A METAL MICRO-RESPIROMETER OF THE BARCROFT TYPE SUITABLE FOR SMALL INSECTS AND OTHER ANIMALS

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(With Two Text-figures)

METAL BARCROFT RESPIROMETER

The metal Barcroft respirometer is an accurate and rapidly operated micro-respirometer, suitable for small insects and other animals which can be maintained in an aerial environment for the period of experiment, and is particularly suitable for experiments in which different gas mixtures are employed. It is based on the principle of the differential (Barcroft) type of manometer, but its chief advantages are its small size and construction from a solid brass block. In consequence, the temperature surroundings of the two chambers are kept uniform by the high conductivity of the metal, and gaseous diffusion is very rapid throughout.

Into a solid brass block *ABCD* are drilled two chambers, the experimental chamber (1) being 1 cm. in diameter and 1 cm. deep, the control chamber (2) being 2 cm. in diameter and 1·75 cm. deep. The chambers taper at the base and are continued as fine capillaries 1·0 sq.mm. in cross-section (*EF* and *GH*) to the base of the block, where they terminate in small brass collars. A bent capillary tube *R* of cross-section 0·2 mm., graduated in cm., containing the manometer fluid, is fitted directly into these collars and sealed with picene wax. The chambers 1 and 2 each communicate with the outside by means of fine capillaries *JK*, *LM*, drilled in the metal and terminating in collars, and intercommunicate by another capillary *PQ*. These capillaries may be opened and closed by taps *U*, *V*, *W*, as shown. The chambers are closed above by glass plates (*S*) cut from microscope slides, which are pressed tightly down on to a thin layer of Apeizon H.V. grease smeared on the surface of the block. If the surface of the block is polished, it is possible to tell by inspection when the chambers are completely sealed off, since any small leak is apparent as an air channel in the grease. We find this type of seal less troublesome than the greased ground-glass join of the standard Barcroft. Not only is it easy to ensure freedom from leaks, but there is also no tendency for the grease to be squeezed out, causing an alteration in the volume of the chamber. In order to reduce any tendency to corrosion, the insides of the chambers are tinned.

Each chamber is furnished with a small hole (*O*) into which is fitted a glass tube containing a solution of potash (10%) and a piece of starch-free filter-paper.* A gauze frame *T* separates the insect from contact with the potash.

The chief disadvantage of the apparatus is the relatively large amount of dead space involved in the capillaries. This is unavoidable, since if the capillaries are made any narrower than 1 sq.mm. in cross-section they are liable to blockage by condensation, and further, the taps do not operate successfully. These in fact constitute the chief weakness in the design, and great care should be taken to ensure that the tapers are accurately machined. On the other hand, the dead spaces, unlike those of the standard Barcroft manometer, are separated from the chambers by very short diffusion paths, and may be treated as parts of the chambers. The difference in volume of the chambers serves to increase the sensitivity of the apparatus.

CALIBRATION

The apparatus is most conveniently calibrated by calculation from its dimensions. We give below a summary of this calculation:

Volume of experimental chamber (1)

Depth to edge of conical part = 0·7 cm.
Total depth = 1·0 cm.
Radius = 0·5 cm.
Length of capillary to base of block = 3·0 cm.
Length of capillary to outer tap = 1·0 cm.
Length of capillary to inner tap = 0·8 cm.
Mean length of space above manometer fluid = 3·0 cm.
Cross-sectional area of capillaries in metal = 0·01 sq.cm.
Cross-sectional area of manometer capillary = 0·002 sq.cm.

$$\text{Total volume} = \pi \times (0{\cdot}5)^2\,(0{\cdot}7 + 0{\cdot}3/3) + 0{\cdot}01\,(3{\cdot}0 + 1{\cdot}0 + 0{\cdot}8) + 0{\cdot}002 \times 3{\cdot}0 \text{ c.c.}$$
$$= 0{\cdot}628 + 0{\cdot}048 + 0{\cdot}006$$
$$= 0{\cdot}682 \text{ c.c.}$$

Volume of gauze = 0·050 c.c. (by weighing).
Volume of insect (approx.) 0·02 c.c.
Net volume of chamber (1) = 612 cu.mm. = V_g in Dixon's formula.

Similarly, for chamber (2), the volume is found to be 4710 cu.mm. = V_g'.

Density of oil P_1 = 0·79 g./c.c.
Manometer vertical θ = 0
Volume of fluid V_f = 0·100 c.c.
Cross-section of manometer capillary = 0·207 sq.mm. (by weight of mercury thread)
Temperature = 20° C.

By Dixon's (1943) formula, uptake in cu.mm.,

$$x = h\left(1 + \frac{0{\cdot}207 \times 13{,}110}{2 \times 4710}\right)\left(\frac{(612 \times 273/293 + 100 \times 0{\cdot}03)}{13{,}110} + \frac{0{\cdot}207}{2} \times \frac{273}{293}\right) = 0{\cdot}181h.$$

* Provided that the apparatus is kept at the small dimensions described the filter paper can be omitted with advantage.

The measurements were carried out by observation with a microscope carrying an eyepiece scale focused on one arm of the manometer. If this movement is h' mm., $x = 0{\cdot}362h$.

Calibration of eyepiece scale 9·8 units = 1 mm.

Hence uptake in cu.mm./hr. $= \frac{60 \times 0{\cdot}362}{9{\cdot}8} \cdot n = 2{\cdot}22n$, when n is movement of column in eyepiece units per minute.

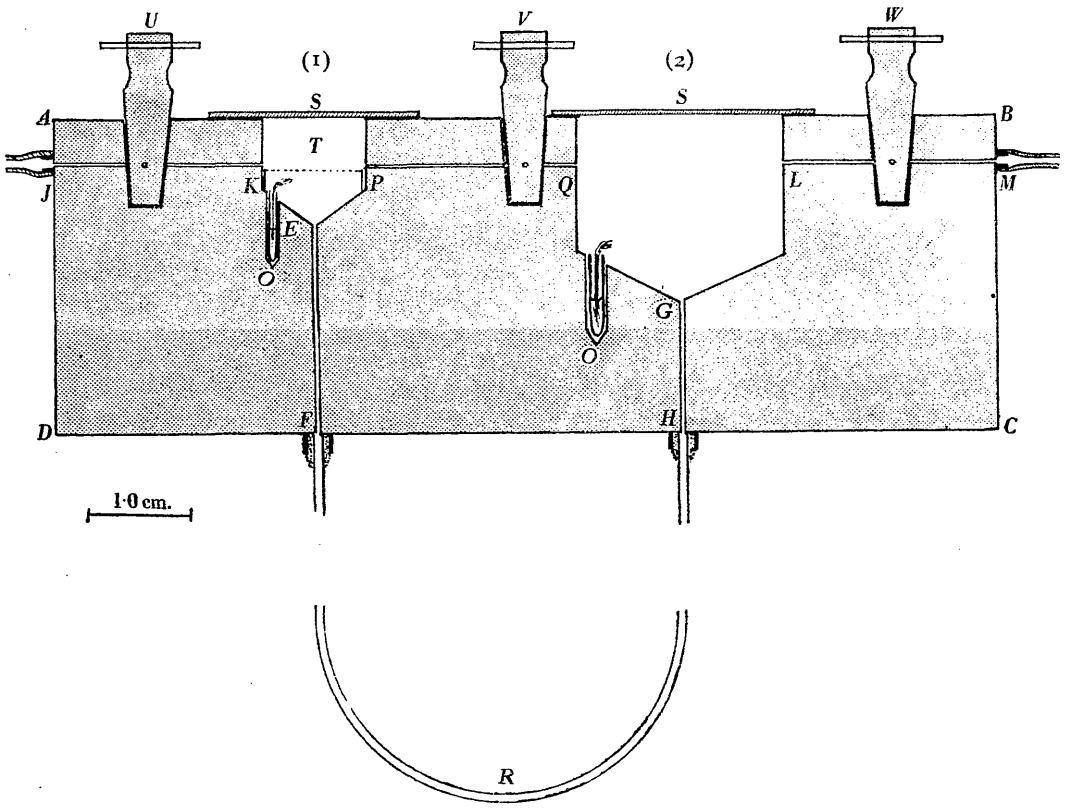

Fig. 1. Diagrammatic section of micro-respirometer. (For explanation see text.)

MANIPULATION

Before fixing the capillary in position the apparatus is cleaned by means of a volatile solvent (ether) which is removed by passing air through the apparatus. The capillary tube of the manometer is cleaned by means of chrome sulphuric acid and distilled ether; any dirt renders the column of paraffin sticky and greatly reduces the accuracy of the apparatus. When filled to a convenient level with paraffin the manometer tube is sealed into position. The apparatus can then be used for an indefinite number of experiments.

Before each experiment the potash in the small tubes and the filter-paper is removed and replaced with fresh and the cover-plates are cleaned and regreased. The gauze T is replaced and the animal inserted in the left-hand chamber. If it is desired to employ a synthetic gas mixture, all the taps are opened and the mixture

passed slowly through. This is best done in two stages, about 70 c.c. being passed at a time, in order to ensure complete exchange in the dead spaces. After passage of gas, taps *U* and *W* are closed, and the apparatus immersed in a thermostat up to the level of the side collars. The level in the manometer limbs should then be equal. When the gas mixture has equilibrated sufficiently (this usually only takes about 2 min.) the centre tap *V* is closed and readings are taken through the microscope. If it is desired to test the efficiency of the taps, the finger can be laid lightly on one of the cover-plates. This will cause a sudden fall of the fluid in this limb, followed by a slower recovery which, however, is complete after about 60 sec., owing to the very rapid temperature equilibration of the apparatus. If the tap *V* is momentarily opened and closed after touching one of the cover-plates, a persistent difference in the manometer levels should be caused. Sustained pressure at *J* or *M* should have no effect on the manometer level. Readings are then taken at intervals and the animal may be observed through the cover-plate.

The thermostating arrangements were found not to be critical on account of the high thermal conductivity of the apparatus. The most suitable arrangement was found to be a large tank of water in a constant-temperature room, but the apparatus worked reasonably well if immersed in a large thermostating device. It was found to be better to have a bath at uniform though steadily changing temperature than one at constant temperature but fluctuating suddenly between narrow limits. In this connexion it should be pointed out that the difference in size of the two chambers does not matter so long as they change equally in temperature, since the coefficient of increase in pressure with temperature is the same in each. If, however, their rate of change were uneven (as would be the case with glass bulbs of low thermal conductivity) the apparatus would become highly temperature sensitive. The low conductivity of the cover-plates is, however, advantageous when the apparatus is not fully immersed, as it damps down any effect of local draught or radiation.

SENSITIVITY AND ACCURACY

The theoretical sensitivity of this apparatus is very high. The scale eyepiece can be read easily to one scale division corresponding to a change in volume of the experimental chamber of 0·037 cu.mm. The comparable sensitivity of the standard Barcroft with a constant of 3–4 and an accuracy of reading of half a millimetre division would be about 3–4 cu.mm. Hence the sensitivity is increased approximately a hundredfold.

The accuracy is limited by two errors. First, by small errors in calibration, and secondly, by slow changes in volume which occur over a long period of time. The errors in calibration are relatively greater than those encountered with the standard Barcroft, owing to the small size of the experimental chamber, but it is unlikely that they exceed 4% in all. The second source of error is the more serious. If the apparatus is set up with the experimental chamber empty, steady small changes are sometimes observed over a long period of time. Table 1 gives the results of some typical control experiments, and it will be seen that the error does not exceed at most 8 div./hr., corresponding to an uptake of less than 0·3 cu.mm./hr., and is usually much less.

The source of this error has not been traced. It is not due to leaks as it is independent of any pressure difference indicated by the manometer. As it is always positive, corresponding to uptake of gas, it may possibly be due to points of corrosion in the apparatus, but no corrosion could be found on examination and the rate of change was somewhat variable. The error is, however, comparable to that caused by the difference in uptake by the small pieces of filter-paper used, and is not therefore very serious.

Table 1

Duration of exp. (hr.)	Rate of change of reading (units/hr.)	Apparent uptake (cu.mm./hr.)
3	1	0·04
25	7	0·26
7	0	0·0
22	2	0·08

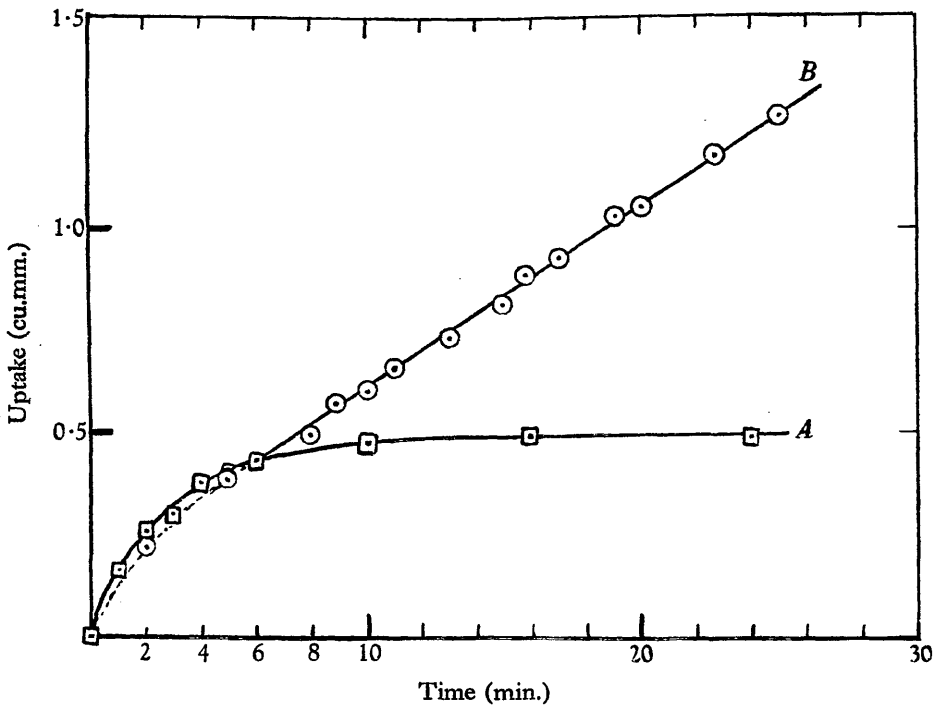

Fig. 2. Oxygen uptake curves. *A*. Control experiment with respirometer empty. Note apparent initial uptake due to vapour-pressure effect. *B*. Experiment with single *Aphelocheirus*. Note apparently greater uptake initially.

The initial equilibration of the apparatus is very rapid. After placing in the water-bath, about 10–15 min. only are required for temperature equilibration, while after small temperature changes caused by handling or passing in a gas mixture the apparatus requires less than 5 min. to become steady. It has been observed that vapour-pressure equilibration takes rather longer. When a damp insect is inserted and the chamber closed, the vapour pressure is close to that of liquid water.

After a short time vapour distils on to the potash-covered filter-paper until the vapour pressure becomes uniform. During this interval the vapour pressure in the experimental chamber is falling, and this gives the impression of an increased initial absorption. This effect is shown in Fig. 2. The effect is still there when no insect is inserted, as the air initially in the chamber is drawn from the immediate surroundings of the water-bath and is saturated. The effect is reduced when more dilute potash is employed, but as long as readings are not taken for about 15 min., when the uptake becomes steady, no error is involved.

When the apparatus has equilibrated it is singularly free from oscillations in the manometer due to temperature changes in the bath or surroundings, so that it is possible to obtain reliable rates of respiration over a very short time interval. For instance, Fig. 2 also gives the results of an experiment where the rate of respiration was 3·5 cu.mm./hr., the whole determination being made in less than 30 min.

This apparatus has been employed by the authors to investigate the respiration of the hemipteron *Aphelocheirus*, and the results of this investigation are now published (Thorpe & Crisp, 1947).

SUMMARY

A Barcroft-type micro-respirometer, made of metal, is described. It is particularly suitable for rapid determination of the respiratory rate of small insects or other animals which can be maintained in air during the course of the experiment. It is especially convenient for experiments in which it is necessary to employ different gas mixtures. Sensitivity is approximately 100 times that of the standard glass Barcroft apparatus. The possible sources of error are considered.

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REFERENCES

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