J. Exp. Biol. (1968), 48, 207–215
With 6 text-figures
Printed in Great Britain

A SIMPLE ELECTROLYTIC RESPIROMETER FOR THE CONTINUOUS RECORDING OF OXYGEN CONSUMPTION UNDER CONSTANT AND NATURAL CONDITIONS

By ANN M. CHASE, D. M. UNWIN and R. H. J. BROWN
Department of Zoology, Downing Street, Cambridge

(*Received* 18 *August* 1967)

INTRODUCTION

During an investigation into rhythmic fluctuations in metabolism, and into the long-term effects of drugs on the respiratory pattern during the development of insects, the need arose for a respirometer with certain specific features. These requirements are: (1) its operation should be independent of barometric and temperature fluctuations, but it should operate with the pressure and the oxygen and carbon dioxide levels as near atmospheric as possible; (2) it should be capable of uninterrupted operation for at least one week; (3) it must have as high a degree of sensitivity and accuracy as possible; (4) it should record continuously and display the results directly as rate of oxygen consumption. Although several respirometers have been described in recent years, none was found to fulfil all the requirements.

The principle of the constant-pressure manometer offers a simple method for determining respiratory rates. The organism is contained in a closed chamber connected to a U-shaped capillary containing a fluid. The carbon dioxide evolved by the organism is absorbed by alkali. As oxygen is utilized, the pressure of the chamber being maintained constant, the volume of gas within the chamber falls. This causes a rise in the level of fluid in the arm of the capillary connected to the chamber. Provided that the capillary bore is uniform, and the temperature and external pressure remain constant, the rate of rise of fluid in the capillary bears a direct relation to the rate of oxygen consumption by the animal. Since the other end of the capillary is open, the measurements will be dependent on the external pressure. By connecting this open side of the capillary to a compensating chamber of equal volume the measurements can be made independent of changes of pressure and temperature. This constitutes the principle of the differential manometer. Differential manometers, such as that devised by Barcroft, have been extensively used in short-term experiments on respiratory rates. However, they are unsuitable for long-term experiments, since their operation entails a declining oxygen level. The principle of using electrolysis to replace the oxygen respired by the organism enables the oxygen to be maintained at atmospheric level. The limit as regards the duration of operation will be set by the saturation time of the carbon dioxide absorber.

Several workers have described electrolytic respirometers. In recent years Capraro (1953), Swaby and Passey (1953), Macfadyen (1955) and Winteringham (1959) have described electrolytic respirometers which use only a single electrode system. The

reduction of gas volume in a closed chamber, as a result of oxygen utilization by the animal, causes an electrolyte to rise up an anode in the chamber. Electrolytic generation of oxygen occurs until either the increase in gas volume is sufficient to cause the meniscus to break contact with the electrode, or until the area of the anode immersed is such that the current passing through it generates oxygen at the same rate as it is being used by the animal. Oxygen production could be measured directly by a voltameter, or indirectly by an integration of the current and the time for which it flows. This simple type of respirometer, where the demand for oxygen is sensed by the oxygen-generating electrode, is generally unreliable for continuous accurate records because of the formation of bubbles at the anode (Macfadyen, 1961). This led Macfadyen (1961), and Heusner, Stussi & Dreyfus (1965) to devise respirometers with separate electrode systems for detecting pressure change and for generating oxygen. If copper sulphate rather than sulphuric acid is used as the electrolyte, oxygen is the only gaseous product, copper being deposited on the cathode. This enables the system to be closed by connecting a compensating chamber to the other end of the capillary which contains the device pressure detecting change. Independence from fluctuations in atmospheric pressure is thereby achieved.

The respirometer to be described uses the same principles as the instruments described by the above authors. Like the Macfadyen (1961) model it is based on a Barcroft type of differential manometer and uses copper sulphate as the electrolyte. Each respirometer unit comprises experimental and compensating chambers connected by a capillary containing the sensing electrode. A reduction in the volume of gas in the experimental chamber causes an electrolyte to move along the capillary. The contact of this fluid with the sensing electrode works a relay, which switches on a direct current to the electrolysis unit in the experimental chamber. Oxygen will continue to be generated until contact is broken at the sensing electrode; the amount of oxygen supplied equals that used by the animal. Provided that the current is maintained constant, the amount of oxygen supplied will be proportional to the time for which electrolysis continues.

DETAILS OF THE APPARATUS

(1) *Respirometer unit* (Figs. 1–3)

The respirometers are constructed of Perspex, since it is relatively cheap and is easy to machine. No toxic effects were found when animals were enclosed for considerable periods within chambers of Perspex. The animal chamber (*a*) and the compensating chamber (*b*), identical in form and size, are machined within a solid block of Perspex. The chambers are closed by lids (*c*) cut from Perspex plate. This enables the animal inside the respirometer to be observed from above, and to be given a suitable light regime. The lids are clamped by a forked brass plate (*d*) screwed down with the aid of a wing nut (*e*). An 'o' ring seal (*f*) ensures that the lids are gas-tight when screwed down. Valves are provided in the lids so that the respirometer can be set up at near atmospheric pressure. The valve comprises a screw (*g*) with a groove cut to allow the passage of gas when the valve is open. When the valve is screwed down a gas-tight seal is provided by an 'o' ring (*h*) under the screw head Within the chamber the animal rests on a Perspex tray (*i*), irregular at the edges to

allow the passage of gases. This tray protects the animal from the copper sulphate spray generated during electrolysis. Vessels to contain alkali (*j*) and copper sulphate (*k*) are drilled from the base of each chamber. The electrodes are 0·005 in. platinum wire passing into the solutions through holes in the Perspex block. They are sealed in with Araldite; this was found to give a permanent seal resistant to copper sulphate and alkali. The electrodes are soldered on to leads (l) at the surface of the block, and the junctions are insulated with Araldite within a Perspex casing (*m*). The whole system is entirely waterproof and can be immersed in a constant-temperature water bath. Oxygen-generating electrodes (*n*) are provided in both chambers. The com-

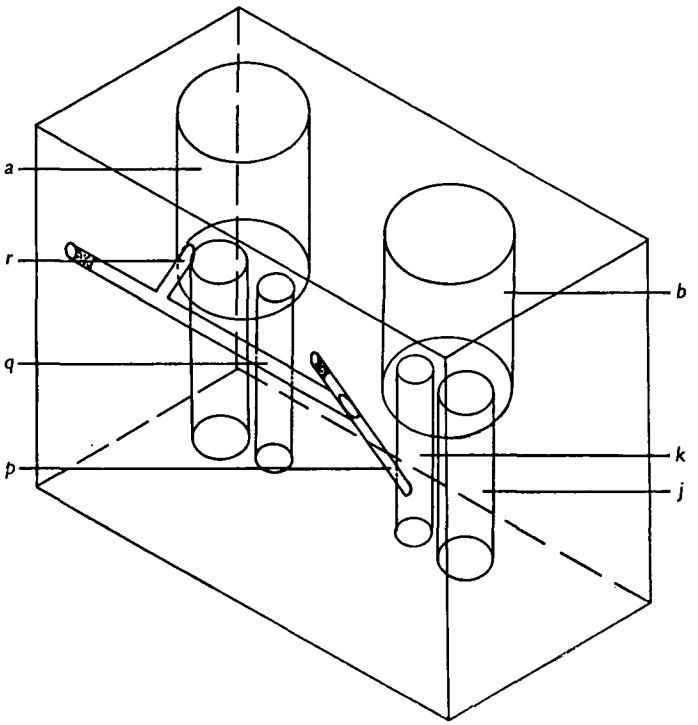

Fig. 1. Simplified diagram of respirometer showing arrangement of chambers within Perspex block, and connecting system of holes. Cut away surfaces shown hatched. Araldite seals shown stippled

a, Animal chamber; *b*, compensating chamber; *c*, lid; *d*, forked brass plate clamping lid; *e*, wing nut; *f*, 'o' ring seal under lid; *g*, valve in lid; *h*, 'o' ring seal operating when valve is screwed down; *i*, Perspex tray on which experimental material is supported; *j*, vessel containing alkali; *k*, vessel containing copper sulphate; *l*, soft solder joint of electrode to its lead; *m*, Perspex casing containing Araldite insulation of lead/electrode joints; *n*, oxygen-generating electrode; *o*, pressure-sensing electrode; *p*, glass capillary; *q*, *r*, holes connecting capillary with the animal chamber; *E*, earth.

pensating electrode is used to adjust the position of the fluid within the capillary. This is necessary especially when the respirometer is set up empty to test for factors affecting the stability of the system such as leakage and the degree of saturation of the carbon dioxide absorber.

The pressure-sensing electrode (*o*) lies within a glass capillary (*p*). The capillary projects into the copper sulphate vessel of the compensating chamber. The hole

into which the capillary is sealed is inclined up towards the outside, but is closed to the exterior where the electrode is soldered to its lead. The hole containing the capillary opens into a wider hole (*q*) drilled within the block parallel to its long side. This in turn is connected with the animal chamber by a short cross hole (*r*).

Six respirometers have been constructed and are operated concurrently.

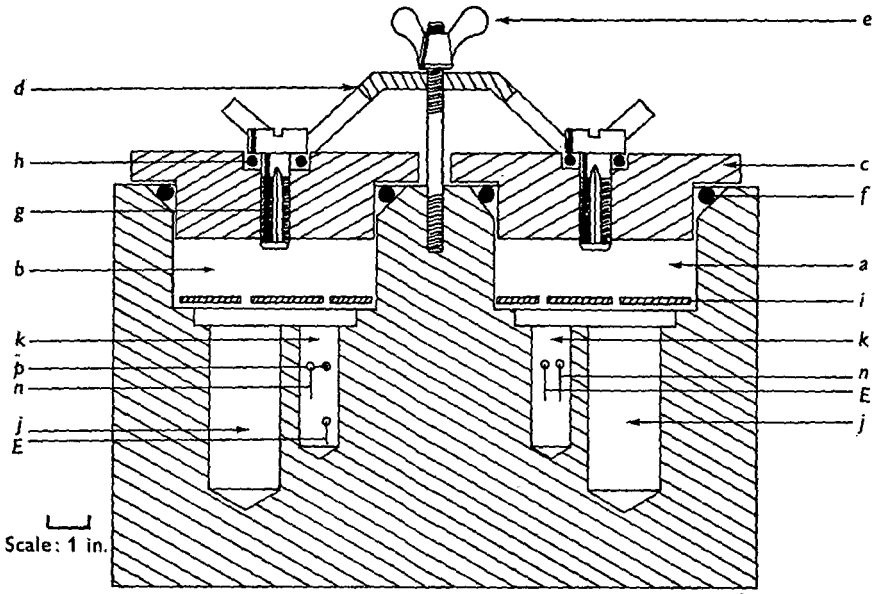

Fig. 2. Section through respirometer with chambers of 12 c.c. capacity. See legend to Fig. 1.

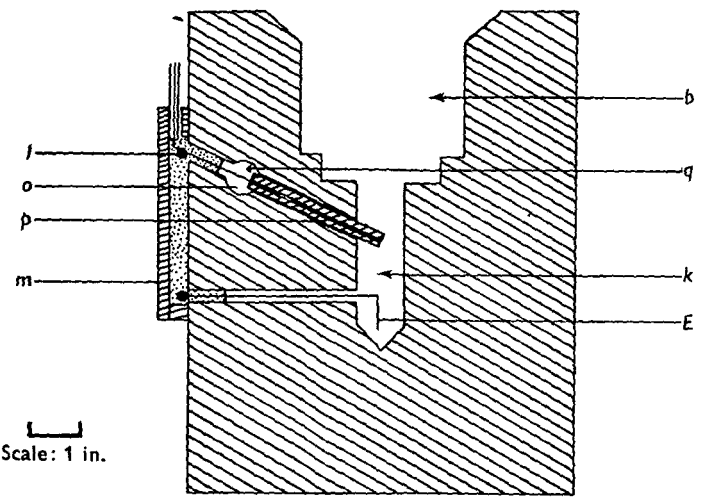

Fig. 3. Section through respirometer at right angles to Fig. 2 passing through compensating chamber to show capillary containing pressure-sensing electrode. See legend to Fig. 1.

(2) *Controlling unit* (Fig. 4)

A small alternating current (50 c/s) is fed into the pressure-sensing electrode (*o*) through a high resistance. *AC* is used to avoid polarization of the electrode, which would reduce the sensitivity. When the solution is in contact with the electrode the voltage between the electrode and earth is negligible. When the contact breaks, an alternating voltage appears between electrode and earth. This voltage is amplified by a two-stage transistor amplifier, whose rectified output is further amplified and operates a relay, which is arranged to switch on electrolysis when contact between the electrode and fluid is made.

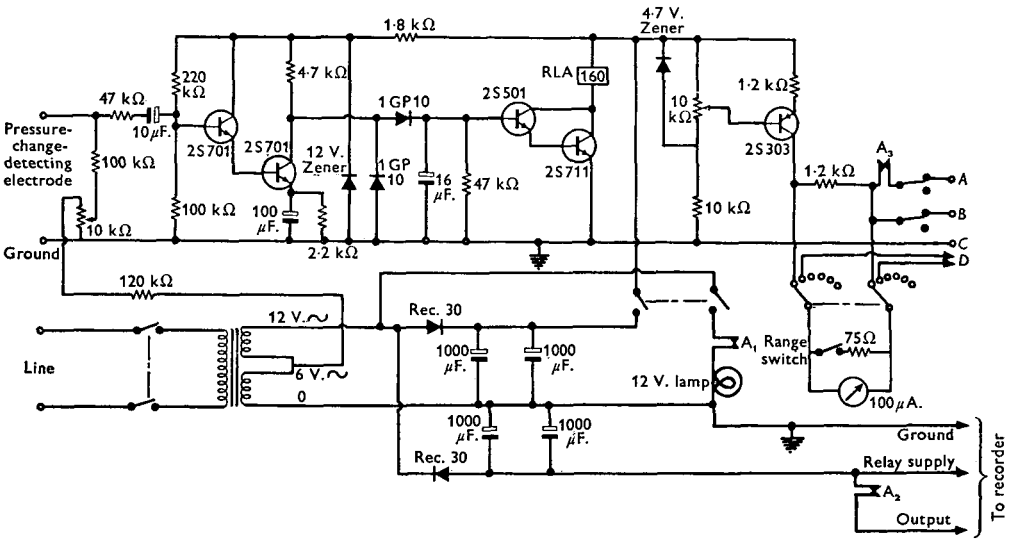

Fig. 4. Circuit diagram of controlling unit. A, Experimental oxygen-generating electrode. *B*, Compensating oxygen-generating electrode. *C*, Ground. *D*, Channel 2.

A constant direct current (from 0 to 2 mA) for electrolysis is derived from the collector of a transistor whose base is returned to a controlled voltage and whose emitter has a resistive load.

(3) *Recording unit* (Figs. 5, 6)

The main relay operated by the pressure-sensing electrode has two functions. When it is energized it allows current to flow in the oxygen-generating circuit, and it also energizes the tape clutch relay (*a*) on the recording carriage (*b*). When the tape clutch relay is energized it grips a tape (*c*), which moves continuously at a rate of 10 in./hr. across the recorder paper (*d*). As the carriage is moved by the tape it marks the paper by pressing down on a typewriter ribbon (*e*) with a sharp-edged wheel (*f*). The paper itself is moved forward continuously at a rate of 0·4 in./hr. (*g*). Every 15 min. a timing signal causes the tape clutch relay to release the tape. Then the carriage is lifted by rotation of the ridged lifting bar (*h*), and reset to zero by movement of the resetting bar (*i*). At the same time the typewriter ribbon is advanced.

The recorder therefore produces a series of approximately parallel lines and each

one has a length proportional to the quantity of oxygen consumed in that 15 min. period.

The recorder uses paper 30 in. wide over which are mounted nine carriages, so that nine simultaneous recording channels are available.

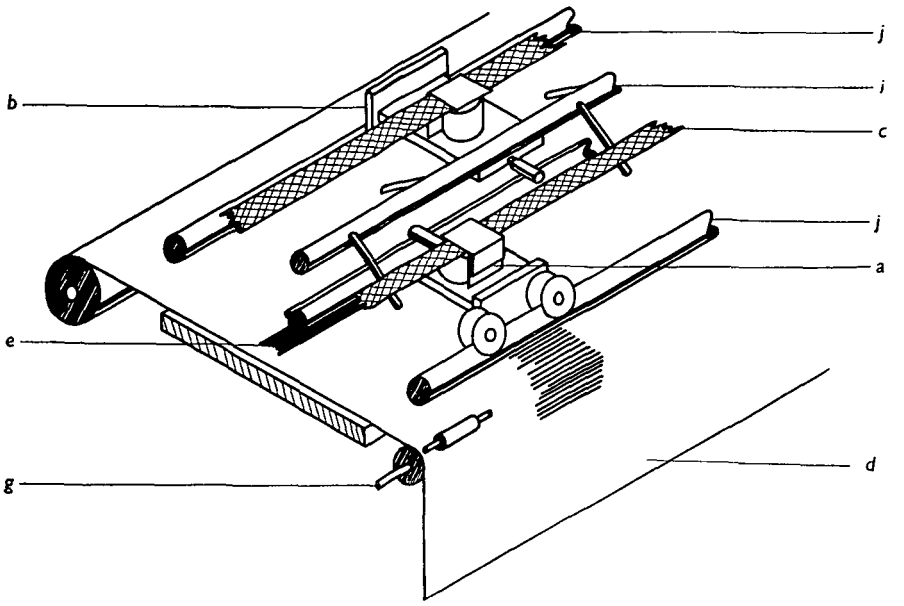

Fig. 5. Simplified diagram showing the essential features of the recording unit. Electric motors driving tape and paper and also the motor and timing system of the resetting mechanisms are omitted.

a, Tape clutch relay; *b*, recording carriage; *c*, tape; *d*, recorder paper; *e*, typewriter ribbon; *f*, sharp-edged wheel; *g*, paper drive mechanism; *h*, lifting bar of resetting mechanism; *i*, resetting bar; *j*, carriage rails.

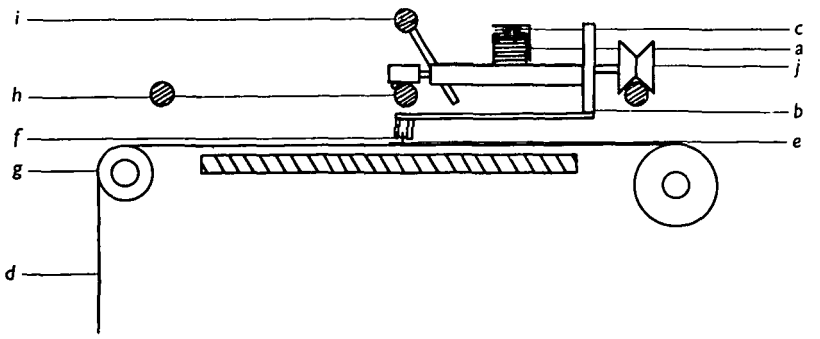

Fig. 6. Section of recorder showing detail of the recording carriage.
See legend to Fig. 5

OPERATION

The respirometer is designed to fulfil the specific requirements listed in the introduction.

(1) Since the system is entirely closed once recording has started, it is not affected

by changes in atmospheric pressure. Immersion in a constant-temperature water bath ensures complete thermal stability once thermal equilibrium has been reached. The valves in the lids allow the respirometer to be set up at near atmospheric pressure. If the valve on the animal chamber is closed with the compensating chamber valve open, the fluid in the capillary will adjust to atmospheric pressure. The volume change involved as the compensating valve is closed is very small. This volume change will be offset by oxygen generation in the animal chamber to restore the meniscus in the capillary to the working position. The final pressure and oxygen level in the animal chamber will be negligibly higher than atmospheric. In most experiments an alkali carbon dioxide absorber is used, which results in an abnormally low level of carbon dioxide. To test the effect of the level of carbon dioxide on the respiratory rate a carbon dioxide buffer has been used. Diethanolamine has proved satisfactory for maintaining the carbon dioxide at atmospheric level. The buffer is prepared as described by Krebs (1951) and gassed with air overnight at the temperature of the respirometer. The stability of the buffer after such treatment was found to be complete. It was tested by setting up a respirometer using the buffer, but with no experimental material. No volume change was found to occur, indicating that the buffer was completely equilibrated with air. The saturated copper sulphate solution used as the electrolyte gives a relative humidity of 90 %. Most animals were found to thrive at this comparatively high humidity.

(2) The duration of uninterrupted operation is determined by the time taken to saturate the carbon dioxide absorbing system. Four per cent alkali is used, since this has the same vapour pressure as the saturated copper sulphate solution used as the electrolyte. A volume of about 1·5 ml. is used in the model described. This will last over a week when the carbon dioxide evolution is of the order of 50–100 μl. per hour. Provided that a rolled filter paper is introduced into the alkali-containing vessel to increase the surface area for absorption it has not been found necessary to stir the solution. The copper sulphate solution has to be renewed at intervals as copper is removed by the electrolytic process. The copper deposited on the cathode is dissolved with nitric acid. It has also been found advisable to clean out the capillary system with detergent from time to time, to ensure free movement of the meniscus.

(3) The sensitivity of the system is determined by the form of the capillary and sensing electrode, the size of the chambers and the nature of the controlling circuit. In the present model, with chambers of about 12 ml. the volume change required to operate the relay is of the order of 0·1 μl. In most conventional manometers the volume change detectable is of the order of 1·0 μl.

The accuracy of the method will depend on several features:

(*a*) the stability of the system. Provided that there is no leakage, a respirometer set up without respiring tissue and with contact just made between the electrode and the meniscus will remain in this state permanently.

(*b*) the stability of the oxygen-generating current. This is ensured by the use of transistors, a common supply being used to all six respirometers. The difference in the electrolysis current when only one channel is operating and when all six are operating, is less than 1 %.

(*c*) the accuracy with which the current is measured. Over the range of currents used the ammeter is known to be accurate to within 1 %.

(*d*) the accuracy of recording. Since the final record is obtained as the length of a line drawn by the recorder pen, representing the portion of time the the recorder relay is activated from the main relay in fifteen minutes, the accuracy will depend on:

(*i*) the delay between the main relay operating and the carriage relay. This is negligible, being of the order of 5 m-sec.

(*ii*) the constancy of the speed of the motor driving the tape which moves the energized carriage relay. A synchronous motor is used, driven from the 50 cyc./sec. mains. Over very short periods of time this is unlikely to have more than a 5% variation. Compensation over longer periods of time means that the error is effectively zero.

(*iii*) the accuracy with which the line is measured. The records are mounted over squared paper, from which it is possible to read off the length to the nearest 0·02 in.

The accuracy of the respirometer was checked against a Flaig differential manometer, with vessels of about the same volume as the respirometer chambers. A *Tenebrio molitor* pupa in 'artificial diapause' was used as experimental material. This pupa had been injected with Actinomycin D at an early stage in the pupal period, a treatment which arrests development. The respiration of this pupa was constant for several days. Readings of its oxygen consumption were taken in the manometer at fifteen minute intervals for 1 hr. It was then transferred to the respirometer and a similar set of measurements was made. This cycle of consecutive readings in the manometer and respirometer was repeated several times. The accuracy of the respirometer was found to be comparable with that of the Flaig manometer.

Flaig manometer	$82{\cdot}9 \pm 0{\cdot}44$ μl./hr.
Respirometer	$83{\cdot}6 \pm 0{\cdot}19$ μl./hr.

(4) The recorder is designed to give maximum resolution of changes in the rate of oxygen consumption, as opposed to changes in the totals of oxygen consumed during long periods of time. It records continuously. Since the slope of the line produced by the pen, as well as the length of the line, bears a relation to the rate of oxygen consumption, resolution of rate changes within the 15 min. periods is possible.

The calibration of the record is simple, since 2·5 in. (the maximum travel of the pen in 15 min.) will be equivalent to 210 μl./hr. when the current is 1 mA. The current is generally adjusted so that oxygen is generated for about half the possible time.

DISCUSSION

Although many different types of respirometer are already in existence, it is felt that the model described in this paper possesses many features which could make it useful in several fields requiring the long-term measurement of respiratory rates under constant conditions. This is especially so for research on circadian and other rhythms, where absolute constancy of conditions is essential and yet the conditions within the respirometer must be as near normal as possible. The ease of construction and operation and the relative cheapness are features which should attract the general biologist. It is designed to permit recording over a wide range of respiratory rates. It has been tested over the range of currents 0·1–1·0 mA, representing respiratory rates of 10–100 μl. oxygen per hour. There is no reason to believe that the range could

not be extended if required. Although the recorder was designed specifically to fulfil the requirements of this respirometer system, it is hoped that it too will find general application where this type of integrated recording is required.

SUMMARY

1. The principles involved in the design of an electrolytic respirometer are set out.

2. A respirometer is described for air-breathing animals which allows the continuous recording over long periods (up to two weeks) of oxygen consumption at rates of the order of 10–100 μl. per hour.

3. Provision is made for maintaining the temperature, pressure, and carbon dioxide and oxygen levels constant and at atmospheric values.

4. A new design of recorder displays the results directly as rate of oxygen consumption.

5. The features of the apparatus are discussed with reference to specific requirements.

We would like to thank Mr R. E. D. Holder for his assistance in the construction of the recorder, Mr R. J. Symonds for assembling the controller unit and Mr M. J. Haggerty for the final drawings of the apparatus. One of us (A.M.C.) is supported by a SRC studentship.

REFERENCES

CAPRARO, V. (1953). A new method for measuring oxygen consumed in the metabolism of small animals. *Nature, Lond.* **172**, 815.

HEUSNER, A. STUSSI, TH. & DREYFUS, E. (1965). Application de la coulometrie a la mésure de la consommation d'oxygène. *Med. Elect. Biol. Engng* **3**, 39–56.

KREBS, H. A. (1951). The use of 'CO_2 buffers' in manometric measurements of cell metabolism. *Biochem. J.* **48**, 349–59.

MACFADYEN, A. (1955). Notes on two pieces of apparatus for the study of metabolism in soil animals. In *Soil Zoology*, pp. 315–32. Ed. D. K. Kevan. Butterworth.

MACFADYEN, A. (1961). A new system for continuous respirometry of small air-breathing invertebrates under near-natural conditions. *J. exp. Biol.* **38**, 323–41.

SWABY, R. J. & PASSEY, B. I. (1953). A simple macrorespirometer for studies in soil microbiology. *Aust. J. Agric. Res.* **4**, 334–9.

WINTERINGHAM, F. P. W. (1959). An electrolytic respirometer for insects. *Lab. Pract.* **8**, 372–5.