A NEW FORM OF DIFFERENTIAL MICRORESPIROMETER*

By BURRIS CUNNINGHAM AND PAUL L. KIRK

(From the Division of Biochemistry, University of California, Berkeley)

(Received for publication, June 17, 1940)

INTRODUCTION

While more than a dozen microrespirometers have been described in the literature of the past decade, only that devised by Heatley, Berenblum, and Chain (7) is an instrument of general utility like the widely used apparatus of Warburg. While the respirometer of Heatley, Berenblum, and Chain may be used on much smaller amounts of tissue than the standard Warburg apparatus, and is designed to permit mixing of solutions as well as exposure to gases of any desired composition, it is unsatisfactory for the work planned in this laboratory because it does not possess the maximum sensitivity desired by us (0.1 λ/hr.)[1] and is, moreover, rather expensive when the costs of thermostat and optical lever system are included.

In addition to overcoming these objections it was also found that our instrument could be so designed that the chamber size and sensitivity could be simply and rapidly altered to meet the requirements of the diverse materials[2]—protozoan organisms, tissue cells in culture, and tissue slices—to be studied.

It is the purpose of this paper to describe the instrument[3] devised by us as well as to give certain data indicative of the reliability of the measurements obtained with it.

Principle

The respirometer was constructed on the well known differential principle, first used for this purpose by Thunberg (9), and since utilized in modified

* Aided by grants from The Rockefeller Foundation and from the Research Board of the University of California.

[1] 1 λ = 1 mm.^{3}, Kirk, P. L., *Mikrochemie*, 1933, **14**, 1.

[2] The respirometer has also been found useful in studies on insect respiration carried out by Dr. Roderick Craig of the Division of Entomology of the College of Agriculture of the University of California.

[3] Mr. C. E. Camenson of the Microchemical Specialties Co., 2112 Berkeley Way, Berkeley, California, cooperated in the construction of this instrument, and is prepared to supply it.

forms by a number of workers, but especially by Fenn (5, 6) and Barcroft (1, 2).

The essential feature of differential respirometers is the presence of two similar chambers, one of which contains the biological system in its medium, the other an equal volume of medium alone. The two chambers are connected by a piece of capillary tubing carrying an index drop of some liquid of relatively low viscosity and vapor pressure. During the period of measurement the chambers and the connecting tubing constitute a closed system. The consumption of gas in one chamber creates a difference in pressure in the chambers. Equilibrium is restored by the movement of the index drop, which can be measured quantitatively. For many types of work this arrangement is advantageous, permits a cancellation of errors arising from the medium alone, and is independent of external temperature fluctuations, provided that such fluctuations induce the same temperature changes in both chambers. Previously described differential respirometers have failed to take full advantage of this last possibility, which permits elimination of the thermostat. Consequently such microrespirometers have required careful temperature regulation.

A disadvantage of the symmetrical differential respirometer is that the displacement of the index drop is only half that which is obtained under similar conditions with a non-differential type. Duryee (4) and Victor (10) have both designed respirometers retaining the closed system feature of the symmetrical differential type, but have made the volume of the chamber containing the biological system negligibly small with respect to that containing none. The displacement of the index drop then approximates the actual change in volume in the respiration chamber. The bulk of the apparatus is thereby considerably increased, since a compensation chamber several hundred times the volume of the respiration chamber is employed. Careful temperature control is necessary since changes in external temperature can hardly be expected to affect both chambers equally, and cancellation of errors arising from the presence of the medium is somewhat less certain, since liquid-gas volume ratios are usually not the same in the two chambers. Consequently, while the apparatus described here may, by a proper choice of fittings, be converted into such a compensated form, it is designed to function primarily as a symmetrical differential type. The symmetrical construction and the placing of the chambers inside a metal block insure a uniform temperature distribution, permit the elimination of the thermostat and make possible a very high degree of sensitivity.

Construction

The respirometer proper and its case were constructed entirely of brass, except for the glass capillaries. The component parts of the respirometer proper and the accessories for its operation can be described under the headings: (1) The chamber block and plugs; (2) the head plate assembly; (3) the respirometer case; (4) accessories.

The construction of the respirometer and its accessories are shown in detail in the accompanying drawing (Fig. 1) in which all dimensions are given in millimeters. A few dimensions could not conveniently be included in the drawing and these will be found in the text below.

1. The Chamber Block and Plugs.—A cylindrical piece of brass 50 mm. in diameter and 35 mm. in length was cut from a brass bar. Two holes, 10 mm. in diameter, were drilled completely through the block parallel to its cylindrical axis. Centers of these holes were 8 mm. from the center of the block face, on opposite sides of the face center. These holes were reamed and polished to the same diameter. The two faces of the block were ground flat against a steel plate, using powdered carborundum and oil. The grinding of one face was continued with fine carborundum and finished with rouge and water against a piece of plate glass.

Three pairs of chamber plugs were constructed of brass cylinders carefully trimmed to fit the holes in the chamber block. The first pair of cylinders were 25 mm. in length, the second 30 mm., and the last 35 mm. In the last pair of plugs (pair C of Fig. 1) circular wells, 4 mm. in diameter and 3 mm. deep, were drilled into the center of one face of each plug. With the last pair of plugs the chamber volume was reduced to 36λ, for handling very small amounts of tissue.

2. The Head Plate Assembly.—A brass disc 6 mm. in thickness was cut from the same brass bar used for the chamber block. Four equispaced holes, 6 mm. in diameter, were drilled to a depth of 4 mm. into the top of this disc. The centers of these holes were located 8 mm. from the center of the disc face. In the center of two opposite 6 mm. holes, 1 mm. holes were drilled through to the opposite lower face of the disc. In each of the other two 6 mm. holes, a pair of 1 mm. holes, 3 mm. apart, were drilled through to the lower face of the disc. The bottom face of the disc was then ground with carborundum and rouge as described under "The chamber block and plugs." The grinding of the contact surfaces of disc and block was completed by grinding the two together with fine rouge, and finally without any abrasive. It was absolutely essential for the proper operation of the respirometer that these two surfaces were ground flat and semi-polished.[4] Failure to achieve this resulted in troublesome "drifting" of the index droplet.

Plugs were inserted into the chamber block and sealed with bakelite varnish at top and bottom. The inside of each chamber was given a very light coat of the same varnish.

Two capillary posts were constructed from brass cylinders 6 mm. in diameter and 15 mm. in length. In each post a 1 mm. hole was drilled through the center of the

[4] Dr. Craig has suggested that the lapping of the surfaces may be more easily achieved if the block and head plate are of metals of different hardness, such as brass and stainless steel.

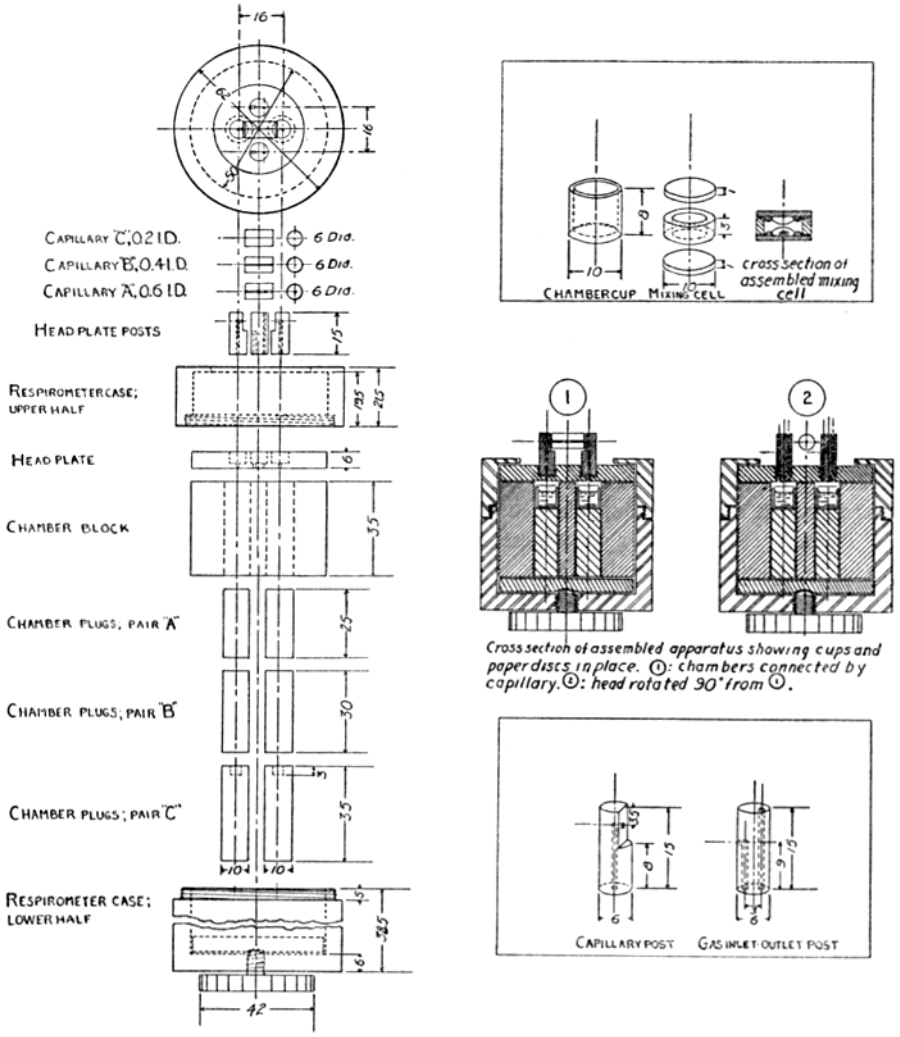

FIG. 1. Scale drawing of microrespirometer and accessories. All dimensions in millimeters. Scale of the two inset drawings is twice that of the remainder of the drawing.

cylinder for a distance of 11.5 mm. after which it was continued at right angles by drilling into the side of the cylinder. The cylinder was filed flat perpendicular to this second hole, to provide a contact surface between capillary and capillary post.

Two gas inlet-outlet posts were constructed similarly, except that one 1 mm. hole

was drilled parallel to the central axis at a distance of 1.5 mm. from it. This extended completely through the post. A second hole was drilled on the opposite side of the center at an equal distance, but only to a depth of 9 mm., after which it was continued at right angles by drilling through the side.

The bottoms of the posts were trimmed to fit the 6 mm. holes in the head plate, as shown in the section drawing of the assembled apparatus. These posts were cemented

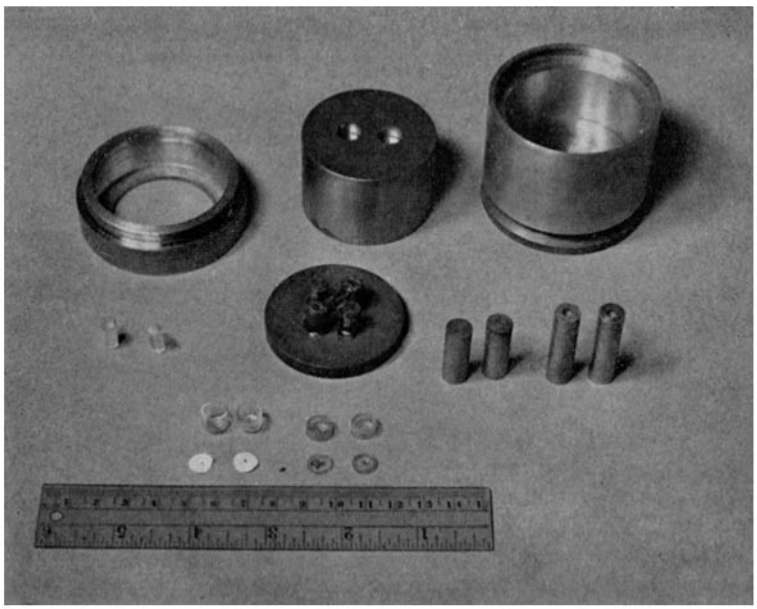

FIG. 2. Microrespirometer and accessories. From left to right: Top row: Respirometer case, upper half; chamber block; respirometer case, lower half.

Second row: Capillaries; head plate assembly; chamber plugs, 25 mm.; chamber plugs, 35 mm.

Third row: Chamber cups; mixing cells with rings and bottom plates assembled.

Bottom row: Absorption discs; mixing bead; top plates of mixing cells.

Note: The respirometer case shown differs in some details from that diagrammed in Fig. 1.

in place with bakelite varnish. The top of each capillary post was given a light coat of shellac and a short piece of small bore rubber tubing was slipped over the end of the post, as may be seen in the photograph (Fig. 2). When the shellac was dry, small holes were drilled through the rubber to connect with the post opening.

Capillaries were constructed from capillary and thermometer tubing cut to the proper length and ground flat on the ends. Before grinding, the capillaries were filled with paraffin to prevent plugging with the grinding compound and scratching of the inside walls.

3. *The Respirometer Case.*—Complete structural details and all dimension data for the

respirometer case are given in the drawing (Fig. 1). The case is essential for the operation of the respirometer only in that it is necessary to provide some sort of clamp for holding the head plate tightly to the chamber block.

4. Accessories.—Chamber cups for holding tissue were constructed of thin walled glass tubing closed and flattened at one end and cut to the proper length. The bottoms were ground to reduce their thickness.

Mixing cells, for mixing solutions inside the respirometer, were constructed of a glass ring and two glass plates, as shown in the drawing. To complete the mixing cells rings of paraffin were placed on one face of each disc. The outside diameter of each wax ring was slightly less than the inside diameter of the glass ring. The ends of the glass rings were notched to permit gas diffusion.

The most satisfactory magnetic mixing beads were constructed from bits of razor blade, about 1 mm. square, and covered with a thin capsule of glass.

Discs for holding the alkali for CO_2 absorption were cut from alkali resistant filter paper. Small holes punched in the centers of these discs assured pressure equilibrium between the gas in the chamber cups and that above the absorption discs.

An electromagnet for operating the mixing beads was constructed by winding approximately 1200 turns of No. 20, B. & S. cotton-covered copper wire around a core composed of a bundle of soft iron wires, the core being 12 cm. in length and 15 mm. in diameter. It was operated by six dry-cells.

It was possible to use a millimeter scale placed behind the capillary, for observing the movement of the index droplet, but it was more convenient and accurate to use a low power microscope fitted with an ocular micrometer. This arrangement is shown in Fig. 3.

The kerosene used as the index liquid should be free of resin-forming unsaturates, as has been pointed out by Schmitt (8). We found it sufficient to treat the kerosene for several days with concentrated sulfuric acid and then to store it over sodium hydroxide pellets in a stoppered container.

Calibration and Testing

1. Calibration.—The volumes of the chambers, of the capillary posts, and of the cups and mixing cells must be determined with considerable accuracy if accurate measurements are to be made with the respirometer.

The volumes of the chambers were determined by filling them with mercury, squeezing out the excess mercury by putting a glass plate over the chamber, and then pouring the residual mercury into a container for weighing.

The volumes of the capillary posts were calculated from the sizes and lengths of the holes through them.

The volumes of glass in the mixing cells and chamber cups were determined by weighing them and dividing the weight by the density of the glass.

The capillaries were calibrated by taking several measurements of the inside diameter with an ocular micrometer and microscope, and calculating the cross-sectional area. The effective cross-sectional area of the capillary in use, however, is somewhat less than this due to the layer of kerosene adhering to the inner wall. A few experiments were conducted to determine the magnitude of this error. The determinations were carried out in the following manner:

A small droplet of kerosene was introduced into one end of a piece of clean, dry capil-

lary tubing. The length of this droplet was measured with an ocular micrometer. The droplet was then permitted to flow slowly along the tube (by tilting the tube slightly) for a measured distance. The tube was then tilted slightly in the opposite direction. When the droplet had reached its original position its length was again measured. The initial and final volumes of the droplet were calculated. The difference represented the amount of liquid adhering to the walls over the measured distance. This is equal to the error caused by the layer of liquid on the walls of the capillary. The volume of liquid on the wall divided by the total volume of capillary traversed by the droplet $\times$ 100 repre-

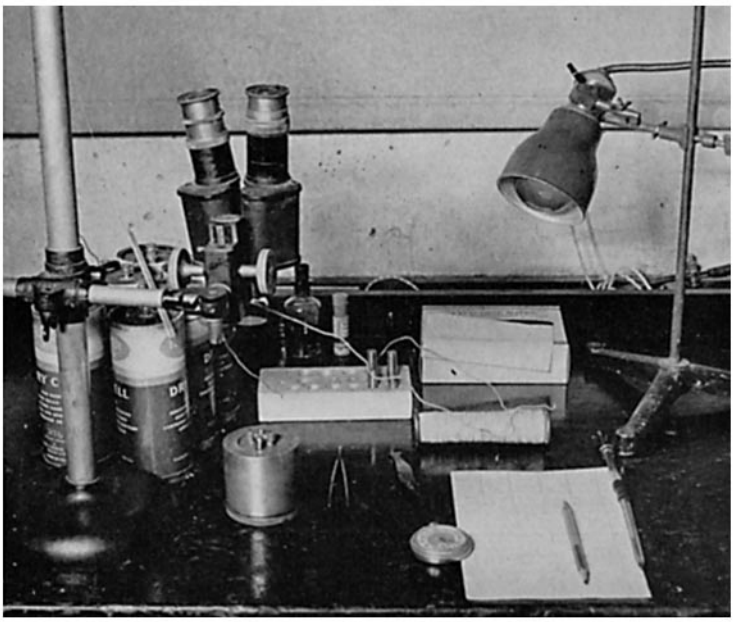

FIG. 3. General view of apparatus in use. Microrespirometer below dissecting microscope. The electromagnet is shown above and slightly to the left of the pencil and pad.

sents the percentage error from this cause. The results of eight such experiments are summarized in Table I.

The mean per cent error from this source is 0.45 $\pm$0.10 for the 0.57 mm. diameter capillary, and 0.42 $\pm$0.07 for the 0.22 mm. capillary. These errors are not significantly different for the two sizes of capillary tubing, as determined by the rather approximate method outlined above. The absolute value of the capillary error is not of significant magnitude in most respiration measurements.

2. Testing.—As a final check on the mechanical reliability of the apparatus it was used to measure the volumes of CO_2 liberated from known amounts of standard bicar-

bonate solution by an excess of sulfuric acid solution. The head plate was lightly lubricated with mineral oil and slipped onto the chamber block from the side, using considerable pressure. The respirometer was placed in the case and tightly clamped by turning the screw at the bottom of the case. A capillary containing an index droplet of kerosene was put in place between the capillary posts and sealed at the edges with a little melted paraffin. 10 minutes were allowed for temperature equilibrium. The position of the bubble was then observed with a low power microscope fitted with an ocular micrometer. If the contact surfaces of the chamber block and head plate had been properly ground the position of the bubble did not shift by more than 0.05 mm. during the ensuing 10 minutes. This served to show whether the grinding of these surfaces had been satisfactory.

The head plate was then removed by a sidewise movement and the bottom plates and rings of the mixing cells put in place in the chambers. A measured amount of

TABLE I

Error Resulting from Index Liquid Adhering to the Wall of the Capillary

Experiment No.	Capillary diameter	Error
	mm.	*per cent*
1	0.57	0.44
2	0.57	0.35
3	0.57	0.48
4	0.57	0.55
5	0.22	0.47
6	0.22	0.35
7	0.22	0.43
8	0.22	0.42

standard bicarbonate was put into each mixing cell. A similar amount of sulfuric acid solution of approximately two and one half times the strength of the bicarbonate solution was placed on the bottom surface of each top plate. The mixing bead was put into the bicarbonate solution in one chamber, which will be called "the reaction chamber," and the top plates dropped into place on top of each ring, with the drop of acid projecting downward. The head plate was put on the respirometer and the apparatus set up as previously described. Previous to placing the capillary in position between the posts, it was tilted slightly to shift the kerosene droplet toward the end of the capillary connected to the reaction chamber. When the index droplet had reached equilibrium the acid and bicarbonate in the reaction chamber were mixed by placing the electromagnet above the chamber and drawing the mixing bead from the lower drop to the upper drop, thus bringing the two in contact and mixing the acid and bicarbonate. 10 minutes were allowed for the reaction to go to completion. The position of the index droplet was again observed and from its displacement the volume of CO_2 liberated was calculated, as discussed under the section, "Theoretical Considerations and Calculations." These experiments served as a final check on the calibrations and mechanical features of the respirometer. The results of fourteen such experiments are summarized in Table II.

Respiration Measurements

After calibrating and testing the apparatus as previously described, it was used for a series of measurements of the oxygen consumption of *Paramecium caudatum*, part of which are summarized in Table III.

These experiments were carried out as follows: A measured volume of a suspension of the organisms was placed in one chamber cup and an equal volume of the same medium, free of organisms, was placed in the other. The cups were put in the chambers

TABLE II

Measurement of Volume of CO_2 Displaced from a Known Amount of Bicarbonate by the Addition of Excess Acid

Experiment No.	Volume of CO_2 calculated (λ)	Volume of CO_2 measured (λ)	Error
			per cent
1	2.60	2.37	−7
2	2.60	2.48	−5
3	3.00	2.72	−10
4	3.15	3.12	−3
5	2.98	2.85	−4
6	2.38	2.46	+3
7	2.95	3.01	+2
8	2.95	3.01	+2
9	2.95	3.01	+2
10	2.16	2.09	−3
11	2.16	1.99	−8
12	2.16	2.08	−4
13	2.16	2.08	−4
14	3.42	3.60	+5
Average per cent error			±4.5
Average per cent recovery			98

and an absorption disc put on top of each cup. The same amount (a few λ) of 0.5 N NaOH was placed on each disc. The apparatus was then set up as previously described and the position of the index droplet was recorded at the intervals indicated in Table III. The O_2 consumption values were calculated from these figures. It was possible to continue oxygen consumption measurements indefinitely, since, as the droplet approached one end of the capillary, rotation of the head plate through 180° caused the droplet to move in the opposite direction. The reproducibility of measurements is also shown by the table. The number of organisms used in these experiments varied from 43 to 231.

Theoretical Considerations and Calculations

The theoretical considerations involved in the testing of the apparatus by liberations of CO_2 may conveniently be discussed with the aid of two idealized

TABLE III

Reproducibility of Results in Measuring the Oxygen Consumption of Paramecium caudatum

Experiment No.	Trial No.	O_2 consumption		
		Mm. displacement/min.		λO_2 at standard conditions per hour
1	1	0.30	*	
	2	0.26	0.26 ±0.04	0.66 ±0.10
	3	0.25		
	4	0.23		
2	1	0.32	*	
	2	0.35	0.33 ±0.02	0.85 ±0.05
3	1	0.20	†	
	2	0.22	0.21 ±0.01	0.54 ±0.02
	3	0.22		
4	1	0.30	*	
	2	0.28	0.29 ±0.01	0.74 ±0.02
5	1	0.14	†	
	2	0.15	0.14 ±0.01	0.35 ±0.02
6	1	0.10	†	
	2	0.08	0.09 ±0.01	0.23 ±0.02
7	1	0.53	*	
	2	0.51		
	3	0.58	0.53 ±0.05	1.35 ±0.13
	4	0.50		

* Measurements made at 10 minute intervals.

† Measurements made at 20 minute intervals.

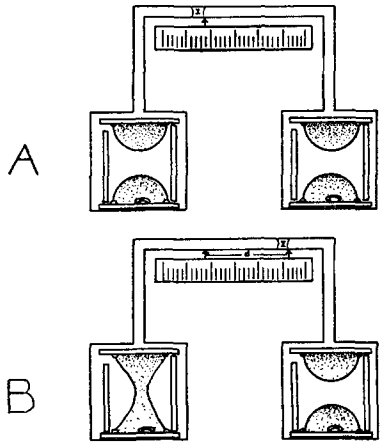

FIG. 4. Illustration of the operation of the apparatus in testing by calibration of CO_2.

diagrams representing the conditions before and after liberations of the gas—Figs. 4 A and 4 B respectively.

The chambers, connected by small openings through capillary posts and capillary constitute a closed system which is separated into two parts by an index droplet *I*. *I* may be regarded as a frictionless piston having a component of motion in the horizontal only, so that at equilibrium the gas pressures at the two ends of *I* are the same.

If, now, gas is liberated in one chamber (by the mixing of acid and bicarbonate in the left-hand chamber as shown in Fig. 4 B), then the pressure in this

chamber rises, forcing the index droplet to the right. This movement compresses the gas in the right-hand chamber. The movement continues until the pressures in the two chambers are the same. Since the displacement of the index droplet is what is measured, it becomes necessary to interpret the observed displacement in terms of volume of gas liberated.

In the discussion of the relationship between observed displacement and volume of gas liberated (or consumed) the following symbols will be used:

1. V_R = volume* of gas in the reaction chamber (the left-hand chamber in Fig. 4). This includes the volume of the chamber proper plus the volume of the capillary up to the index droplet, minus the sum of the volumes of mixing cell, mixing bead, bicarbonate solution, and acid solution.
2. V_C = volume of gas in the compensation chamber.
3. ΔV_{P_0} = volume of gas liberated or consumed measured at P_0 and t.
4. t = temperature of apparatus in degrees Centigrade.
5. Vl_R = volume of liquid in the reaction chamber.
6. Vl_C = volume of liquid in the compensation chamber.
7. P_0 = initial pressure in the system as shown in Fig. 4 A, = barometric pressure at the time the apparatus is set up.
8. P_f = final pressure in the system, as shown in Fig. 4 B.
9. d = observed displacement in millimeters of the index droplet.
10. A = effective cross-sectional area of the capillary in mm.^{2}
    = measured area minus 0.4 per cent.
11. αCO_{2_t} = the solubility coefficient of CO_2 at temperature t and 760 mm. pressure of CO_2, in terms of volumes of gas at standard conditions per volume of liquid.
12. αN_{2_t} = ditto for N_2.
13. αO_{2_t} = ditto for O_2.

* All volumes are expressed in λ (mm.^{3}).

It is clear that if the index droplet I has been observed to move d mm., V_C has been diminished by $Ad\lambda$. The pressure in the compensation chamber has therefore increased from P_0 to $\left(\frac{V_C}{V_C - Ad}\right)P_0$. We shall call this higher pressure P_f. It is equally clear that the pressure in the entire system now has this same value of P_f, since the pressures at the two ends of I are the same. Therefore a gas occupying a volume of $(V_R + V_C)$ at a pressure P_0, now occupies this same volume at a pressure P_f. $(V_R + V_C)$ at P_f would be $(V_R + V_C)\frac{P_f}{P_0}$ at P_0. In other words, if the liberated gas had been allowed to expand freely against a pressure P_0 the total volume of gas would have been increased by $(V_R + V_C)\frac{P_f}{P_0} - (V_R + V_C)$. We shall call this increase ΔV_{P_0}. ΔV_{P_0} represents the volume of gas liberated from

the bicarbonate solution. It becomes necessary then to determine the relation between d and ΔV_{P_0}.

Suppose the ratio of the total volume of the two chambers to the volume of the compensation chamber is n,

$$V_C = \frac{V_R + V_C}{n}$$

then
$$nV_C = V_R + V_C$$

since
$$\Delta V_{P_0} = (V_R + V_C)\frac{P_f}{P_0} - (V_R + V_C)$$

$$\Delta V_{P_0} = nV_C\frac{P_f}{P_0} - nV_C$$

but
$$P_f = \left(\frac{V_C}{V_C - Ad}\right)P_0$$

therefore
$$\Delta V_{P_0} = nV_C\left(\frac{V_C}{V_C - Ad} - 1\right)$$

or
$$\Delta V_{P_0} = nAd\left(\frac{V_C}{V_C - Ad}\right) \tag{1}$$

Since Ad is usually small with respect to V_C the expression: $\left(\frac{V_C}{V_C - Ad}\right)$ is approximately equal to one and equation (1) reduces to

$$\Delta V_{P_0} = nAd \tag{2}$$

ΔV_{P_0}, calculated from d by means of either (1) or (2) may be reduced to standard conditions in the customary manner.

ΔV_{P_0} however, does not represent all the CO_2 produced from the bicarbonate, since an appreciable fraction of the CO_2 remains dissolved in the liquid in the reaction chamber. The quantity of dissolved CO_2 may be calculated, since it is known that a volume of CO_2, ΔV_{P_0}, permeates a space, $(V_R + Ad)$ in which the total gas pressure is equal to Pf. The partial pressure of CO_2 in this space is therefore equal to $[\Delta V_{P_0}/(V_R + Ad)]\ Pf$. If the temperature of the apparatus is t, then the quantity of dissolved CO_2 is given by the formula:

$$CO_2\ (\text{dissolved}) = \left(\frac{\Delta V_{P_0}}{V_R + Ad}\right) Pf\,\alpha CO_{2_t} \cdot Vl_R \tag{3}$$

Since αCO_2 is given in terms of volumes of gas at standard conditions per volume of liquid, it is only necessary to add the reduced value of ΔV_{P_0} to the volume of dissolved CO_2 in order to get the total volume of CO_2 produced by the reaction.

For measurements of greater precision it is necessary to take into consideration an additional source of error. It is apparent that the compression of the gas in the compensation chamber has increased the partial pressure of O_2 and N_2 in this chamber and that more of these gases have gone into solution. At the same time the partial pressures of oxygen and nitrogen have diminished in the reaction chamber, since the same quantities of these gases now occupy a larger volume. There is a smaller volume of dissolved oxygen and nitrogen in the reaction chamber and a greater volume of dissolved oxygen and nitrogen in the compensation chamber than was initially the case. This produces a greater displacement of the index droplet than if the liquid phase were absent.

The partial pressures of oxygen and nitrogen in the compensation chamber have increased to P_f/P_0 times their original values. The partial pressures of oxygen and nitrogen in the reaction chamber have decreased to $\frac{V_R - Ad}{V_R}$ times their original values.

The additional quantity of oxygen forced into solution by compression in the compensation chamber is equal to:

$$\Delta O_2 \text{ (dissolved)} = \alpha O_{2_t} \cdot Vl_C \cdot P_{O_2} \cdot \left(\frac{P_f}{P_0} - 1\right) \tag{4a}$$

where P_{O_2} = initial partial pressure of oxygen.

Similarly for N_2

$$\Delta N_2 \text{ (dissolved)} = \alpha N_{2_t} \cdot Vl_C \cdot P_{N_2} \cdot \left(\frac{P_f}{P_0} - 1\right) \tag{4b}$$

The quantities of oxygen and nitrogen released from solution in the reaction chamber are:

$$\Delta O_2 \text{ (dissolved)} = \alpha O_{2_t} \cdot Vl_R \cdot P_{O_2} \cdot \left(\frac{VR - Ad}{VR} - 1\right) \tag{4c}$$

$$\Delta N_2 \text{ (dissolved)} = \alpha N_{2_t} \cdot Vl_R \cdot P_{N_2} \cdot \left(\frac{VR - Ad}{VR} - 1\right) \tag{4d}$$

The latter two quantities are negative, but since they tend to produce an error in the same direction as the first two, all four Δ's are added, and the sum subtracted from the reduced volume of CO_2 already calculated.

Respiration Measurements

The principles involved in respiration measurements are the same as those already discussed. In this case, however, we have to deal with a system in which the total pressure is decreasing.

ΔV_{P_0} is therefore equal to: $nAd\left(\frac{V_C}{V_C + Ad}\right)$ (5)

A source of error in respiration measurements is introduced by the removal of only one component (oxygen) of the gas mixture in the reaction (respiration) chamber. The partial pressures of oxygen and nitrogen do not remain equal in the two chambers. If the displacement volume $= Ad$, V_C is increased to $V_C + Ad$. The partial pressures of oxygen and nitrogen in the compensation chamber are thus reduced to $\frac{V_C}{V_C + Ad}$ times their initial value.

In the reaction chamber the volume has been reduced to $V_R - Ad$. The partial pressure of nitrogen in the reaction chamber has therefore increased to $\left(\frac{V_R}{V_R - Ad}\right)$ times its initial value. The partial pressure of oxygen, on the other hand, has diminished to:

$$\frac{V_R}{V_R - Ad + nAd} P_{O_2} \quad \text{or} \quad \frac{V_R}{V_R - Ad(n-1)} P_{O_2} \tag{6}$$

The changes in the volumes of dissolved oxygen and nitrogen may then be calculated as already outlined and the correction applied accordingly.

In calculating the initial partial pressures of oxygen and nitrogen, it should be remembered that water vapor constitutes an appreciable fraction of the gas mixture in the chambers.

SUMMARY

1. A microrespirometer suitable for measuring oxygen uptakes from 0.1 to 10λ per hour is described.

2. The sensitivity of the instrument may be readily altered by substituting different sizes of capillary tubing.

3. By means of replaceable brass plugs the chamber volume of this instrument may be varied from 700 to less than 40λ.

4. No thermostat is required for the operation of the instrument at room temperature.

5. It may be charged at one temperature and used at a widely different one.

6. The chambers may be filled with any desired gas mixture.

7. Two solutions may be mixed during the course of an experiment.

8. The entire apparatus may be sterilized.

The authors take pleasure in expressing their gratitude to Mr. Makio Murayama, who prepared the photographs, Figs. 2 and 3.

BIBLIOGRAPHY

1. Barcroft, J., *J. Physiol.*, 1908, **37**, 12.
2. Barcroft, J., Respiratory function of the blood, Cambridge University Press, 1914.
3. Dixon, M., Manometric methods, Cambridge University Press, 1934.
4. Duryee, W. R., *Z. vergleich. Physiol.*, 1926, **23**, 208.
5. Fenn, W. O., *Am. J. Physiol.*, 1927, **80**, 327.
6. Fenn, W. O., *Am. J. Physiol.*, 1928, **84**, 110.
7. Heatley, N. G., Berenblum, I., and Chain, E., *Biochem. J.*, London, 1939, **33**, 1.
8. Schmitt, F. O., *Am. J. Physiol.*, 1932, **104**, 303.
9. Thunberg, T., *Skand. Arch. Physiol.*, 1905, **17**, 74.
10. Victor, J., *Am. J. Physiol.*, 1935, **111**, 477.