is raised and lowered in the burettes not by raising and lowering a mercury reservoir but by means of air pressure, an arrangement which obviates the use of rubber connections between the burettes and the reservoirs and moreover facilitates considerably the analytical manipulations.

¹ The soda or potash lye for CO_2 absorption should never be more concentrated than 10 % which is amply sufficient to absorb the CO_2 from an air sample completely and rapidly.

Fig. 1.

The details of the arrangement for raising and lowering the mercury in one of the burettes is shown in Fig. 2 and parts of it also in Fig. 1.

In Fig. 2 (1) is the burette which is mounted together with the other two and the corresponding reservoirs on a wooden stand (2, comp. Fig. 1). The stems of the burettes and reservoirs are carried through short wide metal tubes (3) in the bottom of the water bath, and a watertight connection which does not expose the glass tubes to any undue strain is brought about by means of soft rubber in the manner shown (4). Below the water bath the burette is connected with the reservoir (9) by means of the U-tube (5) which is provided with the stopcock (6) and the side tube (7). On the side tube is a short piece of stout rubber tubing stoppered with a glass stopper (8). By a screw clip on the rubber a fine adjustment of the level of mercury can be brought about, when the tap (6) is closed. The top of the reservoir (9) is connected by the wide Π -tube (10) and rubber tubing (11) to the three-way tap (12)¹. The Π -tube (10) is interposed to prevent an accidental sucking over of the mercury. The tap (12) is connected through the bottle (13) to a filter pump (14). The same bottle is connected through separate three-way taps with the other reservoirs.

When the tap (12) is open to the atmosphere as shown in Fig. 2 the mercury can rise in the burette to the zero mark at the top, and when by turning tap (12) 90° connection is established with the filter pump, the mercury in the burette can be lowered. The height of the reservoir (9) and the quantity of mercury put into it are so adjusted that the position of equilibrium will bring the meniscus in the burette very near to the zero mark.

Water which is just acidulated by sulphuric acid is introduced into the burettes by taking out the stopcocks (13) and (14) (Fig. 1). In burette 1 about 30 cubic mm. are introduced, and this quantity will last for about 20 analyses (of CO_2 and O_2) corresponding to a loss by distillation of 1.5 cubic mm. during each analysis. In burettes 2 and 3 the quantity of water must be so small that it will always remain in the bulb and not be carried down into the stem, where it would hinder the accurate reading of the mercury meniscus. On the other hand so much water must be present that it is visible as a distinct line along the mercury meniscus when this is anywhere in the bulb. Air is never introduced into any of these burettes directly from the absorption pipettes or from outside but always from burette 1 where it is saturated with moisture.

Burette 2 will in the course of time become a little dirty while 3 will remain clean. The dirt must therefore be caused mainly by oxidation, but we have not been able to detect any diminution in volume when air is left in the burette for a number of minutes.

When a burette has to be cleaned the mercury is lowered to a point just below the side tube (7, Fig. 2) the stopper (8) is removed and a glass tube

¹ The taps (12) are not really placed behind the burettes and reservoirs, but on a line with the taps (6) (see Fig. 1) so as to be easily accessible.

bent at a right angle inserted. Tube (29, Fig. 1) is connected through tap (25) with the filter pump and cleaning fluid (potassium dichromate in dilute sulphuric acid) drawn up. The burette is finally washed with distilled water and dried by a current of air filtered through cotton wool.

Fig. 2.

The CO_2 absorption pipette (10, Fig. 1), the O_2 absorption pipette (11) and the combustion pipette (9) are mounted together on a wooden stand placed in the same water bath with the burettes. The arrangement of the pipettes has been dictated by several considerations. The main point is to avoid any diffusion of oxygen beyond tap (17) before the absorption takes

place, and this end is fully attained in ordinary analyses in which only CO_2 and O_2 are determined. When, after the absorption of CO_2 , connection is established through taps (16, 17 and 18) with the pressure index (20) oxygen will diffuse into the space between taps (16) and (17) but before it can get beyond tap (17) this tap is closed and is not opened again before all (or practically all) the oxygen has been absorbed. If, however, repeated absorptions of CO_2 and corresponding readings become necessary, as in combustion analyses, the possibility cannot be avoided that a trace of oxygen may get beyond tap (17) and escape absorption. Combustion analysis cannot therefore be carried out together with oxygen analysis on the same sample if the greatest possible accuracy of the latter determination is desired.

As the air between tap (17) and the pressure index is always pure nitrogen there is not the slightest danger of contamination of the analysis from this side.

Logically the combustion pipette (4) ought to be placed between the pipettes for CO_2 (5) and for O_2 absorption (6), since combustion is always carried out after the absorption of CO_2 . I did not venture however to have the heavy (about 1 kg.) combustion pipette fused on to the other two and therefore preferred to arrange it as shown.

The CO_2 absorption pipette (5) is a plain cylinder with a cylindrical reservoir (10). It is charged with about 10 % potassium hydroxide, carefully saturated with air at the temperature of the bath. It is filled to such a height that the fluid will stand at the mark when the pressures on both sides are equal.

The O_2 absorption pipette (6) is of a peculiar shape designed to insure a maximum of absorption without any danger of small air bubbles being trapped as often happens in pipettes filled with vertical glass tubes. The absorbing fluid for oxygen is Haldane's potash pyrogallate (23 g. of pyrogallallic acid of the best quality dissolved in 230 cc. saturated potassium hydroxide, specific gravity 1.55). When air is introduced rapidly into the pipette a considerable quantity of the viscous fluid remains above each contraction and flows slowly downwards, thus providing a large absorbing surface which is automatically renewed so long as the fluid is flowing down. This arrangement shortens considerably the time necessary for complete absorption. One charge of the pipette will last for about 150 analyses before the absorption becomes sluggish.

The construction of the combustion pipette (4, Fig. 1) has been altered after the execution of Fig. 1 and the final shape is shown in Fig. 3. A vertical spiral (1) of fine platinum wire (0.1 mm.) is arranged between two stout wires. Current is supplied through a rheostat from a couple of accumulator cells or from the lighting installation. One pole (2) is connected with the mercury in the pipette and the other (3) through a sealed glass tube with the upper one of the two wires (4). The vertical heated wire, which is placed as far down in the pipette as possible, insures a rapid circulation of the inclosed air,

and the combustion can be completed therefore within a very short space of time—one minute or less.

As the available pressure in the burettes is insufficient to drive an air sample over into the combustion pipette the reservoir (5, Fig. 3) is connected through the three-way tap (27, Fig. 1) with the filter pump.

METHOD OF CONDUCTING AN ANALYSIS.

An ordinary analysis involving the determination of CO_2 and O_2 is performed as follows (Fig. 1). We suppose all the fluids to be brought exactly to the zero marks on the burettes and pipettes, the three-way taps (13–16) to be turned into their normal position $\perp$, the index drop of petroleum (20) to stand at 0, the apparatus to be filled with nitrogen and the pressures in the compensating vessel (21) and the apparatus to be equal. We have the sample of air to be analysed in a glass vessel under a positive pressure. The sampling vessel is connected by means of narrow lead or rubber tubing with the horizontal tube to the left (28). The four-way tap (12) is turned so as to connect the tubes (28) and (29) $\perp$, and the connections are washed out with the air to be analysed. The tap (21) is next turned so as to connect the sample with the burette (1) $\neg$. The mercury reservoir (7) is connected with the filter pump through the tap (25). The tap (22) on the burette is opened and the sample drawn in. When the mercury reaches the 50 cc. mark on the burette the tap (22) is closed and (25) opened to the atmosphere. A slight positive pressure should now obtain in the sampling vessel and the burette (1). The lead tube is disconnected from the sampling vessel and while the burette (1) is thus open to the atmosphere the excess of pressure blows itself off. The tap (12) is turned so as to connect the burette (1) with the rest of the apparatus $\neg$. Tap (13) is turned from the normal position $\perp$ into this $\neg$, thereby establishing connection between

Fig. 3.

the burettes (1) and (2). Taps (22) and (23) are opened, and by opening the tap (26) to the filter pump the sample is transferred to burette (2). When the mercury in (2) has reached the 50 cc. mark on the stem the tap (23) is closed and (26) opened to the atmosphere, and thereupon tap (22) is closed and the water on top of the mercury in burette (1) brought to the zero mark just below tap (12). Tap (13) is turned to the position $\top$ and the taps (18) and (19) connecting the apparatus and the compensating vessel with the pressure index are opened alternately, while the index is watched and the level of the mercury in burette (2) adjusted according to its movements. Finally both taps (19 and 18) are opened ($\top \dashv$), the index set at 0, the burette read and the taps (19 and 18) closed again ($\wedge \lambda$).

The tap (16) in the potash pipette is now turned from $\perp$ to $\dashv$ and tap (23) opened in consequence of which the sample is driven over into the pipette (5). When the mercury in (2) reaches up to the zero mark tap (23) is closed and usually the mercury is raised by the adjusting screw clip to a little below the tap (13). The tap (22) on burette (1) is now opened and by alternately opening the tap (25) to the filter pump and to the atmosphere the mercury in (1) is lowered and raised 4 times, about 10–15 cc. each time to wash out the connections and insure the complete absorption of the CO_2 . This being finished the sample is drawn into burette (1). The potash in (5) is set at the zero mark and the tap (16) closed ($\succ$). In just the same way as before the sample is transferred from (1) to (2), connection is cautiously established through the index with the compensating vessel, the level of mercury in (2) adjusted and read.

Tap (17) is turned so as to establish connection with the O_2 absorption pipette (6) $\top$, and by opening tap (23) the sample is transferred to this pipette. As the available mercury pressure is not sufficient to drive the whole of the sample over into (6) the glass bulb (31) is lowered from the hook (32) to (33). The water in bulbs (30) and (31) protects the pyrogallate solution from contact with the atmosphere. As before the mercury in (2) is brought up to a point a little below the tap (13). By means of burette (1) the sample is taken backwards and forwards to hasten the absorption of oxygen. I have found it most effective to keep it constantly moving. After a suitable number of movements (usually 6) the sample is taken back into (1) and the tap (17) closed ($\succ$). Tap (16) is now opened ($\dashv$) and the sample taken once into the potash pipette (5) in order to "fetch" the air between the zero mark and the tap (16). Thereupon it is again taken into (1). The potash is set at zero and (16) turned to the normal position ($\perp$). Tap (17) is opened ($\top$) and the sample again carried forwards and backwards between burette (1) and pipette (6) to absorb the last traces of oxygen. Finally the sample is taken back into (1), the bulb (31) is raised to (32), the absorbing fluid in (6) set at zero and the tap (17) closed. The water on top of the mercury in burette (2) is now set at zero, the tap (13) turned to the normal position ($\perp$) and thereupon the sample is transferred from (1) through the tap (14) to burette (3), where it is measured for the third time.

An analysis of CO_2 and O_2 can be made in about 16 minutes. Before the next analysis is made the taps (18) and (19) are opened to the atmosphere (**T T**) and the nitrogen in burette (3) forced out through (18). The water in (3) is set at zero, the tap (14) turned to the normal position while tap (12) is turned so as to establish connection between tubes (28) and (29) (**J**). When finally taps (19) and (18) are closed (**Λ Λ**) the apparatus is ready for the next analysis.

When a series of analyses is finished the nitrogen is stored in pipette (6), and all taps are closed. Before a new series of analyses is begun all menisci are set and finally the nitrogen is driven from burette (1) through the tap (18), which is turned into position **J**, and the index tube out through the tap (19) (**⊥**). This is done to insure that the whole system is filled with nitrogen.

A combustion analysis is performed as follows: When the air sample has been measured in burette (2) CO_2 is absorbed and a second reading is taken. Thereupon connection is established between (2) and the combustion pipette (4, Fig. 1). Tap (23) is opened and afterwards tap (27) connecting the reservoir (9) with the filter pump. Between the reservoir and the tap (27) a piece of very narrow tubing is intercalated. Otherwise the movements of the mercury between the pipette (4) and the reservoir (9) would become too violent. When the mercury in pipette (4) has been lowered sufficiently the reservoir is turned off from the pump and the atmosphere and current put on to the platinum wire for one minute. Hydrogen and carbon monoxide will burn readily when the wire is just visibly glowing, but methane will require a bright red heat. A few cc. air can be drawn backwards and forwards between burette (1) and pipette (4), but with the very low percentage of combustible gases which may be present in an ordinary analysis (0-0.01 %) this will scarcely be necessary. After 1 minute's heating the current is shut off and tap (27) opened to the atmosphere. The sample is drawn into burette (1), the meniscus in pipette (4) set, tap (15) closed, the sample transferred to burette (2) and measured. If there is a contraction in volume an absorption of CO_2 is thereupon undertaken.

The volume of air between tap (15) and the zero mark in (5) cannot be subjected to combustion. It is however only about 0.3 % of the total sample. If therefore the percentage of combustible gases does not exceed 0.3 the error committed will be imperceptible. With a higher percentage it can easily be allowed for.

DIVISION OF BURETTES. CALCULATION OF ANALYSES.

The stems of burettes 2 and 3 have been selected by myself from a large number of glass tubes and are very nearly cylindrical in bore. Their internal diameter is about 2 mm. and they are divided in ten thousandth parts of 50 cc. The distance between two divisions is approximately 1.6 mm. and it is easy by means of a lens to judge $\frac{1}{10}$ or even $\frac{1}{20}$ of the division. The burettes have been divided and marked with a view to facilitate as much as possible

the calculation of the analyses and especially to make it possible to perform all calculations by means of a simple slide rule.

On burette 2 the point corresponding to 50 cc. has been marked 100, and when the CO_2 percentage in the air analysed is less than 1 (100 divisions) the sample taken should correspond as nearly as possible to this volume. If it is desirable, however, a larger volume can be taken, up to 180. Supposing the first reading of the burette to be $100 + a$ and the second after absorption of the CO_2 to be $100 + b$ we have the CO_2 directly in hundredths % as $a - b$. If the initial reading differs considerably from 100 we must apply a correction which is $-\frac{a(a-b)}{10000}$.

Example. Initial reading 170.0, second reading 20.0. Correction $-\frac{70 \cdot 150}{10000} = -1.05$. The corrected figure for CO_2 is therefore $170 - 20 - 1.05 = 148.95$. When the initial reading is between 90 and 110 and the CO_2 is 50 or less the correction can be omitted altogether, viz. $\frac{10 \cdot 50}{10000} = 0.05$. Usually initial readings between 97 and 103 can be secured.

In burette 3 the point corresponding to 79.000 % of 50 cc. is marked 0. If the initial reading is $100 + a$ and the third reading (after absorption of the oxygen) is c we have the nitrogen in hundredths % = $7900 + c - \frac{79}{100} a$.

Example: $a = 2.4$

$c = 11.7$

$\frac{79}{100} a = 1.9$

$\text{N}_2 = 9.8$ Nitrogen percentage 79.098

If the first reading differs considerably from 100 this figure must be corrected by $-\frac{\text{N}_2 \cdot a}{10000}$.

Example:

First reading	175.4	CO_2 $148.3 - \frac{148.3 \times 75.4}{10000} = 147.2$
Second reading	27.1	
Third reading	120.3	
$\frac{79}{100} a$	59.5	N_2 $60.8 - \frac{60.8 \times 75.4}{10000} = 60.35$

Or in per cent. $\text{CO}_2 = 1.472$ %, $\text{N}_2 = 79.6035$ % and $\text{O}_2 = 18.9245$ %.

In gas exchange work it is never necessary to figure out the actual percentages, because differences only are dealt with.

Example: Analyses of

atmospheric air		outgoing air	
1. 99.4		1. 100.1	
2. 96.05	CO_2 3.35	2. 42.0	CO_2 58.1
3. 3.95	N_2 4.4	3. 12.9	N_2 12.8
$\frac{79}{100} a$ +.45	$\text{CO}_2 + \text{N}_2$ 7.75	$\frac{79}{100} a$ -.1	$\text{CO}_2 + \text{N}_2$ 70.9

Differences

CO_2 out. - CO_2 in. $58.1 - 3.35 = 54.75$ hundredths of 1 %
 N_2 out. - N_2 in. $12.8 - 4.4 = 8.4$ " "
 O_2 in. - O_2 out. $70.9 - 7.75 = 63.15$ " "

The oxygen deficit must be corrected in the usual way, assuming the quantity of nitrogen in the outgoing and ingoing air to be the same. The

correction on the ingoing oxygen (O_i) is $O_i \frac{79.00 + N_0}{79.00 + N_i}$. As the fraction $\frac{79.00 + N_0}{79.00 + N_i}$ differs very little from unity we can without introducing any perceptible error transform to $\frac{79.00 + N_0 - N_i}{79.00}$ or the corrected ingoing oxygen $O_i + \frac{O_i}{79.00} (N_0 - N_i)$.

For the purposes of the correction O_i can be taken as constant and $\frac{O_i}{79} = 0.265$.

In the example considered the correction on the oxygen deficit is

$$0.265 \times 8.4 = 2.2,$$

the corrected deficit $63.15 + 2.2 = 65.35$ and the respiratory quotient

$$\frac{54.75}{65.35} = 0.838.$$

ACCURACY OF RELATIVE ANALYSES FOR GAS EXCHANGE WORK.

In gas exchange work the only practical requirement is that analyses of the same air made within a short space of time (say one day) should agree, but whether the apparatus will give identical results when the same air is analysed repeatedly over a prolonged period is irrelevant. That the apparatus fulfils the conditions of gas exchange work has been thoroughly tested by making regular double determinations of the ingoing atmospheric air and a large number of samples of the outgoing air in actual experiments with a Jaquet respiration chamber. The following series of examples have been selected at random from a very large number and show the accuracy attained in respiratory exchange work when the analyses are performed as rapidly as possible (between 3 and 4 per hour).

Table I. Double determinations.

Date	Atmospheric air			Outgoing air		
	CO ₂ %	N ₂ %	O ₂ deficit %	CO ₂ %	N ₂ %	O ₂ deficit %
	0.0	79.0	0.0	0.	79.	0.
8. v. 1917	335	445	78	5775	1315	709
	335	44	775	5785	1295	708
10. v.	32	425	745	5625	1315	694
	33	395	725	563	1315	6945
11. v.	35	42	77	552	125	677
	33	41	74	553	126	679
14. v.	32	405	725	5785	094	6725
	32	—	—	5775	0935	671
15. v.	315	42	735	600	0775	6775
	33	42	75	600	078	678
17. v.	30	41	71	543	089	632
	305	41	715	544	088	632
18. v.	31	40	71	542	127	669
	32	385	705	541	126	667
21. v.	31	465	775	505	192	697
	315	39	705	506	190	696
22. v.	325	485	81	481	1895	6705
	315	45	765	4805	1895	670
24. v.	35	47	82	499	183	682
	30	46	76	4995	184	6835
25. v.	33	45	78	506	1675	6735
	31	45	76	5055	1685	674
Average difference between double determinations	0.0014	0.0012	0.0025	0.0008	0.0009	0.001

Table II. Double determinations.

Date	Atmospheric air			Outgoing air		
	CO ₂ %	N ₂ %	O ₂ deficit %	CO ₂ %	N ₂ %	O ₂ deficit %
	0.0	79.0	0.0	0.	79.	0.
7. ii. 1918	34	44	78	4655	138	6035
	325	445	77	466	1375	6035
8. ii.	315	51	825	4785	1525	631
	315	525	84	4795	1545	634
9. ii.	305	485	79	4575	149	6065
	31	49	80	456	152	608
10. ii.	30	525	825	4565	1535	610
	31	51	82	457	1545	6115
11. ii.	295	53	825	4685	1235	592
	30	545	845	469	1225	5915
12. ii.	38	57	95	485	102	587
	375	58	955	486	101	587
14. ii.	36	43	79	500	0705	5705
	355	43	785	501	0705	5715
15. ii.	34	455	795	4575	1115	569
	34	465	805	457	1105	5675
17. ii.	39	495	88	4605	158	6185
	395	485	885	460	158	618
	32	44	76	5125	0865	599
	32	445	765	512	087	599
Average difference between double determinations }						
	0.0005	0.0009	0.0009	0.0008	0.0010	0.0010

Table III. Double determinations.

Date	Atmospheric air			Date			
	CO ₂ %	N ₂ %	O ₂ deficit %		CO ₂ %	N ₂ %	O ₂ deficit %
1918	0.0	79.0	0.0	1918	0.0	79.0	0.0
5. iv.	325	555	88	16. iv.	31	485	795
	32	555	875		305	48	785
6. iv.	33	54	87	17. iv.	335	535	87
	325	535	86		33	535	865
7. iv.	32	54	86	19. iv.	30	55	85
	325	555	88		305	545	85
8. iv.	315	48	795	20. iv.	31	525	835
	31	48	79		305	53	835
9. iv.	32	40	72	21. iv.	30	525	825
	32	42	74		295	525	82
10. iv.	32	435	755	22. iv.	305	525	83
	315	43	745		31	515	825
11. iv.	315	465	78	23. iv.	30	525	825
	32	455	775		305	54	845
12. iv.	31	45	76	24. iv.	295	52	815
	315	445	76		30	51	81
13. iv.	315	49	805	25. iv.	315	515	83
	315	48	795		32	51	83
14. iv.	31	455	765	26. iv.	305	515	82
	315	455	77		30	515	815
15. iv.	31	48	79	Average difference }			
	315	485	80				
					0.0005	0.0006	0.0007

The atmospheric air analysed was taken from a Copenhagen street about 20 cm. in front of a window in the laboratory. For the analyses given in Table I two separate samples were taken with an interval of 1-2 hours. Each

sample was taken in less than half a minute, and between the analyses from 2-10 analyses of outgoing air were made. For the analyses in Tables II and III one sample only was taken in six separate portions of about 20 cc. each during the two hour experiment, but one of the analyses was made before and one after a series of 3-6 samples of outgoing air.

The analyses show irregular variations from one day to another both in the CO_2 and in the nitrogen percentages, and these variations are sufficiently large to make it necessary to analyse the ingoing atmospheric air each experimental day, if perfectly reliable results of the gas exchange determinations are to be secured.

The variations observed in the carbon dioxide percentage are real and due to the gas exchange of the town, but the variations in nitrogen are to a large extent of instrumental origin and represent variations in the amount of water present in the measuring burettes.

Further details and a discussion on the composition and possible variations of the atmospheric air have been reported in a separate publication [1920].

ABSOLUTE DETERMINATIONS OF OXYGEN AND NITROGEN.

While in a series of analyses of the same air extended over a prolonged period the CO_2 percentage indicated by the apparatus will remain constant indefinitely, the nitrogen and oxygen may show irregular variations amounting to several thousandths of 1 %. When it is desired to avoid these and to make the determinations absolute, as for instance in investigations of the composition of pure atmospheric air, certain further arrangements and precautions are necessary.

1. The measuring burettes must be very carefully calibrated. The calibration is performed as follows. The burettes are disconnected from the tubing below, cleaned and dried completely by a current of air. A tap is put on to each by means of thick rubber tubing. The junction is made rigid by applying a bandage soaked in gypsum. The lower tube of the tap is drawn out to a point (not too fine) and marked with a line about 5 mm. above the point¹. A basin containing mercury is arranged below the burette to be calibrated, and by means of burette 1 mercury is drawn up to the desired mark on the stem (care must be taken to avoid air between the tap and the burette). The basin is lowered until the line on the tap is just level with the mercury, the tap is then closed the burette read and the basin weighed. Thereupon the process is repeated, the mercury being drawn up to the zero mark at the top. The difference between the two weighings represents the mercury necessary to fill the burette.

¹ When a second apparatus of this type is built, the lower ends of the measuring burettes will be ground to a standard size, and a tap with a corresponding ground joint provided for calibration purposes.

I reproduce as an example the calibrations made in 1918:

Volumes in cc. at 20°.

Burette 2			Burette 3		
Date	T.	0 to 100.0 on stem	Date	T.	0 to 0.0 on stem
13	14.97°	50.0052	13	14.60°	39.4900
14	14.60°	50.0052	13	14.79°	39.4890
14	14.80°	50.0045	14	14.90°	39.4901
Average 50.0050			39.4897		
Boring of tap 12 0.021			0.021		
50.0260			39.5107		

$$\frac{1}{100} \times 50.0260 = 39.5205$$

$$\text{Difference } 0.0098 = 0.0195 \%$$

I have found that during the first year after the mounting of the apparatus the volumes of the burettes did not remain constant, but changes amounting to several hundredths of 1 % took place. During the second year and a half the changes observed were very slight, and the volumes have now, probably, become constant.

2. When the dry burettes have been connected with the reservoir and filled with pure and dry mercury a measured quantity of water acidulated with sulphuric acid is introduced into each and preferably 10 cubic mm. into burette 2 and 8 into 3 by which the relation between their volumes is not altered.

3. The water bath must be kept at a constant temperature, lower than that of the room—preferably about 15–16°. To accomplish this it is cooled by means of a current of tap water and heated by a small flame regulated through a sensitive regulator. The lower temperature of the bath will prevent the distillation of water from the burettes to the tubing above.

4. The potassium hydroxide solution must be saturated with air and the pyrogallate solution with nitrogen at ordinary barometric pressure and the temperature of the bath.

5. For absolute determinations the potassium pyrogallate must be tested regarding its absolute absorbing power and the formation of carbon monoxide. The absorbing power can be tested by analysing air with a small proportion of carbon monoxide (1–2 %). When after the absorption of oxygen the residual mixture of nitrogen and CO is put to the test of combustion any residue of oxygen left will cause a contraction of the volume and a formation of carbon dioxide. I have never yet performed this test. I have not the slightest doubt that with a good sample of pyrogallic acid the absorption of oxygen is complete, because the readings become absolutely constant after a very short period of absorption.

When the potassium hydroxide used to make up the pyrogallate solution is not concentrated very appreciable amounts of carbon monoxide are produced by the absorption of oxygen, and I have therefore tried to find out

whether a small quantity of the gas might not possibly be produced also by a concentrated solution. This is done by adding air to the residual nitrogen and subjecting the mixture to combustion analysis. As an average of two series of such determinations I have demonstrated the production of 0.0025 % CO by each analysis. The nitrogen percentage read off must therefore be reduced by subtraction of 0.0025.

6. Even with all the precautions mentioned it is not possible to obtain absolutely constant results over a long time, because a slight accumulation of dirt in burette 2 cannot be avoided, while nothing happens in burette 3 where the mercury is in contact with nitrogen only. Burette 2 must therefore be cleaned frequently and a fresh supply of water introduced after careful drying. The results obtained with a burette which is not quite clean or does not contain the exact quantity of water can be corrected by making control analyses from a stock of standard air alternating with the real analyses. Air which is compressed to 3–5 atmospheres can be stored indefinitely in a strong absolutely dry glass bulb of about 2 litres capacity when it is dried completely before being introduced. I have pumped in the air by means of a bicycle pump through glass tubes with calcium chloride and phosphorus pentoxide. The bulb is arranged as shown in Fig. 4 (1). The reservoir (2) which holds a little over 50 cc. can be connected with the analysis apparatus through the tube (3).

When a control analysis of the standard air shows a deviation which is outside the limits of accidental error (above ± 0.001 % for the nitrogen) the experimental analyses made on the same day should be corrected accordingly.

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Fig. 4.

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