A Semi-Microrespirometer

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A sensitivity greater than that of the standard Barcroft apparatus may be obtained by scaling down the apparatus. Various modifications to such an apparatus, designed to reduce the errors inherent in the estimation of small pressure changes, have been reported. Perhaps the most widely adopted of these is the introduction, on the reaction chamber side, of a micrometer syringe by means of which the pressure in the reaction vessel can be equilibrated with that in the compensation vessel (e.g. Adam, 1920; Tyler & Berg, 1941; Scholander, 1942; Dixon, 1951). In general, the reaction and compensation vessels were of the same capacity.

The purpose of the present paper is to describe a micro-Barcroft apparatus in which the compensation vessel is several times larger than the reaction chamber and the micrometer syringe is placed on the compensation side. In this way the pressure changes in the reaction vessel are magnified approximately in the ratio of the capacities of the two chambers, and can be estimated with a smaller error.

APPARATUS

In Fig. 1, *A* is the reaction chamber, which had a capacity of about 1 ml. It had two compartments, the smaller for the tissue, the larger for the KOH, and was connected to the manometer *B* (Pyrex capillary, bore 0.4 mm.) by small-bore, thick-walled polythene tubing. The reaction chamber could thus be shaken independently of the rest of the apparatus. The manometric fluid was the fraction of commercial paraffin boiling between 160° and 190°, coloured

with a little Sudan III. This fluid has a low vapour pressure and a high mobility. *D* is a bridge and three-way tap to equalize the pressure on either side of the manometer after temperature equilibration had been attained. The compensation chamber *E* was connected to the manometer by a B 14 standard ground-glass joint allowing compensators of different sizes to be fitted. The apparatus was connected through a levelling bulb *F* and a length of small-bore Portex (vinyl-vinylidene chloride co-polymer) tubing (Portland Plastics Ltd., Hythe, Kent) to an Agla syringe and micrometer *G*. The syringe and Portex tube were filled with mercury, initially, up to one of two datum lines; the upper (just above *F*) was used when gas uptake was expected, the lower (just below *F*) when gas was

Fig. 1. Diagram of the apparatus. For explanation see text.

Fig. 2. Section through the reaction chamber assembly. For explanation see text.

evolved. The whole apparatus, with the exception of the syringe and the micrometer, was immersed in a water bath. At 30°, temperature equilibration was attained in 20 min.

The details of the reaction chamber assembly are given in Fig. 2. The chamber V was drilled from Perspex rod (1 in. diam.) and excess of material was removed to hasten temperature equilibration. The chamber was carried on the brass table T which could be raised or lowered by means of the screw S . A raised rim on T ensured accurate and reproducible alignment of the chamber with the upper brass plate P . The whole was held in the rigid brass frame R . The polythene tube W was joined to the reaction-chamber assembly by the connector Y . Thus the whole assembly could be rapidly dismantled and detached from the apparatus for cleaning and charging. These operations had no noticeable effect on the calibration of the instrument.

The menisci of the manometric fluid were viewed through a simple reading microscope (5 in. objective and $\times 10$ cross-line micrometer eyepiece) and kept on the hairline by manipulation of the micrometer. A reading could be taken in a few seconds; duplicate readings agreed well, the agreement being dependent on the capacity of the compensation vessel. As a rule they did not differ by more than ± 3 graduations with a compensation vessel of 100 ml., and by ± 1 graduation with one of 10 ml. capacity.

The experiments for which this apparatus was designed did not require the instrument to be filled with a gas mixture differing from that of air. For that reason no provision was made for filling the system with any other gas mixture. However, the apparatus is capable of modification without affecting the underlying principles.

THEORY

Let x = the amount of gas evolved, in $\mu\text{l.}$ at s.t.p. (if the gas is absorbed x will be negative);

V = total volume of the reaction vessel;

V_F = the liquid volume in the reaction vessel;

V_G = the gas volume in the reaction vessel;

ΔP = the change of pressure in the reaction vessel (if gas is absorbed ΔP will be negative);

T = the absolute temperature of the water bath;

P = the atmospheric pressure;

p = the vapour pressure of water vapour at T ;

P_0 = the standard pressure;

α = Bunsen's absorption coefficient of the gases in the reaction vessel (a composite figure depending on the nature and partial pressures of the gases concerned);

V_{Hg} = volume of mercury required to adjust manometer levels (if gas is absorbed V_{Hg} will be negative).

The same symbols with dashes are used for the compensation vessel.

Then the amount of gas initially present in the reaction chamber is

$$V_G \frac{273}{T} \frac{(P-p)}{P_0},$$

and the final amount after the manometer has been adjusted is

$$V_G \frac{273}{T} \frac{(P-p+\Delta P)}{P_0},$$

whence

$$\begin{aligned} x &= V_G \frac{273}{T} \frac{(P-p+\Delta P)}{P_0} - V_G \frac{273}{T} \frac{(P-p)}{P_0} \\ &= V_G \frac{273}{T} \frac{\Delta P}{P_0}. \end{aligned} \quad (1)$$

The amount of gas initially present on the compensating side is

$$V'_G \frac{273}{T} \frac{(P-p)}{P_0}, \quad (2)$$

and that finally present

$$(V'_G - V_{\text{Hg}}) \frac{273}{T} \frac{(P-p+\Delta P)}{P_0}. \quad (3)$$

Since no gas is absorbed or evolved in the compensating vessel, (2) = (3), whence

$$\Delta P = \frac{V_{\text{Hg}} (P-p)}{V'_G - V_{\text{Hg}}}.$$

Substituting for ΔP in (1)

$$x = \frac{V_G}{(V'_G - V_{\text{Hg}})} \frac{273}{T} \frac{(P-p)}{P_0} V_{\text{Hg}}. \quad (4)$$

During the experiment the partial pressures of the gases in both vessels change, resulting in secondary changes in pressure due to gases either diffusing out of or dissolving in the liquids in the vessels. This effect is not taken account of in equation (4). The respective pressure changes are:

$\frac{V_F \alpha \Delta P}{P_0}$ in the reaction chamber and $\frac{V'_F \alpha' \Delta P}{P_0}$ in the compensation chamber.

Rather than introduce correction factors incorporating these quantities, it was considered preferable to adjust the ratio of the liquid volumes in both vessels in such a way that the secondary pressure changes in the two vessels cancel out. This can be done by arranging for V_F and V'_F to be such that the ratio of the pressure changes in the two vessels equals the ratio of the gas volumes; i.e.

$$\frac{V_F \alpha}{V'_F \alpha'} = \frac{V_G}{V'_G} = \frac{V_G}{V' - V'_F},$$

whence

$$V'_F = \frac{V_F \alpha V'}{V_F \alpha + V_G \alpha'}. \quad (5)$$

It may be noted that if α and α' are equal, or if they differ by a negligibly small amount, equation (5) reduces to

$$\frac{V'_F}{V_F} = \frac{V'}{V}.$$

Thus in these circumstances the identity to be satisfied is

$$\frac{V'_F}{V_F} = \frac{V'}{V} = \frac{V'_G}{V_G}.$$

This treatment is still not rigid since the adjustment of the liquid volumes is based on fixed gas volumes in both vessels, while the essence of the method is that V'_G can be varied at will. From equation (4), it is seen that for a given experiment

$$x = \frac{k V_G V_{\text{Hg}}}{V'_G - V_{\text{Hg}}},$$

where k has a value of about 0.8. Therefore

$$V_{\text{Hg}} = \frac{x V'_G}{k V_G + x}.$$

Expressing Γ_{H_2} as a percentage of V'_G ,

$$\frac{100V_{H_2}}{V'_G} = \frac{100x}{kV_G + x}$$

Thus the percentage error introduced by neglecting V_{H_2} compared with V'_G is independent of the ratio V_G/V'_G but can be conveniently related to V_G . Furthermore, V_G can be so fixed that the percentage error introduced above is kept down to a negligible value.

RESULTS

Two series of tests have been carried out on the apparatus. In the first, the observed volume of CO_2 liberated by the action of acid on Na_2CO_3 was compared with the theoretical volume. A volume (12.7 μ l.) of Na_2CO_3 solution of known concentration was placed, as a single drop, in the large compartment of the reaction chamber along with a drop (also 12.7 μ l.) of $N-HCl$ and a lead shot coated with Perspex. The solutions were mixed by shaking the reaction chamber so that the lead shot mixed the reagents. The recoveries were quantitative (Table 1).

In the second test the rate of O_2 uptake by yeast suspensions as measured in the micro-apparatus was compared with the rate as measured in the standard Warburg apparatus. The results are recorded in Table 2.

Table 1. *Evolution of carbon dioxide from sodium carbonate*

Carbon dioxide was liberated by the action of $N-HCl$ (12.7 μ l.) on Na_2CO_3 solutions (12.7 μ l.) of various concentrations.

V'_G/V_G ratio	Vol. of CO_2 at s.t.p. (μ l.)		Observed as % of theoretical
	Theoretical	Observed	
100	0.28	0.30	107
	0.64	0.64	100
	1.26	1.26	100
	1.64	1.60	98
10	2.67	2.67	100
	5.04	4.92	98
	10.80	10.54	98
	15.49	15.62	101

Table 2. *Respiration of a yeast suspension*

Rates of O_2 uptake by two different samples of yeast were measured by the standard Warburg apparatus and on the microrespirometer ($V'_G/V_G = 10$).

Sample	Wt. of yeast (mg.)	Time (min.)	O_2 uptake (μ l./mg. wet wt./hr.)	
			Micro	Warburg
A	0.52	20	-59	—
	0.49	20	-58	—
	3.8	30	—	-61
	6.5	15	—	-64
B	0.83	20	-44	—
	0.81	20	-39	—
	4.1	20	—	-42
	5.9	20	—	-43

DISCUSSION

The main purpose of this paper is to draw attention to the principle of the apparatus, but the following points might prove of use in the design and construction of a similar apparatus.

(1) Changes in the bath temperature which affect both vessels simultaneously and to the same extent will not upset their pressure relationship (except in so far as one side might take longer to reach equilibrium than the other), but differential temperature effects will drastically alter the pressure relationship. Such effects can be minimized, at the design stage, by mounting the reaction and compensation vessels as close together as possible and providing for great accuracy and sensitivity of temperature control.

(2) Although there seems to be no theoretical limit to the size of the ratio V'_G/V_G , it was found in practice that the apparatus became unstable when the ratio was greater than about 130:1 and the index fluid moved spontaneously. This movement was always in the same direction, namely consistent with an increase of pressure in the compensation chamber. It appears to require 4 or 5 days for the apparatus to settle down. This drift, the cause of which has not been definitely established, has been noticed by earlier authors (e.g. Adam, 1920; Schmitt, 1933; Tyler & Berg, 1941).

(3) A further limit to the magnitude of the ratio V'_G/V_G is imposed by the fact that as the ratio increases so does V_{H_2} for a given value of x . Thus in the present apparatus, at a ratio greater than about 150:1, the greater theoretical sensitivity of the apparatus was outweighed by a marked sluggishness of response to the addition or withdrawal of mercury.

The maximum practical value for V'_G/V_G in the present case was about 100 and the optimum about 50.

SUMMARY

1. A micro-Barcroft apparatus is described in which the reaction and compensation vessels are of different capacities and in which the pressures on either side of the manometer are equilibrated by altering the volume on the compensating side.

2. The theory of the apparatus is given.

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