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AN OXYGEN ELECTRODE MICRORESPIROMETER

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INTRODUCTION

The oxygen consumption of small nematodes has often been measured with methods using many individuals. Santmeyer (1956) emphasized that this reduces the technical difficulties involved but limits the interpretation of results to certain general comparisons. The Cartesian diver technique (Linderstrom-Lang, 1937) has been used to measure the oxygen uptake of single nematodes (Nielsen, 1949; Wieser & Kanwisher, 1960, 1961; Teal & Wieser, 1966). This paper describes an oxygen electrode respirometer which possesses certain advantages over the above techniques.

The membrane-covered, polarographic electrode described by Clark (1956) has features which make it suitable for some biological applications. In particular, it is capable of continuously and directly measuring the partial pressure of oxygen over prolonged periods. Furthermore, only an inert, non-conductive membrane, permeable to gases, comes into contact with the animal. However, the reduction of oxygen at the cathode causes a fall in the oxygen tension of an enclosed volume of water. As it is usually preferable not to compensate for this, the electrode is mainly used in situations in which this effect is negligible. The Clark-type electrode and associated equipment, manufactured by Radiometer of Copenhagen, is particularly suited to the measurement of oxygen uptake of small animals since it consumes little oxygen itself. Moreover, since the rate of oxygen consumption by the electrode does not exceed the diffusion rate of the gas in water, stirring is not necessary to maintain its calibration.

The apparatus has been found to be very sensitive and reliable. It has been used to measure the oxygen consumption of individual adult males of the marine nematodes *Enoplus brevis* and *E. communis* (Atkinson 1973*a*, *b*). It is hoped that it will also be found useful for other small animals; the enoplids ranged from 60–380 μg in body weight.

APPARATUS

Essentially, the respirometer consists of an oxygen electrode within a stainless steel housing, which is surrounded by a constant-temperature water jacket. The membrane of the electrode forms part of an enclosed animal chamber which has a volume of 250 mm^3. The top of the chamber can be removed and then re-sealed after placing an animal in one part of the cell. A slowly rotating micro-stirrer, magnetically controlled, reduces the diffusion gradient between the nematode and the electrode, but does not disturb the animal. The fall in the oxygen tension due to the oxygen consumption

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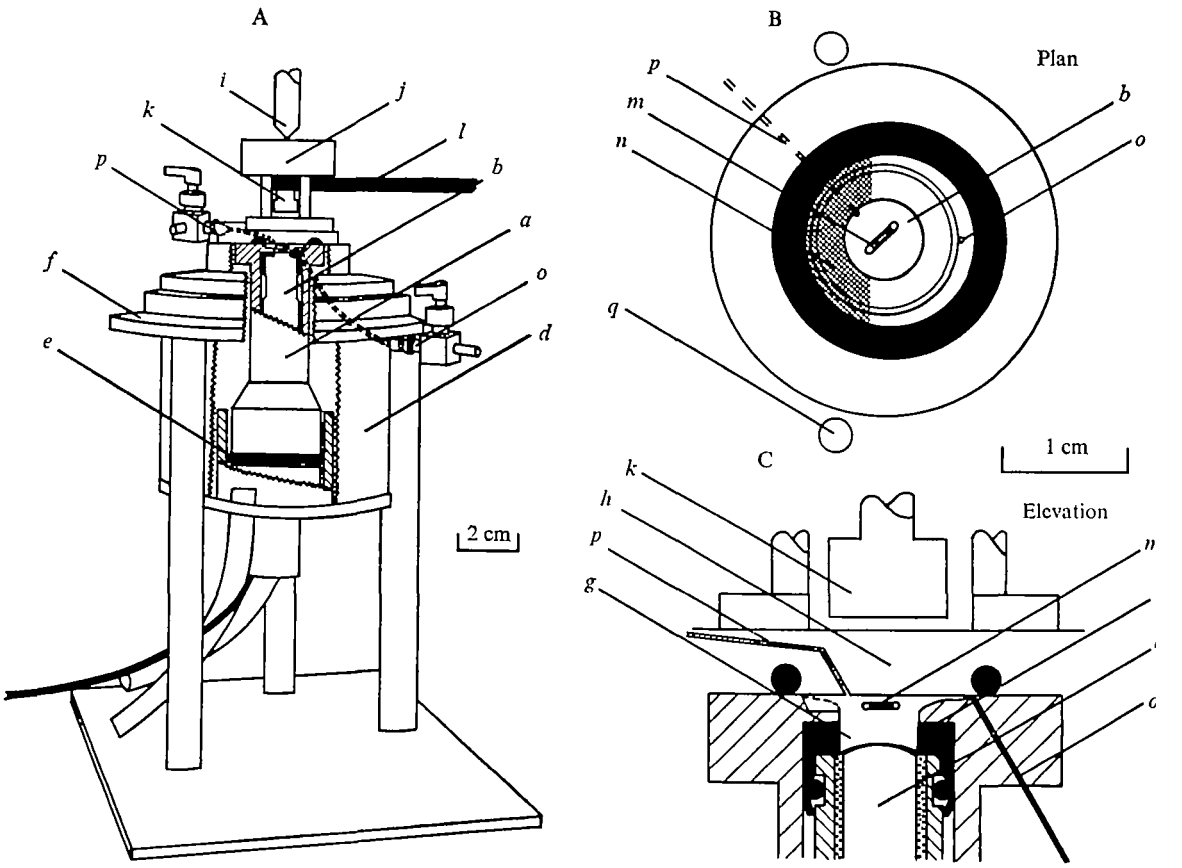

Fig. 1. A: Simplified longitudinal section of the respirometer. B and C: The animal chamber. *a*, Stainless steel electrode housing; *b*, oxygen electrode; *c*, oxygen electrode cap; *d*, water jacket; *e*, large 'O' ring sealing electrode housing to jacket; *f*, brass stand; *g*, animal chamber; *h*, Perspex top to chamber; *i*, centre screw of 'gear extractor' clamp (not shown), legs act against flange (*f*) of brass stand; *j*, tripod; *k*, rotating magnet; *l*, drive band to magnet from constant speed motor (not shown); *m*, magnetic follower; *n*, net envelope; *o*, inlet with valve; *p*, outlet with valve; *q*, one of two pins locating Perspex top to animal chamber.

of the individual is monitored by the oxygen electrode. After amplification its output is displayed on a meter, and recorded continuously by a potentiometric pen recorder.

The steel electrode housing (*a*, Fig. 1 A) is a modification of that provided by Radiometer (thermostated cell type D 616) for use with the oxygen electrode E 5046 (*b*). The glass face sealing one end of the bore of this standard housing was removed, and a stainless steel washer cemented in its place. When the oxygen electrode was mounted into the housing, its cap (*c*, Fig. 1 C) formed a gas-tight seal against this steel facing. The housing was fixed into a ring of Tufnol (Tufnol Ltd.), which had been sealed to one end of a Perspex cylinder 8 cm in diameter; these formed the roof and walls of the water jacket (*d*) respectively. Its base was made of Tufnol, with a central cylinder which sealed against a larger 'O' ring (*e*) fitted to the rear of the steel housing. The water jacket was mounted into a brass stand (*f*).

When sealed in place, the electrode face formed part of the animal chamber (*g*). The Perspex top of the cell (*h*) bore a groove which accepted a Vitron O ring (Edwards Ltd.); this gave a gas-tight seal against the upper surface of the steel electrode housing (*a*) when pressure was applied from a clamp (*i*) through a tripod (*j*). The centre of this tripod was fitted with a ball race for an axle carrying a magnet (*k*). This was rotated at 25 revolutions per minute by a constant-speed motor via a drive band (*l*). The magnetic stirrer (*m*, Fig. 1) contained within the respirometer chamber was approximately 5 mm long, and was made of fine iron wire, encapsulated in glass. Physical contact between it and the nematode was prevented by a net envelope (*n*) of 60-mesh per cm, which restricted the nematode's movement to one section of the chamber. The cell could be flushed with water, the flow being controlled by two gas-tight Teflon valves (Hamilton and Co.) at the inlet (*o*) and the outlet (*p*) of the chamber. The nematode was visible to the naked eye through the transparent Perspex cell top.

All measurements of oxygen consumption were made at $15 \pm 0{\cdot}1$ °C. The respirometer cell was maintained at constant temperature by a Radiometer water bath VTS 13, which pumped water at constant temperature through the jacket surrounding the electrode. A mixture of water and glycerol was pumped from a refrigerated reservoir through the coils in the VTS13 water bath. This had a cooling effect on the water bath, which was opposed to constant temperature by a more powerful heater controlled by a mercury-contact thermometer. The circulating water contained 4·2 g/l KNO_3; this acted as an earthed, conductive shield around the electrode, and prevented electrostatic disturbance.

The oxygen electrode

The Radiometer oxygen electrode type E5046 is based on the design of Clark (1956). The current flowing as the result of the reduction of oxygen at the cathode is small, approximately 10^{-11} amp/Torr at 38 °C. This is due to two factors: the diameter of the exposed surface of the platinum cathode is 20 μm, and the polypropylene membrane is relatively impermeable to oxygen. The high-gain stable amplifier of the Radiometer pH meter PH27 was connected to the oxygen monitor PHA927b, which adjusts the sensitivity range of the system to give an output for measurement of 0·1 mV/Torr. The oxygen tension was read directly from a calibrated meter, and a continuous recording was obtained by connexion to a suitable pen recorder.

In general, the oxygen electrode was assembled for use as described in the Radiometer manual. The plastic cap used to seal the electrode into the steel electrode housing was made pliable by exposure to ultrasonic vibration for 5 min at 16 kHz, and a peak-to-peak intensity of 8 μm. It was fitted to the face of the assembled electrode jacket, which was then screwed into the steel housing until the applied pressure was sufficient to seal the cap to both the electrode and the steel washer. The electrode was left in place until its sensitivity had diminished; usually the useful life of a membrane was 2 months. At low oxygen tensions the plastic cap was replaced by a baked Vitron O ring (Edwards Ltd.), held in a groove machined into the face of a modified electrode jacket. A gas-tight system was more easily obtained at low oxygen tensions with seals of this type.

Calibration of the oxygen electrode

The respirometer was equilibrated at 15 °C for 1 h before the oxygen electrode was calibrated as recommended. A freshly made solution of 20 mg/ml Na_2SO_3 in 0·01 M borax (Koltoff & Lingane, 1952; Davies, 1962) was placed on the electrode face and covered with a glass coverslip. When there was no further fall in the electrode output, the oxygen monitor and pen recorder were adjusted to zero. The solution was removed carefully with filter paper, and the cell was thoroughly washed with distilled water. It was only necessary to repeat this part of the calibration procedure occasionally, as the zero of the electrode was very stable; possible contamination of the animal with sulphite was, therefore, minimized. Water from the water bath was gently squirted on to the electrode face from a syringe several times, and the top of the chamber was then sealed in place. The stable oxygen tension recorded was adjusted to the partial pressure of oxygen on the assumption that oxygen contributed 20·94 % to the ambient pressure after due adjustment for the presence of water vapour. The chamber was then dried as before, and flushed with sea water before use. This second calibration procedure was adopted before each measurement of the oxygen consumption of an individual animal. It was found that the calibration was stable during the few hours required for the measurement of oxygen consumption.

The oxygen content of the sea water in the animal chamber

The fall in oxygen tension in the animal chamber enables the oxygen consumption of the animal to be determined, but clearly it is necessary to know the volume of the chamber and the solubility of oxygen in the sea water used.

The volume of the chamber, as set up for measurement of oxygen consumption, was determined by a dye-dilution technique, conveniently whenever it was necessary to remove an ageing membrane from the electrode. The chamber was filled with a strong Amaranth solution, which was then flushed clear and the washings were collected. They were pooled, diluted to a known volume, and the concentration of the dye was determined spectrophotometrically at 524 mμ against known dilutions. On each occasion, the median three of six similar readings were averaged; it was found that re-sealing the chamber had little effect on the volume, which remained 250 ± 5 mm^3.

The salinity of the sea water, collected from the shore at Bamburgh, Northumberland, did not vary, and was considered sufficiently similar to L'Eau de Mer Normale, 19·38‰ Cl, to allow this standard to be adopted. When in calibration with water-saturated air at one atmosphere total pressure at 15 °C, the solubility of oxygen in sea water at this chloride value was taken to be 5·73 ml O_2/l at N.T.P. (Strickland & Parsons, 1968). The small error in assuming that the reading of the oxygen electrode at 15 °C was strictly proportional to the concentration of the oxygen in sea water, as well as to the partial pressure, was ignored. It was calculated that a change in oxygen tension in the respirometer of 1 Torr at 15 °C would be caused by a change in oxygen concentration of the sea water of approximately 0·009 mm^3 O_2 at N.T.P.

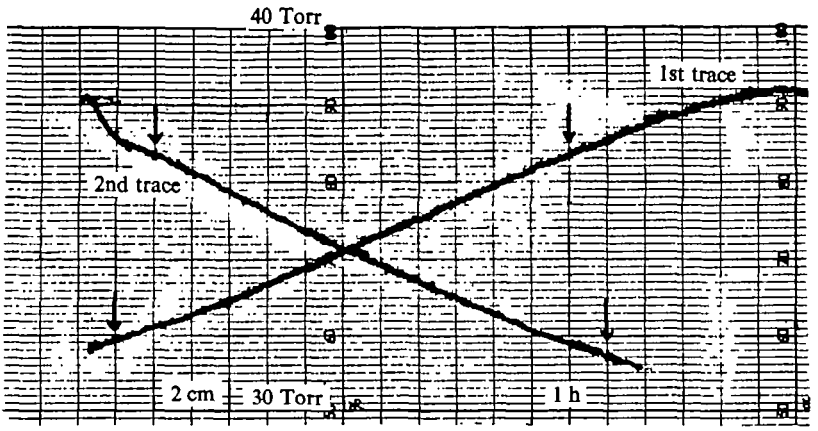

Fig. 2. Fall in oxygen tension within respirometer due to the oxygen consumption of one *Enoplus brevis* male at an oxygen tension of approximately 35 Torr. Arrows indicate measurement for two periods of an hour with fresh sea water introduced between recordings.

EXPERIMENTAL PROCEDURE

The electrode was first calibrated as described; then 150 ml of sea water was passed through a porosity-5 glass sinter (Gallenkamp and Co.) fitted to an evacuated flask, to remove most suspended organisms. A gas mixture was bubbled into it until it attained the required oxygen tension, and the sea water was then allowed to flow into the respirometer. The chamber was sealed, and the magnetic stirrer was started. The oxygen tension was recorded for a period of 1 h, and the experiment was continued only if a constant value was recorded; instability was usually due to a defective membrane. A blank run was carried out on each day of experiments.

For a measurement of oxygen uptake, a further 150 ml of sea water was treated as described, and allowed to flow into the chamber. A nematode was gently transferred to the respirometer with a fine, camel-hair brush, the free edge of the Nylon net being lifted and the animal stroked into place under it. The magnetic stirrer and the pen recorder were switched on, and a period of equilibration of 15–30 min was allowed. During this period the high level of activity of the nematode, presumably due to handling, fell to a level similar to that prior to its transfer. The fall in oxygen tension within the respirometer was then recorded for 1 h. The chamber was flushed for 5 min with a further volume of freshly filtered sea water, to restore the tension to its original value and to help reduce the build-up of suspended micro-organisms within the respirometer. The oxygen uptake of the nematode was then measured for a second period of 1 h. A typical pair of recordings obtained for an *Enoplus brevis* male at 35 Torr are shown in Fig. 2. The mean of the two rates, usually in reasonable agreement, was used in the calculations.

The following precautions probably increased the accuracy and reliability of the apparatus. (1) Since the elastomer seals contain oxygen (de Csernatory, 1966), large fluctuations in the oxygen tensions within the chamber probably caused a change in the quantities they held. Experiments were not carried out involving large changes

in the oxygen tension while an animal was in the respirometer. (2) In order to maintain a constant oxygen tension in the apparatus, the chamber was constantly flushed with distilled water when not in use. Benzyl penicillin (100 units/ml) and streptomycin sulphate (0·1 mg/ml) were added to the distilled water, and the oxygen tension was adjusted to that required for a series of experiments. The antibiotics reduced any possible build-up of micro-organisms within the apparatus, but the nematodes were not subjected to these chemicals since the chamber was thoroughly flushed before use.

DISCUSSION

Holter & Zeuthen (1966) only considered respirometers capable of measuring changes of 0·1 mm^3 O_2/h with an accuracy of at least 5 %, in a review of techniques available for measuring very small rates of oxygen utilization. The microrespirometer herein described can therefore be included in this grouping. At 135 Torr the nematode produced a change in oxygen tension of 10–15 Torr, corresponding to an uptake of 0·09–0·135 mm^3 O_2/h; at 35 Torr the consumption was approximately 0·036 mm^3 O_2/h, which could be estimated to 0·1 Torr or 0·0009 mm^3 O_2.

It is not possible to define the absolute accuracy of the apparatus in use because of other sources of variation. Errors in estimating the body size of enoplids and differences in the oxygen requirements of individuals are more likely to make major contributions to the relatively small, weight-independent variation found for the oxygen consumption of male enoplids (Atkinson, 1972).

Greater sensitivity could probably be attained by increasing amplification of the output from the electrode or by reducing the volume of the animal chamber. The acceptable level of oxygen consumption by the electrode itself and the noise level in the signal would probably determine the maximum sensitivity. However, the permeability of the cell materials to oxygen and the absorption of the gas by the elastomer seals may be limiting factors in smaller cells.

SUMMARY

1. A constant-temperature microrespirometer, based on a Clark-type oxygen electrode, is described. The apparatus was designed for use with small, individual nematodes weighing 60–380 μg body weight.

2. The experimental procedure for accurate and reliable measurements of oxygen consumptions of the order of 0·02–0·2 mm^3 O_2/h is given.

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