

XXXII. A CONSTANT-VOLUME DIFFERENTIAL MANOMETER.

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FOR the gasometric study of physiological reactions, two types of manometer have been used, simple and differential: the simple manometer generally used is the blood-gas manometer of Haldane and Barcroft [1902], the instrument adopted by Warburg; whilst the differential principle is widely used in this country in the Barcroft apparatus [Barcroft, 1908], the application of which to the study of tissue metabolism has been described by Dixon and Elliott [1930]. In our experience the simple manometer is accurate and convenient for nearly all purposes. It is more economical in vessels than the differential manometer, in which each reaction vessel has its own compensation vessel, for several simple manometers can be used with a single thermo-barometer. Further, when a "blank" determination has to be made simultaneously with the main experiment, it is preferable to make it separately in a simple manometer rather than in the compensation vessel of a differential manometer. On the other hand, the simple manometer has certain limitations. Atmospheric pressure fluctuations may be so violent that the use of a separate thermo-barometer does not afford sufficient compensation, although we have never encountered such conditions in London. Again, the use of the simple manometer is restricted in cases where it is necessary to measure small differences between two large amounts of evolved or absorbed gas. For example, in the measurement of respiratory quotients in bicarbonate media [Dickens and Šimer, 1931], it was necessary to use a liquid of sp. gr. 4 as manometer fluid. It is obvious that under these conditions the amounts of gas which can be usefully evolved are limited by the length of the manometer limb, and that the sensitivity of the instrument is decreased by the use of a dense manometer fluid. If, however, the two amounts of gas, the difference between which is to be measured, be released simultaneously one in each vessel of a differential manometer, the amounts which can be released are no longer limited by the density of the manometer fluid, and this fluid can be chosen to suit the differences between the amounts and not the amounts themselves.

In the calculation of the gaseous exchange from the pressure readings, the Haldane-Barcroft manometer, as a constant-volume instrument, has the advantage of great simplicity. The pressure changes due to the evolution or absorption of a given gas have merely to be multiplied by a "vessel-constant" [Warburg, 1926], depending on the volumes of the vessel and of its contents and the solubility of the gas, in order to convert them accurately into mm.³ of gas at N.T.P. The Barcroft differential manometer, on the other hand, works neither at constant pressure nor at constant volume. The menisci in both limbs move, and it is necessary to use a rather complicated formula [Warburg, 1926] to

calculate the gaseous exchange from the observed pressure change. It was with the idea of enabling the simple, accurate, vessel-constant method of calculation to be used with the differential manometer that the constant-volume differential manometer described in this paper has been evolved.

Principle of the constant-volume differential manometer.

This manometer differs from those previously described in that both vessels are kept at constant volume. Each vessel is attached rigidly to its manometer limb, but each limb can be moved up and down independently of the other, as the lower ends are connected only by a length of rubber pressure-tubing filled with manometer fluid (Fig. 1). By raising or lowering one vessel with respect to

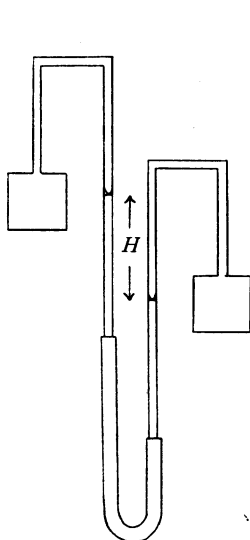


Fig. 1.

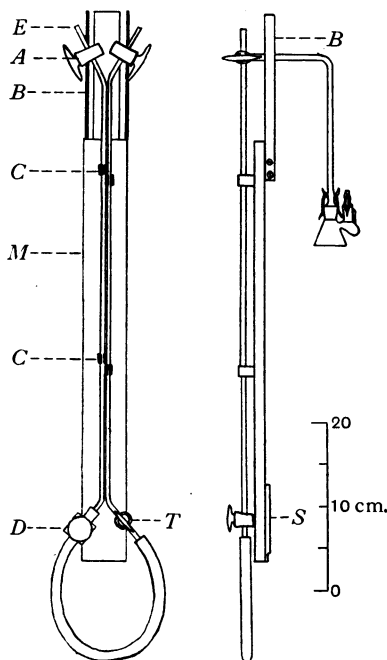


Fig. 2.

the other, the meniscus in each limb is kept at a given mark on that limb, so that each vessel is kept at constant volume. The difference in pressure between the two vessels is then given in terms of height of manometer fluid by the vertical distance between the marks on the two limbs.

In order to illustrate the method of calculation used, two cases will be discussed.

I. One vessel employed for temperature compensation only. If the vertical distances between the menisci, adjusted to their respective marks, be respectively H_0 and H_1 before and after the evolution of a given amount of a gas in the reaction vessel, then the observed pressure change $h = H_1 - H_0$ is related to the volume x mm.³ occupied by that amount of gas at N.T.P. by

$$x = k'_v \cdot h \quad \dots\dots(1),$$

where k_v^t is the vessel-constant of the reaction vessel for the particular gas under the experimental conditions, and is given by

$$k_v^t = \frac{v_F a_v^t + v_G \cdot \frac{273}{t + 273}}{P_0},$$

where v_F = mm.³ liquid in reaction vessel.

v_G = mm.³ gas in reaction vessel and manometer tubing as far as mark.

a_v^t = Bunsen absorption coefficient of the gas at t° .

P_0 = pressure of one atmosphere expressed in mm. of manometer fluid (for Brodie fluid P_0 is approximately 10,000).

II. Different amounts of the same gas are released simultaneously in both vessels. It is arranged that both vessels shall have the same vessel-constant k_v^t for that gas: methods of ensuring this are described later. If the amounts of gas evolved in vessels A and B would occupy volumes x_A and x_B respectively at N.T.P., and if as before h denotes the observed pressure change, then

$$\pm (x_A - x_B) = k_v^t \cdot h \quad \dots\dots(2).$$

Construction.

The instrument is shown in Fig. 2. Each vessel is attached by a ground joint to its manometer. The manometer with attached vessel can be raised or lowered as required, the manometer limb sliding in the spring clips C , and the horizontal tubes being kept at right-angles to the mounting board M by the vertical slots formed by the guides B and the wooden centre-piece. The board M has the brass sleeve S for fixing on to the usual Warburg apparatus by which the vessels are shaken in the well-stirred thermostat (depth 40 cm.). The taps A are T-bored so that the vessels, the manometer limbs and the outside air may be connected in pairs or together. Brodie fluid is used for the manometer liquid, which may be prevented from moving by closing the tap T . The level of the fluid in the manometer limbs is adjusted by means of the screw-clip D which compresses the rubber tubing; metal plates are soldered on to the clip so as to increase the area over which the rubber is compressed. If the rubber were inextensible, it would not be necessary to alter this adjustment once it had been made, but in practice a slight re-adjustment of the screw-clip is necessary for every reading.

The graduations are etched on the glass limbs. One limb (the left) bears a single ring at its mid-point, to which the level of manometer fluid is always adjusted. The other limb carries a scale of mm., 0 to 300, reading from top to bottom, the fluid level in the limb being kept at the 150 mark at the mid-point of the limb. It is important that the limbs should be almost touching, and the scale on the inner side of the capillary; it is then possible to read accurately on the graduated scale on the one limb the level of the ring on the other. A single reading thus determines the relative positions of the two menisci; and the difference between this reading and 150 gives the difference in pressure between the two vessels, expressed in mm. of manometer fluid. The procedure in taking a series of pressure readings is thus exactly the same as with the simple manometer, with the added advantage that no thermo-barometer corrections are necessary.

One or two points in the construction are to be noted. During the raising and lowering, whilst the vessels always remain completely immersed in the thermostat, varying lengths of capillary tube are exposed. It is therefore necessary that the amount of air in the part of the capillary tube involved shall be so small that the change of pressure caused when it is moved up and down is negligible. In order to give a sufficient margin of safety even when the readings involve the full

displacement of the scale, the whole of the tube between the vessels and the tap *A* is made of capillary tubing of such a bore that the increase of pressure when the air in the vertical portion is raised from room temperature to 38° is less than 1 mm. Brodie fluid. It was necessary to use tubing of 0.3 mm. bore, and this was found to be perfectly satisfactory in practice. The manometer limbs are of 1 mm. capillary tubing.

The vessels used may be of any of the usual types [see for example Krebs 1928]; for filling with gas mixtures of known composition the stoppers should be of the type described by Warburg and Kubowitz [1929]. We would emphasise the importance of making the ground joints really tight before the beginning of the experiment by working them in until they will no longer turn. Accidental displacement of the stoppers before this is done is prevented by rubber bands stretched over hooks on the stoppers (Fig. 2).

It should also be noted that either limb with its vessel may be detached separately from the board and removed from the thermostat; this enables fluid to be tipped from bulbs into the vessels one at a time. In this flexibility it has a distinct advantage over the Barcroft manometer. Either limb may be opened to the air by the T-tap; the instrument may then be used as a simple manometer in conjunction with a thermo-barometer. The taps also enable either vessel to be completely shut off: this has an important application, as will be seen later.

The apparatus is described here in its simplest form; it is evident that rack and pinion adjustment of the manometer limbs or similar mechanical additions could be fitted.

Method of use.

The vessels are filled with a suitable gas by connecting the leading tubes by means of a T-piece to the tubes *E* of the manometers. It is desirable to pass the gas under rather higher pressure than usual owing to the narrow capillary tubes. The arrangement for filling the manometer with gas is shown in Fig. 3. The

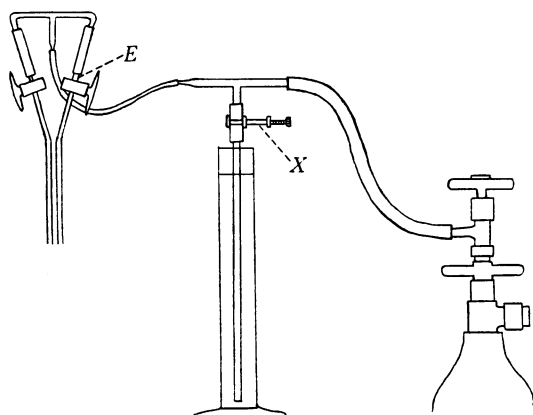


Fig. 3.

pressure from the gas cylinder is increased until gas bubbles pass gently through the water in the safety device. The clip *X* is then screwed up. When enough gas has passed, *X* is unscrewed, the stoppers in the manometer vessels closed, the manometer taps turned and the gas supply disconnected. This filling with gas may be done with the apparatus in or out of the thermostat. After this stage the carrying out of the experiment calls for no special comment.

Calibration. The vessels and capillaries as far as the mark are calibrated by filling with mercury. The vessels are first filled and then slowly pushed on to the ground joint. Owing to the fineness of the capillaries it will be found convenient either to apply suction at *E* while the vessel is being attached, or to warm the vessel slightly after it has been attached, and so to link up the mercury with a thread in the wide capillary.

The vessels should be made of nearly the same size; several methods are then available for securing equality of vessel volumes. A glass bead of suitable volume may be added to the larger vessel, but a more satisfactory permanent adjustment is easily secured by alteration of the size of the side-bulb by blowing, followed by suitable grinding in of the ground joints as a fine adjustment. A certain latitude is permissible; for example, with two 26 cc. vessels containing 3 cc. liquid a difference in vessel volume of 0.1 cc. causes a difference of level of only 2 mm. of Brodie fluid when 1000 mm.³ CO₂ at 30° are evolved in both vessels. A correction to allow for this slight change may readily be made, and since the correction is proportional to the total pressure change for ordinary releases of the order of 200 mm.³ the correction will lie within the limits of error of reading.

The equality of the vessels is conveniently checked by this method by the liberation of CO₂ from a measured amount of NaHCO₃ by means of excess acid. 3 cc. of a 0.2 % solution of NaHCO₃ previously saturated with the gas mixture are weighed into each of the clean dry vessels. The acid (0.4 cc. *N* HCl) is pipetted into the side-bulbs and the vessels are connected with their manometers and filled with oxygen or nitrogen containing 5 % CO₂. This amount of NaHCO₃ liberates some 1600 mm.³ CO₂ at N.T.P. Any change of pressure on acidification of both vessels is noted, and may be applied as a correction in the way already mentioned.

As a further control on the use of the instrument we have liberated known small amounts of CO₂ by adding measured amounts of standard acid (0.2 cc. 1.02 *N*/20 H₂SO₄, volume checked by weighing) from the side-bulbs to an excess of the bicarbonate solution (1.5 cc.) contained in the main part of the vessels, the gas space being filled with a gas mixture containing 5 % CO₂. This method is convenient for controlling the accuracy of calibration and for demonstrating that no measurable error is introduced by the raising and lowering of the fine capillaries in the thermostat (temp. 38°). For the latter purpose care was taken to arrange the menisci at the beginning of the experiment at very different levels; the acid was then rinsed into the bicarbonate in the vessel occupying the highest position and after time had elapsed for temperature equilibrium a reading was taken. The acid and bicarbonate were then mixed in the other vessel, when the level regained its former value. This was repeated many times, first one vessel then the other being at the highest point of its travel at the beginning of the experiment. The results of one such series are shown below together with a simultaneously made series using the simple Warburg manometer. Volume of CO₂ in mm.³ at N.T.P. evolved from 0.2 cc. 1.02 *N*/20 H₂SO₄ (calculated CO₂ = 227 mm.³):

Simple manometer: 225, 226.5, 231, 227.5, 226, 225, 231, 227.5, 225, 226.5, 226, 229.5, 226, 228.5, 226, 227. *Mean:* 227 (highest 231, lowest 225).

Differential manometer: 224.5, 224.5, 225.5, 226.5, 226, 227.5, 228, 227.5, 228, 229.5. *Mean:* 227 (highest 229.5, lowest 224.5).

The satisfactory agreement shows that the error is little greater than that of the scale reading (± 0.5 mm.), and that the submerged length of capillary in the differential manometer introduces no measurable error.

In order to indicate the possibilities of the instrument, we describe an

experiment in which the anaerobic metabolism of rat testis was studied. Pieces of tissue, of equal wet weight, were placed in 3 cc. of glucose bicarbonate Ringer in each vessel of the manometer, a gas mixture containing 5 % carbon dioxide in nitrogen was passed for 4 minutes, and the apparatus shaken in the thermostat at 38° for 10 minutes. The pressure in the vessels was allowed to fall to atmospheric; the vessels were then closed again to the atmosphere and connected to the manometer limbs. An exact reading was taken; the right-hand tap was then turned so that the vessel was shut off and the limb opened to the air. An excess of acid was tipped from a bulb into the closed right-hand vessel. The evolution of gas in the left-hand vessel was followed, using the apparatus as a simple manometer in conjunction with a separate thermo-barometer. After 90 minutes, 226 mm.³ of carbon dioxide had been evolved. The meniscus was then brought to the 150 mark on the right-hand limb, the tap *T* was closed, and the right-hand vessel was connected to the limb by turning the tap *A*. Excess acid was tipped from the bulb into the left-hand vessel. After a few minutes the tap *T* was opened, so that the Brodie fluid now showed the pressure difference between the two vessels. After 10 minutes this became constant. Provided that the tissue had itself produced or consumed no carbon dioxide, so that the total CO₂ in the vessels remained unchanged, the final reading should be the same as the initial reading. As will be seen from the protocol, within the errors of reading, this was indeed so.

Protocol.

Ringer solution: 0.2 % glucose. Bicarbonate content = 580 mm.³ per cc.

Vessels: 3 cc. Ringer in main part. 0.5 cc. 0.5 *N* HCl in bulb; $k_{\text{CO}_2}^{38} = 2.20$.

Tissue: Rat testis. Rinsed 5 mins. in Ringer and afterwards in two changes of salt solution. 150 mg. wet weight placed in each vessel. Dry weights 11.9 mg. and 12.5 mg.

Time: 0 min. Initial reading 148.5. Right-hand vessel closed and limb opened to air; contents of vessel acidified.

Time mins.	Reading	<i>h</i> (corrected for thermo- barometer)
1	146.5	0
20	174	29.5
40	197.5	53
60	220.5	74.5
90	251	102.5

CO₂ evolved = 102.5 × 2.20 = 226 mm.³ $Q_{\text{N}_2}^{\text{N}_2} = 12.5$. Right-hand meniscus adjusted to 150 mark. Tap *T* closed. Right-hand vessel connected to limb. Acidify left-hand vessel contents. Tap *T* opened at 94 mins.

Time mins.	Final reading
100	144.5
110	144.5

Difference between final and initial level	= - 4 mm.	}	corrected pressure change = - 0.5 mm.
Correction for pressure change on acidifying 3 cc. Ringer in each vessel of same apparatus	= - 3.5 mm.		

We are at present developing an improved method for the measurement of respiratory quotient, respiration and glycolysis of tissues in bicarbonate media using the manometer here described.

SUMMARY.

A differential manometer is described in which both vessels are maintained throughout at constant volume.

The pressure change is given as the difference of single scale readings, and the corresponding gas volume is derived from this pressure change by multiplication by a simple vessel constant.

The instrument thus combines the differential manometer's advantage of compensation with the simple manometer's simplicity of reading and calculation.

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