

## A PIPETTE FOR THE MICRO ESTIMATION OF RESPIRATORY GASES.

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### ABSTRACT.

A pipette of new design is described for the estimation of carbon dioxide and oxygen in 0.2 ml. or smaller samples of gas. Procedures described include the use of saturated calcium chloride solution instead of mercury for work with carbon dioxide, and the use of chromous sulphate as an oxygen absorbent.

### INTRODUCTION.

Gas analysis in the pipette to be described is carried out by drawing a small sample of gas into a capillary tube of uniform bore where its length is measured; the sample is then subjected to the action of a specific absorbent after which its length is again measured in the capillary, the diminution representing the proportion of the particular gas absorbed. Procedures for the estimation of carbon dioxide and oxygen are given but of course the apparatus would be equally suitable for any other gas for which a satisfactory absorbent is available.

Timiriazeff<sup>1</sup> was perhaps the first to apply the method to biological investigation. Krogh<sup>2</sup> constructed a tonometer on the same principle, which was later simplified and applied to a variety of uses by Jordan (see Jordan and Hirsch<sup>3</sup>). These pipettes have the common advantage, for certain experiments, of using less than 0.2 ml. of gas for a test, and in experienced hands all have about the same accuracy. The special advantages of the one here described are: (1) it is practically impossible to lose the analytical sample halfway through the test; (2) the danger of spoiling an experiment by adding an extra bubble of outside air to the test gas is virtually eliminated; (3) carbon dioxide is estimated with the same ease as oxygen, with comparable accuracy and without the use of

<sup>1</sup> Timiriazeff. *Ann. d.sc.nat. Sér 7. Botanique*, 2, 111 (1885).

<sup>2</sup> Krogh. *Skand. Archiv f. Physiol.*, 20, 279 (1908).

<sup>3</sup> Jordan and Hirsch. "*Uebungen aus der vergleichenden Physiologie.*" Springer, Berlin, 1927, p. 27.

mercury; (4) a supplementary procedure is described by means of which as many tests as desired may be made on portions of a single sample, e.g. of alveolar gas; (5) a new and convenient oxygen absorbent is used.

#### REAGENTS.

Approximately 0.4M violet chrom alum,  $\text{KCr}(\text{SO}_4)_2 \cdot 12 \text{H}_2\text{O}$ , in 0.1 N sulphuric acid. This is most simply made up as follows. Dilute 1 ml. of concentrated  $\text{H}_2\text{SO}_4$  to 350 ml. with water. In 250 ml. of this dissolve without heat 50 gm. of chrom alum. Keep cool, preferably in a refrigerator. If the reagent turns emerald green in colour discard it. The passage of this chromic salt through a Jones reductor reduces it to the chromous condition, making it an extremely effective oxygen absorbent. The application of the reagent to gas analysis is described by Stone<sup>4</sup> who states that it is 25 to 50 times as effective as other well-known oxygen absorbents, over which it possesses the additional advantage of lack of causticity. Like all oxygen absorbents it must be protected from air, and the technique described below overcomes this difficulty.

Amalgamated zinc, 20 mesh. The preparation as described by Stone and Beeson<sup>5</sup> is as follows. 480 gm. of 20 mesh granulated zinc are stirred in 200 ml. of 3N (25 percent) hydrochloric acid for 30 seconds. Then 200 ml. of 0.013 M mercuric chloride solution (10 ml. of a saturated solution at 25°C. diluted to 200 ml.) are added to the zinc acid mixture. Soon after the addition of the mercuric chloride the evolution of hydrogen practically ceases. The stirring is continued for 3 minutes longer, after which the amalgamated zinc is washed thoroughly by decantation with distilled water. 480 gm. of zinc is sufficient for the Alcoa modification of the Jones reductor; half this quantity suffices for the conventional model. Amalgamated zinc usually retains its reducing power indefinitely. Stone and Beeson state, however, that certain pre-

<sup>4</sup> Stone. *Journ. Amer. Chem. Soc.*, 58, 2591 (1936).

<sup>5</sup> Stone and Beeson. *Ind. Eng. Chem., Anal. Ed.*, 8, 188 (1936).

parations, which at first were entirely satisfactory, suffered a loss of efficiency that could not be explained.

An alternative oxygen absorbent which is satisfactory is Stokes reagent made by mixing the following saturated solutions in the order given.

- 30 per cent sodium potassium tartrate . . . . 5 parts.
- 40 " " ferrous sulphate . . . . . 1 part.
- 60 " " potassium hydroxide . . . . . 1 part.

For a series of tests it is convenient to make up about 100 ml. at a time and to store it in a glass stoppered cylindrical separatory funnel of 125 ml. capacity. Here the slimy mass which appears on the addition of the ferrous sulphate may be removed by repeated inversion after the potassium hydroxide is added. The oxygen in the separatory funnel is of course absorbed, but this does not matter. The absorbent is delivered from the funnel into the gas pipette. It is not necessary to cover the Stokes solution with a layer of oil, but agitation is avoided, and the last few ml. of solution which have changed colour are discarded. It will not keep over night.

Saturated calcium chloride solution. Dissolve, with heat, 500 gm. of  $\text{CaCl}_2$  in 500 ml. water and acidulate with a drop of 10 percent hydrochloric acid. Cool and use the supernatant fluid. The merits of calcium chloride as a substitute for mercury in gas analyses have been discussed by Peters and Van Slyke<sup>6</sup>. In a gas pipette mercury is impracticable because it frequently runs out of the capillary when the apparatus is inverted, and because the capillary would have to be laboriously dried before every test. It is essential that the fluid used be one in which none of the gases in the mixture to be analyzed shall be soluble. Water can usually be used in contact with mixtures containing  $\text{O}_2$  and  $\text{N}_2$ , which are not very soluble in it. Carbon dioxide, however, is so soluble in water that contact with respired air cannot be permitted for even a brief time without significant absorption of  $\text{CO}_2$ . If, however, the water

<sup>6</sup> Peters and Van Slyke. "Quantitative Clinical Chemistry." Williams and Wilkins, Baltimore, 1932, vol. 2, p. 85.

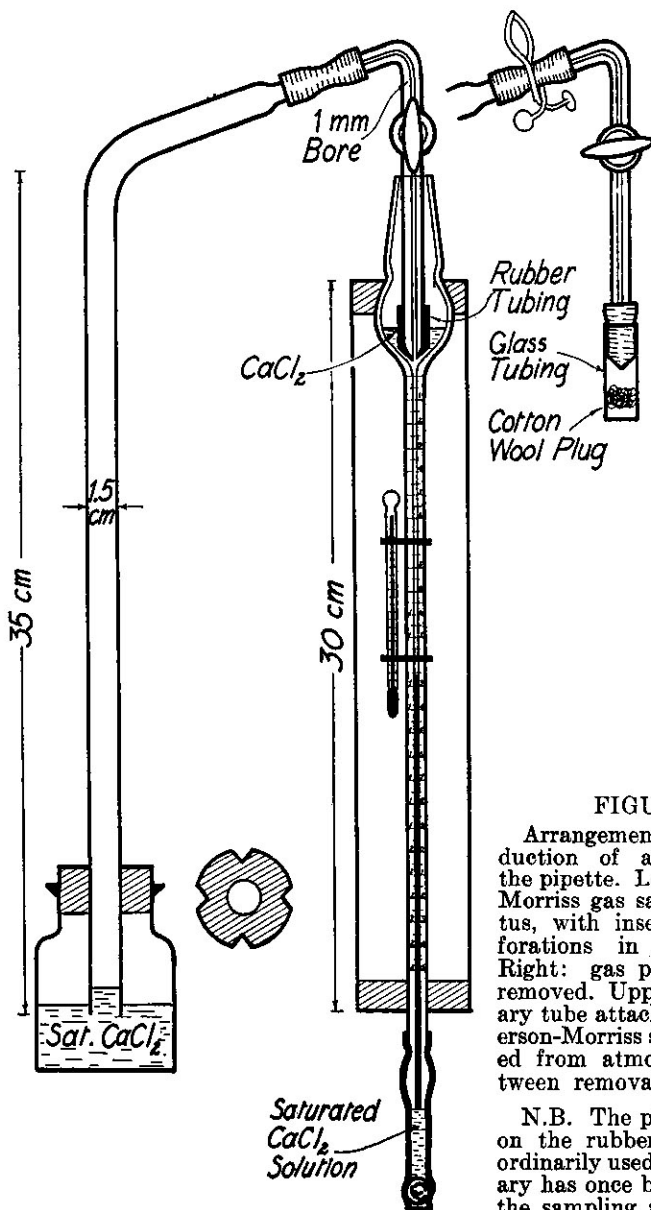


FIGURE I.

Arrangement for the introduction of alveolar gas to the pipette. Left: Henderson-Morriss gas sampling apparatus, with inset showing perforations in cork stopper. Right: gas pipette with top removed. Upper right: capillary tube attachment to Henderson-Morriss sampler protected from atmospheric air between removal of samples.

N.B. The pinchcock shown on the rubber tubing is not ordinarily used after the capillary has once been attached to the sampling apparatus.

is saturated with calcium chloride and slightly acidified, the solubility coefficient of  $\text{CO}_2$  at room temperature is lowered from about 1.0 to 0.06. If the calcium chloride solution is brought into equilibrium with alveolar air, it can remain in contact with respiratory gases for days without measurable absorption of  $\text{CO}_2$ . This is the condition in the Henderson-Morriss apparatus after the first few samples have been taken.

Acetic acid, 5 per cent. This is used to wash the pipette after the use of alkaline solutions.

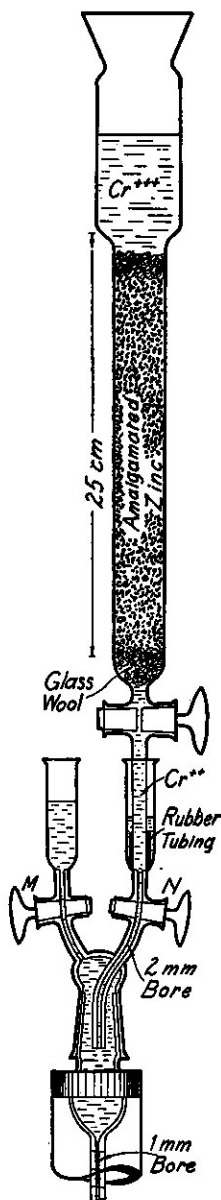
Two per cent sodium hydroxide. This is used as an absorbent for  $\text{CO}_2$ .

#### APPARATUS.

The gas pipette (Fig. I, II, III) consists of a capillary tube of 1 mm. bore, with a central portion 25 or 30 cm. long bearing 1 mm. graduations. To one end of the capillary is attached a short piece of rubber tubing, plugged with a glass bead. The other end is expanded into a chamber of 15-20 ml. capacity over which fits a top with ground glass joint of some 25 mm. diameter. (Standard Taper 24/40 is satisfactory). The top bears two capillary outlets with stopcocks each of which opens above into a chamber of 6-7 ml. capacity. These outlets (M and N of Fig. II and III) have a 2 mm. bore; they differ from one another in that capillary N extends some 4 cm. down into the chamber, while M has no such appendage. The pipette throughout most of its length is enclosed by a water-jacket containing a thermometer.

The alveolar gas sampler illustrated at the left of Fig. I is that of Henderson and Morriss<sup>7</sup>. It consists of a vertical tube of 50-100 ml. capacity held by a cork, from which segments have been removed, in a small wide-mouthed bottle containing a saturated solution of calcium chloride. The distal end of the tube projects just below the surface of the solution, and thus forms a Mueller valve. The other end of the tube is contracted to 7 or 8 mm. inner diameter and connects, by means of a piece of rubber tubing, either with a short glass mouth-piece (not illustrated), or with a delivery tube of 1 mm. bore pro-

<sup>7</sup> Henderson and Morriss. *Journ. Biol. Chem.*, 31, 217 (1917).



vided with a stopcock. The rubber connection is closed by means of a pinch-cock. The collecting tube should not project more than a few mm. beneath the surface of the calcium chloride solution, in order to avoid any serious obstruction to the flow of air. For the same reason the segments removed from the stopper must be large enough to afford, for the escape of air, openings totalling in area the cross section of the collecting tube.

The Alcoa modification of the Jones reductor is illustrated in Fig. II. A layer of glass wool is placed in the constricted end of the tube above the stopcock, and the tube filled with 20 mesh amalgamated zinc to make a column some 20 cm. or more in height. A thin layer of glass wool at the top completes the construction. When not in use the apparatus is filled with distilled water, several portions being used to rinse the zinc. The zinc column should be kept immersed at all times, the level of the fluid never being allowed to expose the top of the zinc, whether the apparatus is used or stored. If the zinc is allowed to become dry it is well to run 200 to 300 ml. of normal sulphuric acid through the apparatus before using it again. When

FIGURE II.

Arrangement for the introduction of oxygen absorbent to the pipette. Above: Jones reductor. Below: gas pipette with top attached and both stopcocks open.

N.B. The pipette at this stage is filled with water.

the apparatus is to be used the water in it is replaced by drawing chrom alum solution through until the chromous solution appearing at the bottom has reached a uniform blue tint. A little hydrogen which is liberated by use does not interfere with the operation of the reductor except to slow the rate of delivery somewhat, but it may be removed from the interstices of the zinc at the conclusion of a series of tests by attaching the reductor to a suction pump and pulling water through.

#### PROCEDURE.

##### I. The Collection of samples of respiratory gases.

*Preliminary analysis of oxygen in atmospheric air.* The pipette, with top and rubber tubing removed, is clamped vertically on a stand. The rubber tubing, whose end is closed by a glass bead, is filled with distilled water, slipped on the capillary, and the water column forced up the latter and into the bell. The pipette is now rotated to a horizontal position permitting the excess water in the bell to flow clear of the capillary opening, and by manipulation of the rubber tube a column of air about 20 cm. long is drawn in. The pipette is again placed vertically and 3 or 4 cm. of water drawn in on top of the column, after which the pipette is removed from the stand and laid horizontally on a table. The rubber tubing is moved until the ends of the air column are equidistant from the ends of the graduations, and the length and temperature recorded. Repeat several times, moving the column a little after each reading (see "Precautions" below). The pipette is now replaced vertically to the stand and the top put on. If chromous sulphate is to be used as an absorbent fill the bell with water through stopcock N; otherwise fill it up with Stokes solution and proceed with the absorption of oxygen. (Note. The  $\text{CO}_2$  in atmospheric air amounts to only 0.03 per cent and may be neglected).

*Carbon dioxide and oxygen in alveolar air.* During a normal expiration the pinchcock at the top of a Henderson-Morriss sampler is opened, the mouth piece inserted between the lips, and the expiration prolonged forcibly. Just at the

end of the expiration the pinchcock is closed. The respirations must remain natural throughout the procedure, and the tendency to catch a short breath just before the forcible expiration must be prevented. The expiration is made entirely through the mouth and at the end the pinchcock is closed sharply in order to prevent even the smallest inspiratory motion. The Mueller valve serves a double purpose. It prevents entrance of room air into the tube, and any inspiration at the beginning or end of the expiration can be detected by observation of the calcium chloride column in the tube.

The glass mouth piece, if one was used, is now removed from the rubber tubing, and the latter filled above the pinchcock with saturated calcium chloride solution. The stopcock of the capillary tube shown in Fig. I top right is opened, and the bent end inserted in the rubber tubing and pushed down to the pinchcock. This causes calcium chloride to displace air completely and drip out the end of the capillary, where it is prevented from becoming a nuisance by the glass tubing and cotton wool plug shown in Fig. I. The glass stopcock is now closed and the pinchcock removed from the rubber tubing, being of no further use in the test.

To prepare the gas pipette for the reception of a sample clamp it vertically on a stand with the top removed, fill the rubber tubing with calcium chloride solution and connect it. The capillary is now filled with solution and a little has run up into the bell. The capillary of the Henderson-Morriss apparatus, whose end is tapered to a cone and covered with thick-walled rubber tubing, is now inserted into the bell under the fluid surface in direct communication with the capillary of the pipette. A light downward pressure with the fingers makes a tight seal. By opening the stopcock and manipulating the rubber tubing of the gas pipette a column of alveolar gas 20 cm. long is slowly and steadily transferred to the capillary. The first sample is discarded by closing the stopcock, loosening the seal, and expelling the gas into the bell. The second or third sample is kept, the procedure being to close the stopcock, remove the alveolar sampling apparatus, pull



down some 3 cm. of solution on top of the gas column, record the length with the pipette placed horizontally, and proceed with the analysis of  $\text{CO}_2$ . At least six tests can be made with one sample of alveolar gas, the limiting factor being the rising column of fluid in the tube above the bottle (Fig. I lower left). If one or two small droplets of calcium chloride get into the column of gas they can be readily measured and their length subtracted from the total.

*Composition of the tracheal air of an insect.* This experiment and the two following are suggested by von Buddenbrock and von Studnitz<sup>3</sup>, whose directions are for oxygen estimation only, with the Jordan pipette. Invert the gas pipette with top removed, so that the bell dips beneath the surface of calcium chloride solution in a suitable vessel. Attach a piece of rubber tubing to the other end and suck through calcium chloride until the whole apparatus is filled. Close the rubber tubing with a screw clamp placed in the position occupied by the glass bead in Fig. I lower right. Now bring any large insect under the solution and quickly open the tracheal system so that the released bubbles float up into the bell. They are then drawn up into the capillary, the length recorded, and the analysis proceeded with.

*The rate of oxygen utilization and respiratory quotient of insects.* The pipette with top removed is clamped vertically to a stand, and the rubber tube filled with calcium chloride and attached. Any excess solution reaching the bell is carefully removed with filter paper, so that the solution just reaches the top of the capillary. Several small insects, e.g. flies, beetles, etc., are put in the bell, the top attached, and stopcocks M and N both closed. After several hours or next day, the pipette is tipped slightly and a little solution forced up into the bell. A column of gas is then withdrawn for analysis and sealed with solution by restoring the pipette to a vertical position. To make the experiment quantitative the insects are weighed and the volume of air in which they were confined

<sup>3</sup> von Buddenbrock and von Studnitz. "Vergleichend-physiologisches Praktikum." Springer, Berlin, 1936, p. 82.

is determined by filling the bell with water and emptying it out into a graduate.

*The maximum utilization of oxygen by insects.* Fill the capillary with 2 per cent sodium hydroxide solution, and allow a little to flow up into the bell. The insects are put in as before except that they are confined in wire gauze to prevent contact with the hydroxide. Continue the experiment until the insects are dead or obviously dying. Collect a sample as before and estimate the percentage of oxygen in it.

## II. The Estimation of Carbon Dioxide.

Carbon dioxide is determined by absorbing it with sodium hydroxide and observing the decrease in the length of the column. Unfortunately sodium hydroxide and calcium chloride react to form a white precipitate and for this reason most of the latter is removed before the hydroxide is added. Rinse

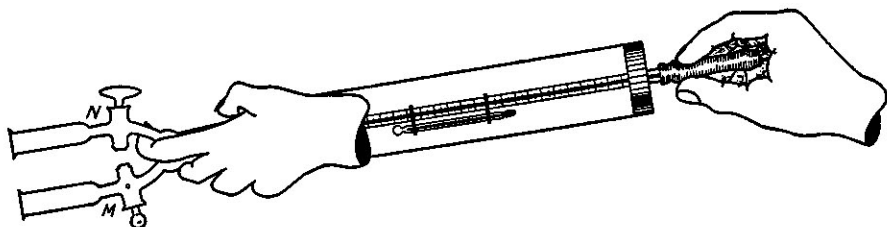


FIGURE III.

Position for expelling the column of gas from the capillary into the bell for absorption of  $\text{CO}_2$  or  $\text{O}_2$ . Although cock N is open, ensuring atmospheric pressure inside at all times, the bubble cannot be lost. Cock M is closed. To withdraw the bubble into the capillary the pipette is inverted. Note that the bubble is manipulated by sliding the rubber tubing up and down rather than by squeezing it.

the bell twice with distilled water, attach the top with a seal of grease and fill the bell with water through stopcock N. Be sure that no bubbles are left either in the bell or capillaries of the top. Close stopcock M and with the pipette held as in Fig. III expel the gas into the bell. Leave stopcock N open and turn the pipette upright again, permitting the bubble to rise. Put a little water in the chamber above N and, by

squeezing the rubber tubing, pump the calcium chloride solution out of the capillary into the bell, where it mixes with the water. Invert the pipette still leaving stopcock N open and withdraw the bubble into the capillary. The length diminishes by loss of about half of the  $\text{CO}_2$  through contact with water. Open stopcock M, empty the apparatus and refill it with 2 per cent sodium hydroxide. Close stopcock M and, as in Fig. III, expel the gas into the bell. A slight turbidity due to precipitated calcium hydroxide does no harm. Keeping the same angle rotate the pipette for 10 seconds, then invert it and withdraw the gas into the capillary. Take several readings of the length and record the temperature. Repeat in order to make sure that all the  $\text{CO}_2$  has been absorbed. This should produce no change in length. Open stopcock M and empty the apparatus leaving the gas in the capillary, protected from the air by some 3 cm. of fluid at the top.

### III. The Estimation of Oxygen.

If chromous sulphate is to be the absorbent used, fill the pipette with water through stopcock N. Empty and refill with water. Close stopcock M and pipette off any water in the chamber above it. A little chrom alum is now passed through the reductor to clear out any oxygen in the tube below the stopcock. It is desirable, but not necessary, to have a column of fluid uninterrupted by air in this tube. One or 2 ml. of water is placed in the chamber above stopcock N and the delivery tip of the reductor brought into contact with the capillary as in Fig. II. A piece of rubber tubing over the tip of the reductor makes a seal which is maintained by the weight of the reductor. It is convenient to clamp the pipette and the reductor on the same stand. Open fully stopcock N and that of the reductor, and by manipulation of stopcock M permit the chamber above M to fill with displaced water. The 5 or 6 ml. of chromous sulphate introduced in this way is adequate to absorb the oxygen. Close stopcock M and remove the reductor. Invert the pipette several times to make the solution inside homogeneous, and then expel the gas

column into the chamber as in Fig. III. Rotate the pipette at the same angle for one minute then invert it and withdraw the bubble. Record the length of the gas column and again expel the bubble into the bell, this time for 10 seconds. Repeat until a minimum reading is obtained.

If Stokes solution is the absorbent used it is not necessary to rinse out the sodium hydroxide as a preliminary. After  $\text{CO}_2$  absorption bring the separatory funnel containing Stokes solution over stopcock N and fill the empty bell. Force the gas column into the bell for one minute as above (Fig. III), and repeat with 10-second additional exposures until a minimum reading is obtained. At the conclusion wash with water and 5 per cent acetic acid.

#### IV. Precautions.

Experiments are most frequently spoiled by air bubbles which remain in the bell and mix with the analytical sample. Before the top is attached they can be readily removed with a camel hair brush. When the apparatus is filled and the top on, the best procedure is to blow gently into the chamber above stopcock M until a large bubble collects in the bell. Close stopcock M and rotate the apparatus in such a manner as to roll the bubble over the whole inner surface where it will pick up any smaller bubbles. Fill the chamber above N and open M to let the large bubble out. Persistent trouble with bubbles suggests a greasy surface, for which the remedy is to wash with ether and leave overnight in contact with sulphuric-dichromate.

When the gas is brought into the capillary it is advisable to wait one minute for temperature equalization before reading the length of the column. The temperature of the water jacket is found to rise gradually during the first two or three tests of a series, due to the heat of the hand. Subsequently it shows little fluctuation.

If the temperature of the pipette varies by  $1^\circ\text{C}$ . or more during any one test it becomes necessary to apply a volume correction to the readings. This could be done by subtracting

from each reading the vapor pressure of water at the appropriate temperature and reducing the dry gas volume to 0°C. Since the pressure does not change significantly during the test it could be assumed at a convenient value, e.g., 760 mm. Temperature corrections, however, are considerably simplified by reference to the accompanying table, which is based on the thermal coefficient of expansion of air. To use this table at the end of a test proceed as follows. From the highest temperature recorded for any reading subtract each of the lower temperatures. Look up in the table the number which corresponds to the length of each gas column and its temperature difference, and add this value to the length reading. After each reading except the highest is corrected in this way the calculations for percentage of oxygen, etc. may be made. For example:

	cm.	Difference in temp.	Value to be added	Corrected result
Initial length of column...	20.13 at 20°	5°	.37	20.50
After CO <sub>2</sub> absorption.....	19.01 at 22°	3°	.21	19.22
After O <sub>2</sub> absorption.....	16.43 at 25°	..	...	16.43

$$\text{CO}_2 = (20.50 - 19.22) = 1.28 \text{ cm.} \quad \frac{1.28}{20.50} = 6.24 \text{ per cent.}$$

$$\text{O}_2 = (19.22 - 16.43) = 2.79 \text{ cm.} \quad \frac{2.79}{20.50} = 13.60 \text{ per cent.}$$

It may be noted that in this example neglect to apply a temperature correction would cause a decrease of 11 per cent in the carbon dioxide results, and 6 per cent for oxygen.

Grease the ground glass connection of the bell each time the top is to be put on so that there will be a clear film of lubricant without air bubbles. When stopcocks fail to turn with perfect ease they should be cleaned with ether and old grease removed from the capillaries with commercial pipe cleaners. A minimal amount of grease is then applied in a

ring about each end of the stopcock, and worked in by turning the core in its casing. A well lubricated cock appears transparent throughout. In warm laboratories a vaseline-rubber-paraffin mixture such as "Lubriseal" of the Arthur H. Thomas Company, Philadelphia, makes the best lubricant.

Table for the correction of gas volumes. Explanation in text.

Length of gas column centimetres.	Values to be added to correct for differences in temperature. Centimetres and degrees Centigrade.					
	1°	2°	3°	4°	5°	6°
10	.04	.07	.11	.15	.18	.22
11	.04	.08	.12	.16	.20	.24
12	.04	.09	.13	.18	.22	.26
13	.05	.10	.14	.19	.24	.29
14	.05	.10	.15	.21	.26	.31
15	.06	.11	.17	.22	.28	.33
16	.06	.12	.18	.24	.29	.35
17	.06	.12	.19	.25	.31	.37
18	.07	.13	.20	.27	.33	.40
19	.07	.14	.21	.28	.35	.42
20	.07	.15	.22	.29	.37	.44
21	.08	.15	.23	.31	.39	.46
22	.08	.16	.24	.32	.40	.48
23	.08	.17	.25	.34	.42	.51
24	.09	.18	.26	.35	.44	.53
25	.09	.18	.27	.37	.46	.55

In the directions above it is advised that the gas column, after being exposed to an absorbent, withdrawn and read, be repeatedly exposed for as many 10-second periods as are necessary to obtain a constant reading. After several such exposures, however, the length of the column increases slightly and if this happens it will necessarily introduce errors. It is therefore best to keep the additional 10-second exposures as small in number as possible.

The whole procedure is based on the assumption that the capillary of the gas pipette is of uniform bore throughout, and it is well to test this with every pipette, by measuring the length occupied by a column in different graduated regions.

Sudden movements of the rubber tubing can stretch or shrink the column of gas sufficiently to introduce noticeable errors. In general the column tends to be a little longer when it is being pulled towards the rubber tubing than when it is being pushed toward the bell. For this reason it is unsafe to rely on a single reading. In a typical case duplicate readings of 22.45, 22.48, 22.46, 22.43 cm. were obtained for the length of the column, for which the probable error is  $\pm 0.03$  per cent.

#### RESULTS.

In a typical set of analyses of oxygen in atmospheric air the results were 20.88, 20.93, 20.33, 21.35, 20.55 per cent. The average is  $20.81 \pm 0.12$ ; expressed as a percentage the probable error is  $\pm 0.6$ . Krogh<sup>2</sup> reports that this last value can be reduced to  $\pm 0.1$  per cent, an accuracy which has only occasionally been attained in the present work.

Duplicate analyses on a sample of alveolar gas gave the following results:

	CO <sub>2</sub>	O <sub>2</sub>	N <sub>2</sub> etc. by subtraction
Individual Tests.....	7.16 6.15 5.76 5.65 6.22	12.57 14.10 14.21 13.23 13.47	80.27 79.75 80.03 81.12 80.31
Average.....	6.19	13.52	80.29
Probable error $\pm$ .....	0.17	0.22	0.16
Percent P. E. $\pm$ .....	3	2	0.2

Comparing these values with those for inspired air it is seen that the nitrogen has increased from 79.02 to 80.29 per cent. Since the blood and tissues are already in equilibrium with the nitrogen of the air this gas is neither absorbed nor excreted in the lungs, consequently the amount of nitrogen which leaves

with the expired air is the same as that which entered with the inspired air. The apparent increase is due to a reduction in the total gas volume during respiration. To compare the readings before and after respiration they must be referred to equivalent quantities of nitrogen, and to do this all the percentages of gas in the expired air are divided by 80.29 and multiplied by 79.02. The resultant figures are compared with those of inspired air in the following table.

	Nitrogen etc.	Oxygen	Carbon Dioxide
Inspired air (theoretical).....	79.02	20.95	0.03
Expired air.....	79.02	13.31	6.09
Difference.....	.....	7.64	6.06

The respiratory quotient in this experiment is  $\frac{6.06}{7.64} = 0.80$ .