

A MODIFIED CAPILLARY MICRORESPIROMETER

By F. J. BERGERSEN AND B. E. MERZ

(From the Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra, Australia)

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ABSTRACT

Modifications which enable simpler operation of the Cunningham-Barth-Kirk microrespirometer are described and an example of its use is quoted. Added advantages include easy positioning of indicator drop in the capillary and thermal equilibration at atmospheric pressure.

INTRODUCTION

Cunningham and Kirk (1940) developed a differential microrespirometer consisting of chambers of equal size drilled in a rectangular brass block and joined by a capillary tube within which the movement of a drop of kerosene or other low viscosity liquid could be measured. The apparatus was modified by Barth and Kirk (1942). This respirometer can be used for measuring gas changes of the order of 0.1 to 10 μ l. per hour with errors of less than 5 per cent. There are, however, difficulties in using the apparatus, notably the haphazard positioning of the indicator drop in the capillary during assembly, the changes in pressure which occur in the chambers during thermal equilibration and the uncertainty of the grease seal between the chambers and the lucite head plate. The modifications to be described involve the capillary mountings and head plate and chamber seals and are designed to minimise these drawbacks to an otherwise very useful piece of equipment.

Description of the Respirometer

The main block design (Fig. 1, A) is the same as that described by Barth and Kirk (1942). It consists of a 3 inch length of rectangular brass ($1\frac{1}{4} \times \frac{3}{4}$ inches) with two holes $\frac{3}{8}$ inch in diameter, $\frac{3}{4}$ inch deep and $1\frac{5}{16}$ inches between centres, drilled symmetrically in one narrow face of the block to form the chambers. The top of the block is machined to leave a rim $\frac{1}{32}$ inch high and $\frac{1}{16}$ inch wide around the top of each chamber. Threaded studs are placed near each end for the assembly of the apparatus. Rubber gaskets (Fig. 1, g) surround the rims of the chambers and are just a little thicker than $\frac{1}{32}$ inch.

The head plate (Fig. 1, B) has been modified and consists of a piece of brass strip $\frac{3}{4} \times \frac{1}{8} \times 3$ inches, drilled at the ends for the assembly bolts: a $\frac{1}{16}$ inch hole communicates with each chamber. The capillary tube (Fig. 1, C) is mounted on the head

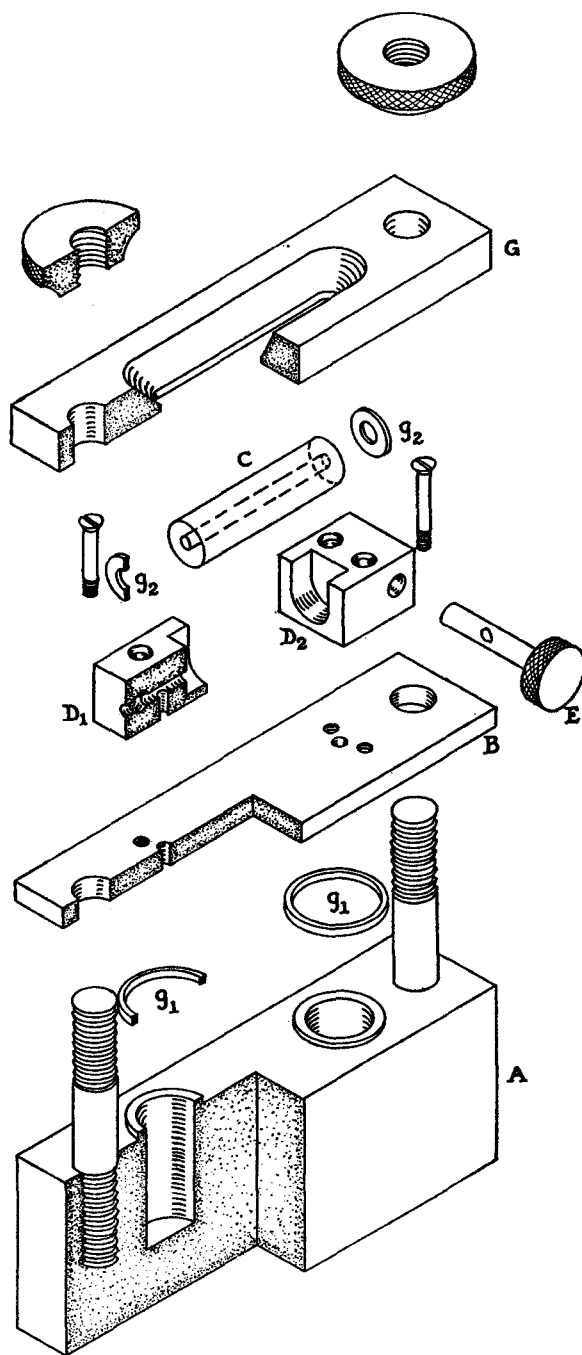


FIG. 1. Isometric drawing of the modified differential microrespirometer. For explanation of the lettering see the text.

plate between rubber gaskets (Fig. 1, g_2) in the recesses of shaped brass blocks (Fig. 1, D_1 and D_2), which are secured to the head plate by countersunk screws, the joint being sealed with a little gasket cement. These blocks are drilled with $\frac{1}{16}$ inch holes which correspond to the holes in the head plate, and communicate with horizontal $\frac{1}{16}$ inch holes drilled right through the blocks in positions corresponding with the horizontal axis of the capillary tube. These horizontal holes are traversed by tapered holes (tapered pin reamer 3/0, major diameter 0.117 inch), within which turn tapered stainless steel stopcocks (Fig. 1, E). These permit pressure equilibration between each chamber during the positioning of the indicator drop.

The head plate is clamped to the chamber block by means of a $\frac{3}{4} \times \frac{1}{4} \times 3$ inches

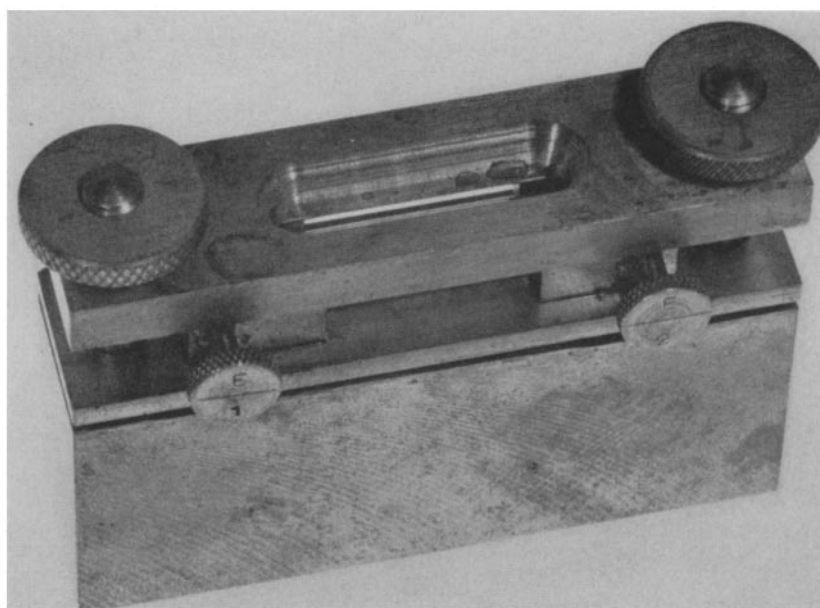


FIG. 2. The assembled microrespirometer.

brass strip (Fig. 1, G), drilled for the assembly bolts, and slotted to expose the capillary for reading from above. When the knurled nuts on the assembly bolts are tightened, this brass strip bears on the tops of the capillary mounting blocks, pressing the head plate on to the gaskets around the chamber rims. If the nuts are tightened until the head plate makes contact with the rim of the chambers, reproducible chamber volume is ensured, together with a rubber seal between head plate and chamber block; (this seal may be further aided by a smear of silicone grease on the rubber surfaces). The assembled instrument is illustrated in Fig. 2.

The thick walled glass capillary tubes are $1\frac{1}{4}$ inches long, and cut from uniform bore tubing of known bore diameter: the ends are ground flat and exactly at right angles to the axis of the tube. The capillaries are mounted, or changed by slackening the screws holding one of the shaped blocks to the head plate. The gasket cement is wiped off the adjacent faces with a suitable solvent and a fresh film applied before

assembly. The capillary tube is squeezed between the greased rubber gaskets which must be thick enough to make considerable pressure with the fingers necessary in order to reinsert the screws into the threaded holes in the head plate. The capillary tubes should be frequently cleaned in chromic acid. The indicator drop (kerosene washed with concentrated H_2SO_4 and stored over NaOH pellets) is inserted in the capillary by means of a very fine glass pipette passed through the hole in the capillary

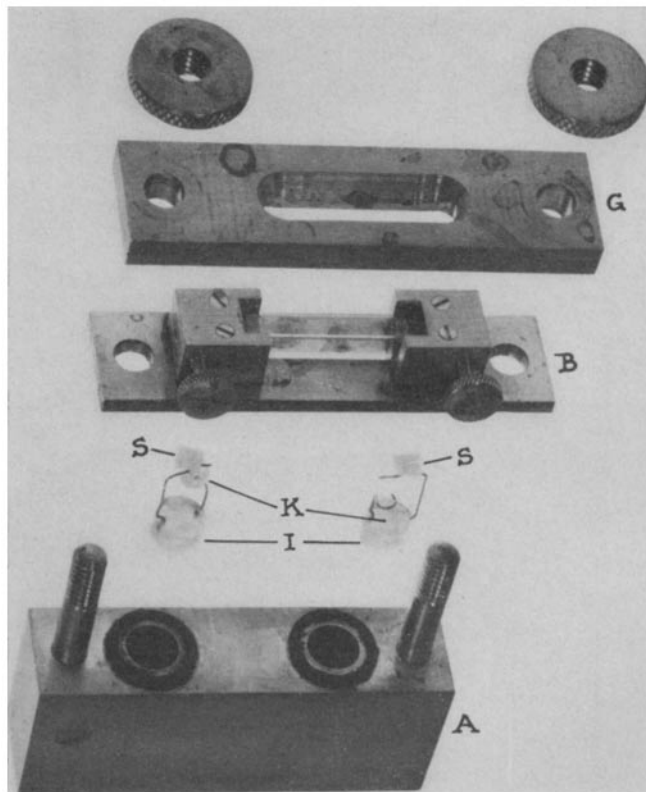


FIG. 3. A view of the various components. *A*, chamber block; *B*, head plate assembly; *G*, clamping strip. *I*, perspex inserts with KOH-impregnated filter paper squares, *S*, and glass balls, *K*,—the left one shown on its wire support and the right one in the mixing position.

mounting after first removing the stopcock. (The latter should be thoroughly greased before replacing). The indicator drop should be run back and forth through the capillary several times by tipping the head plate, in order to wet the entire length of the tube with the liquid.

The insides of the chambers are protected by a thin layer of paraffin wax (applied dilute in xylol). Matched "perspex" inserts of various designs may be used in the chambers (*a*) to increase the sensitivity of the respirometer by reducing the chamber

volume and (b) to hold the materials being studied. The inserts illustrated in Fig. 3 consist of two depressions in a perspex disc with a wire frame for holding a piece of filter paper soaked in KOH (for CO₂ absorption) and a glass ball, which when dislodged by tipping, mixes the substrate and cells placed in separate drops in the depressions in the disc.

One of the authors has used four of these microrespirometers mounted on a rail in a thermostatically controlled water bath. Successive readings of indicator drop movement were made with a Cambridge measuring microscope, the respirometers being slid along the rail to bring them beneath the microscope lens.

Procedure for Use

The materials are introduced into the chambers and the respirometer is assembled with the stopcocks open. It is then placed in the thermostat until thermal equilibrium is reached when the position of the indicator drop is adjusted by tilting the respirometer and allowing the drop to run towards the compensation chamber. For most purposes the starting position of the drop should be 3 to 5 mm. from the end of the capillary; if this distance is reduced there is a risk of the drop adhering to the external orifice of the capillary, or even being drawn right out by surface tension forces. The stopcocks are then closed and readings commenced. For maximum accuracy amounts of tissue, size of inserts, and capillary dimensions should be selected so that readings may be made at 10 minute intervals. Shorter intervals tend to decrease accuracy owing to non-uniform drop movement, which is exceedingly difficult to avoid.

Absorption of gas in the reaction chamber of the respirometer is calculated from the formula

$$\Delta V_{p_0} = nAd \left(\frac{Vc}{Vc + Ad} \right)$$

in which ΔV_{p_0} is the change in volume of gas in the reaction chamber at the initial pressure (barometric pressure when the chambers were closed); n is the ratio of the total volume of the two chambers to the volume of the compensation chamber; A is the effective cross-sectional area of the capillary (actual area minus 4 per cent, Cunningham and Kirk, 1940); d is the displacement of the indicator drop; Vc is the volume of the compensation chamber up to the indicator drop at its initial position. If gas is being evolved the sign of the lower line of the equation is reversed to $Vc - Ad$.

When accurate absolute measurements are required, it is necessary to correct for changes in amounts of dissolved gases in each chamber as the partial pressures change owing to the uptake of oxygen by the tissue. This effect is very small as long as the volumes of liquids used are also very small.

The sensitivity and accuracy of the modified instrument have proved to be substantially the same as those claimed by Cunningham and Kirk (1940).

There was usually a variation between replicates of about 5 per cent and preliminary tests showed quite good agreement with measurements made on similar material in Warburg respirometers. For example, bacteroid preparations from subterranean clover nodules gave Q_{O_2} 's of 195 $\mu\text{l.}/\text{hour}$ for 10^9 cells in the microrespirometer and 221 $\mu\text{l.}/\text{hour}$ in the Warburg.

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REFERENCES

- Barth, L. G., and Kirk, P. L., 1942, A simplified differential microrespirometer, *J. Gen. Physiol.*, **25**, 663.
Cunningham, B., and Kirk, P. L., 1940, A new form of differential microrespirometer, *J. Gen. Physiol.*, **24**, 135.