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AN ELECTROLYTIC, DIGITAL RECORDING, MULTICHANNEL MICRO-RESPIROMETER

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SUMMARY

A 20-channel micro-respirometer is described which replaces oxygen consumed by the respiring organism by that generated by the electrolysis of saturated copper sulphate solution. Electronic circuitry checks each respirometer once every second and provides a square-wave pulse 0·1 sec long and 1·0 mA in amplitude if oxygen is required. A single pulse produces 5·7 nl of oxygen at S.T.P. Electromechanical counters record when a pulse is required for oxygen generation. Total errors introduced by the electronics amount to less than 0·1 %.

INTRODUCTION

In recent years there has been an evolution in the design of electrolytic respirometers towards increasing sensitivity, automation and usefulness in long-term experiments. An historical review of the use of electrolytic respirometers has been given by Macfadyen (1961), and Heusner (1970) has provided an extensive reference list together with data on the optimal design of such respirometers.

One of the main problems with electrolytic respirometers has been the difficulty in accurately recording very small quantities of oxygen evolved by electrolysis. Records produced by previously described electrolytic respirometers (e.g. Phillipson, 1962; Chase, Unwin & Brown, 1968) are usually in the form of traces which have to be accurately measured. To simplify this measurement problem, digital methods have been introduced by Heusner (1970) and Dunkle & Strong (1972). These authors used capacitor discharge to generate oxygen, and having calculated the quantity of oxygen evolved by a single discharge of the capacitor through an electrolyte, used electromechanical counters to count the number of discharges per unit time.

These circuits employing capacitor discharge are, however, temperature-sensitive as to the switching point of the undischarged capacitor and also to the saturation voltage of the charging device. The present authors have therefore returned to the constant-current principle but electronically present it to each respirometer in turn, as a brief square-wave pulse of fixed amplitude and time, and use electromechanical counters to record the number of such pulses which are used in oxygen generation in ch respirometer. Twenty such channels are available in the prototype, which, together with the associated glassware for the respirometers, cost under £100 to make. These respirometers are designed to record the oxygen uptake of specimens up to about 10 mg in weight.

THE MICRORESPIROMETER

This is conceptually similar to the respirometers of Dunkle & Strong (1972) and Heusner (1970), being an asymmetric thermobarometer to monitor pressure variations and a small respiratory chamber of about 2·5 ml capacity (Fig. 1). The asymmetric nature of the respirometer increases the sensitivity of the device but requires very stable temperature control of the water bath in which the respirometers are immersed. Symmetrical respirometers, such as those designed by Macfadyen (1961) and Chase *et al.* (1968) based on the Barcroft differential manometer (Dixon, 1951), can equally easily be used with the oxygen generating and recording unit and are less susceptible to temperature fluctuations. With symmetrical respirometers two channels per respirometer are used one for each side of the manometric fluid.

Asymmetric respirometers were constructed as follows (the letters inside parentheses refer to Fig. 1). The bottom of a small 2·5 ml screw-top glass vial (Camlab, U.K.) was removed either by orthodox glass-cutting techniques or by gently warming the vial base in a microbunsen and then plunging the hot base into cold water. With practice this latter method resulted in a clean break around the periphery of the vial's base, which was then knocked out and the edges smoothed with carborundum paper. This modified vial (*D*) contains the respiring organism and the carbon dioxide absorbent (*G*). The manometer (*O*) consisted of a short length (about 10 cm) of thick-walled capillary tubing of 2 mm diameter bore. Heusner (1970) has indicated that this diameter is the optimal size as strong capillary forces are formed when the bore is smaller than about 3·14 mm^2. The anode, which lies within this capillary, is a platinum wire (0·15 mm diameter) (*M*) sheathed by and sealed in a thin glass capillary tube (*N*). It is important that this sheathing is a very loose fit in the manometer capillary to allow the free diffusion of oxygen up into the respiratory chamber. The upper end of the manometer was cemented into the modified vial using an epoxy resin. A stainless-steel tube (*R*) (taken from a hypodermic needle) bent into a U was included in this cemented joint as was the flying lead (*Q*) to the glass sheathed anode positioned within the manometer. The stainless-steel tube is for releasing the pressure in the respiratory chamber. This pressure-release tube is closed by a short length of polythene tubing (*E*) which is sealed by squeezing between the heated jaws of a pair of forceps.

The manometer together with the attached respiratory chamber was embedded along with a stout copper wire cathode (*J*) and another stainless-steel tube (*I*), for releasing pressure, into a 'Quickfit' ground-glass cone (*P*) (size 19/26, CNB 19) using an epoxy-resin-based filler (*H*). The outer surface of the filler was sealed with epoxy resin, which was warmed to increase its ability to flow. The flying leads to the electrodes were also anchored with epoxy resin. The electrolyte, saturated copper sulphate solution (*L*), was contained in a test-tube (*K*) with a 'Quickfit' ground-glass socket (size 19/26 MF 24/2). Temperature stability was enhanced by enclosing the respirometer within a large boiling tube (200 × 30 mm) (*T*).

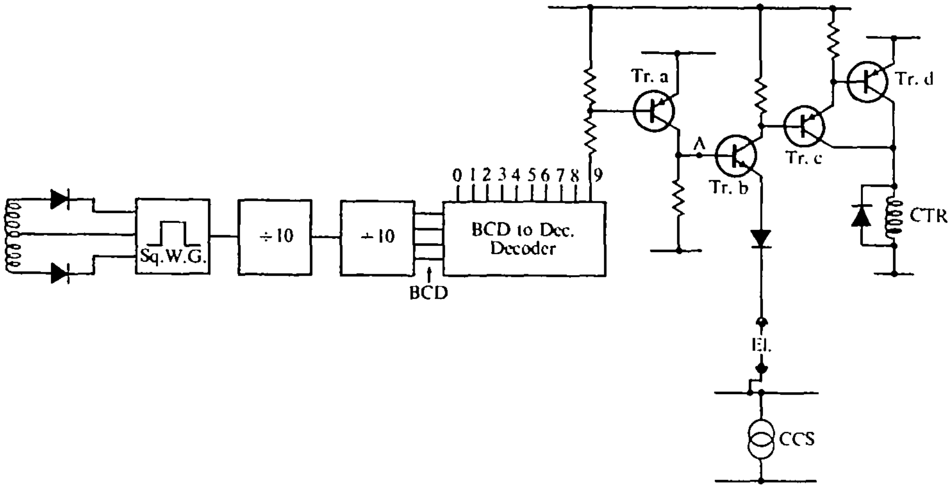

Fig. 2. The basic circuitry of the timing and oxygen generation units. Only one set of electrodes is shown. See text for description of the circuit. Sq.W.G., Square-wave generator; BCD, binary-coded decimal; CTR, counter; El, electrodes; CCS, constant current source.

Some ozone may be produced in electrolytic respirometers (Woodland, 1973). If spurious results are obtained and ozone toxicity is suspected Woodland suggests nickel di-*N*-butyldithiocarbamate as a suitable catalyst to reduce ozone levels within respirometers. It is possible that some crystallization of the saturated copper sulphate solution may occur at the anode. In practice, however, no such crystallization has been observed in continuous experimental runs lasting up to 8 days.

THE CIRCUIT

The circuit, basic units of which are shown in Fig. 2, was designed to operate on the British domestic electricity supply of 240 V and 50 Hz.

Sequential control signals switch a 1·0 mA constant current source to ten respirometer units giving each unit a current pulse of 0·1 sec duration every second. To generate these 0·1 sec pulses the output of a secondary winding on the mains transformer is full-wave rectified and squared to give a pulse output at twice mains frequency. This is then divided by 100 using two T.T.L. decade counters. The binary-coded decimal output from the second decade counter is decoded into decimal to provide the ten 0·1 sec timing pulses to the respirometer circuits.

The operation of each of the respirometer units is as follows. Transistor *a* (Fig. 2) inverts and amplifies the signal from the decimal decoder providing the correct voltage swing to operate the current switch, transistor *b*. When the tip of the platinum anode is immersed in the electrolyte (saturated copper sulphate solution) and the current switch is in the 'on' state a 1·0 mA current will pass from the constant current source via the respirometer unit to the digital counter. Transistors *c* and *d* provide the necessary amplification to operate the electromechanical counter. A counter therefore registers one count every time its associated respirometer unit passes an applied current pulse.

In the prototype 20 respirometer unit outputs are provided by duplication of the

Table 1

	Temperature 8 °C	19 °C
No. of counts required to generate 1·0 μl O_2 at the temperature given		
Observed (mean ± S.E.)	171·1 ± 1·29	163·4 ± 0·95
Theoretical (172·4 at 0 °C)	167·50	161·08
Volume of O_2 generated per pulse, reduced to S.T.P.		
Observed	5·679	5·720
Theoretical	5·804	5·804
Difference between observed and theoretical estimates (%)	2·02	1·40

circuit from the output of transistor *a* (point A in Fig. 2), including a second constant current source. The full circuit diagrams together with additional technical notes are given in the appendix.

CALIBRATION AND ERROR SOURCES

Using Faraday's law, each 1·0 mA pulse was calculated to produce 5·8039 nl of oxygen at S.T.P. The theoretical gas volume per pulse at any temperature (V_t) can then be calculated as

$$V_t = 5{\cdot}8039 \times (273+t)/273.$$

The usual corrections are made for pressure when expressing the results at S.T.P.

Some calibration experiments were carried out to see how closely the observed gas evolution approached that estimated theoretically. An Alga micrometer syringe was mounted on a hypodermic needle affixed with epoxy resin to a screw cap fitting the respirometer chambers. A series of 5 μl gas samples were withdrawn from the respirometer chamber and the number of counts for the apparatus to make up the gas deficit were recorded. This was carried out at two temperatures and the results are shown in tabular form (Table 1).

The observed values are about 2 % below the theoretical values. Resistance of the circuit, electrodes and electrolyte was suggested as a possible cause of the errors in the apparatus of Dunkle & Strong (1972). In this present circuit, however, the large voltage excesses (normal working voltage 3–4 V, available voltage 24 + V) available to the constant current supply overcome any slight resistances in the respirometer unit. The circuit has been tested to the limits of available test equipment. The desired 1·0 mA square-wave pulse 0·1 sec long is better than 0·998 mA in amplitude and 0·0999 sec long. There are slight spikes at the beginning and end of the square wave caused by the transistor switching from one respirometer unit to the next. These deviations last less than 50 μsec and introduce errors of about 0·005 %. There is a slight leakage across the electrodes in the 'non-pulse state' of less than 9 nA (error of 0·009 %). The platinum and copper electrodes in saturated copper sulphate solution act as a cell producing a small back E.M.F. A diode in the circuit from transistor *b* to the electrode (Fig. 2) prevents this back E.M.F. leaking and causing a spurious gas emission. The E.M.F. is easily overcome by the excess voltage available to the constant

Table 2. *Typical data of the respiration rates of a variety of invertebrates in the electrolytic digital microrespirometer at* 17·5 °C

Species	Live wt.	Mean O_2 uptake in µl/h ± S.E.	No. days in respirometer	No. readings
Sitophilus sp. (grain weevil)	3·3 mg	5·400 ± 0·140	3	8
Tribolium sp. (pupa – grain beetle)	2·6 mg	1·133 ± 0·027	3	6
Tribolium sp. (adult – grain beetle)	4·0 mg	2·196 ± 0·679	8	5
Littorina neritoides	4·55 mg with shell 4·80 mg with shell	Together 1·707 ± 0·055	3	15
L. saxatilis	13·0 mg with shell	1·869 ± 0·592	5	6
Daphnia magna (in water)	0·1 mg dry wt	0·773 ± 0·017	3	4

current source when the respirometer unit is in the conducting state. The only remaining possible error source is in the frequency of the mains voltage. If studies are carried out for several days then slight changes in the frequency are compensated for every 24 h to maintain the accuracy of clocks, etc., using synchronous motors. If used for shorter periods than 24 h and slight errors are important measurements should be made during off-peak times. These errors are, however, small (in the order of 0·01 %) and can usually be ignored. It seems likely that the error of 2 % found in the calibration experiments is due to slight inaccuracies in the micrometer syringe and its use.

Table 2 shows some typical experimental results obtained with the respirometer and also gives an idea of the size range of animals whose respiration can be measured. All the results are for animals devoid of food but at a similar relative humidity to that of their culture. The data for the two species of *Littorina* are for aerial respiration and it is interesting to note that both species displayed prolonged rhythmicity in their metabolic activity. Also included in the table is an aquatic animal used to explore the possibilities of using the respirometer with non-terrestrial animals. A single specimen of *Daphnia magna* (length 2–2·5 mm, dry weight approximately 0·1 mg) was kept in a small volume of distilled water in a small glass chamber inside the respirometer. The results compare favourably with those for *D. pulex* (Richman, 1958) but is about 10 times larger than the result obtained by McArthur & Baillie (1929) for *D. magna* (see Richman (1958), p. 282, for comments on this result).

The large standard error associated with the results for the adult *Tribolium* is a result of a reduction in the respiration rate from an initial level of 5·23 µl/h to about 1 µl/h after 24 h. This may be a metabolic adaptation to the absence of food.

As it has been described, the respirometer can only measure oxygen consumption over a period of time. Where respiratory rates are important records of oxygen consumption at regular time intervals are required. The apparatus can be connected to some of the commercially available timer–printer units, although this may be expensive. If time-lapse cinephotography equipment is available it can easily be arranged to photograph all 20 counters once every 15 min or 1 h, depending on the requirements of the investigator.

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APPENDIX

CIRCUIT NOTES

The full circuit diagrams are shown in Fig. 3 (power supply) and Fig. 4. Only one pair of respirometer circuits is shown. Nine similar pairs are connected to the outputs C to K of the 7441 Integrated Circuit, giving 20 channels in all.

As a respirometer receives current for only one-tenth of a 1 sec period, leakage current in or out of the respirometer in the remaining $\frac{9}{10}$ sec must be negligible. To achieve this the diodes (all OA 202 types) which block any reverse current through the oxygen-generating electrodes, and those which clamp the lower voltage point of the constant current supplies, must be low-leakage types, $I_R < 10$ nA. As diodes specified for low-leakage applications can be difficult to obtain in small quantities (22 required), it was found easier when constructing the prototype instrument to select from a batch of 50 Mullard OA 202 diodes those with the lowest I_R at 25 °C. The required quantity being easily obtained with leakage currents of < 2 nA. Resistors of 2·4 kΩ are included in series with the oxygen-generating electrodes to protect the respirometer switching circuit against excessive current flow if a respirometer connexion should accidentally touch chassis or earth. As the resistors drop 2·4 V of the available respirometer supply they may be removed from the circuit if a fully insulated connector is used.

To calibrate the constant current supplies the second decade counter is reset to zero using the switch in the decade counting circuit, enabling a steady current reading to be obtained on the first and the eleventh respirometer outputs. The 2 kΩ linear variable resistors in each constant current circuit can then be adjusted to give the required output current. The 1 kΩ variable resistor in the precision voltage reference circuit sets the current through the voltage reference diode (IN 825A) to 7·5 mA or 5·1 Volts across the 680 Ω resistor in series with the variable resistor. A continuous 10 mA current output is provided by the 10 mA supply circuit for rapid oxygen production to quickly lower the level of the electrolyte to the operating point. The 28 V lamp glows when the current is flowing.

To obtain a maximum of 24 V for the respirometers and to compensate for losses

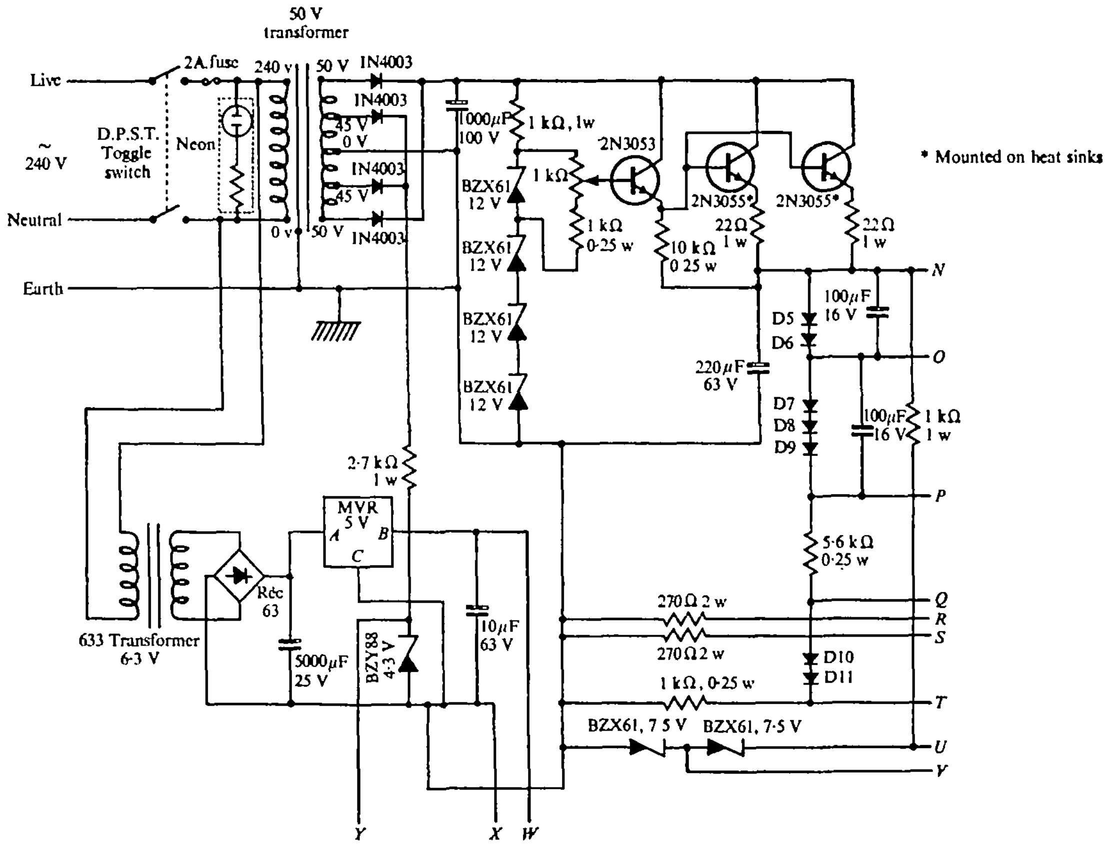

Fig. 3. Full circuit diagram of the power supplies showing the origins of the power rails (*N*–*Y*). D5–D11, IN4001; MVR; modular voltage regulator.

within the switching circuit and constant current supplies, a stabilized supply of 44 V, output *N* (set by the 1 kΩ variable resistor in Fig. 3) is provided from which a diode-resistor potential divider to chassis gives the following intermediate voltages:

$O = 42{\cdot}6$ V, $P = 40{\cdot}5$ V, $Q = 11{\cdot}2$ V, $T = 10$ V, $U = 15$ V and $V = 7{\cdot}5$ V.

A separate transformer and rectifier circuit is used with a regulator module to provide a stabilized 5 V output (*W*) for the integrated circuit decade counters and the decimal decoder.

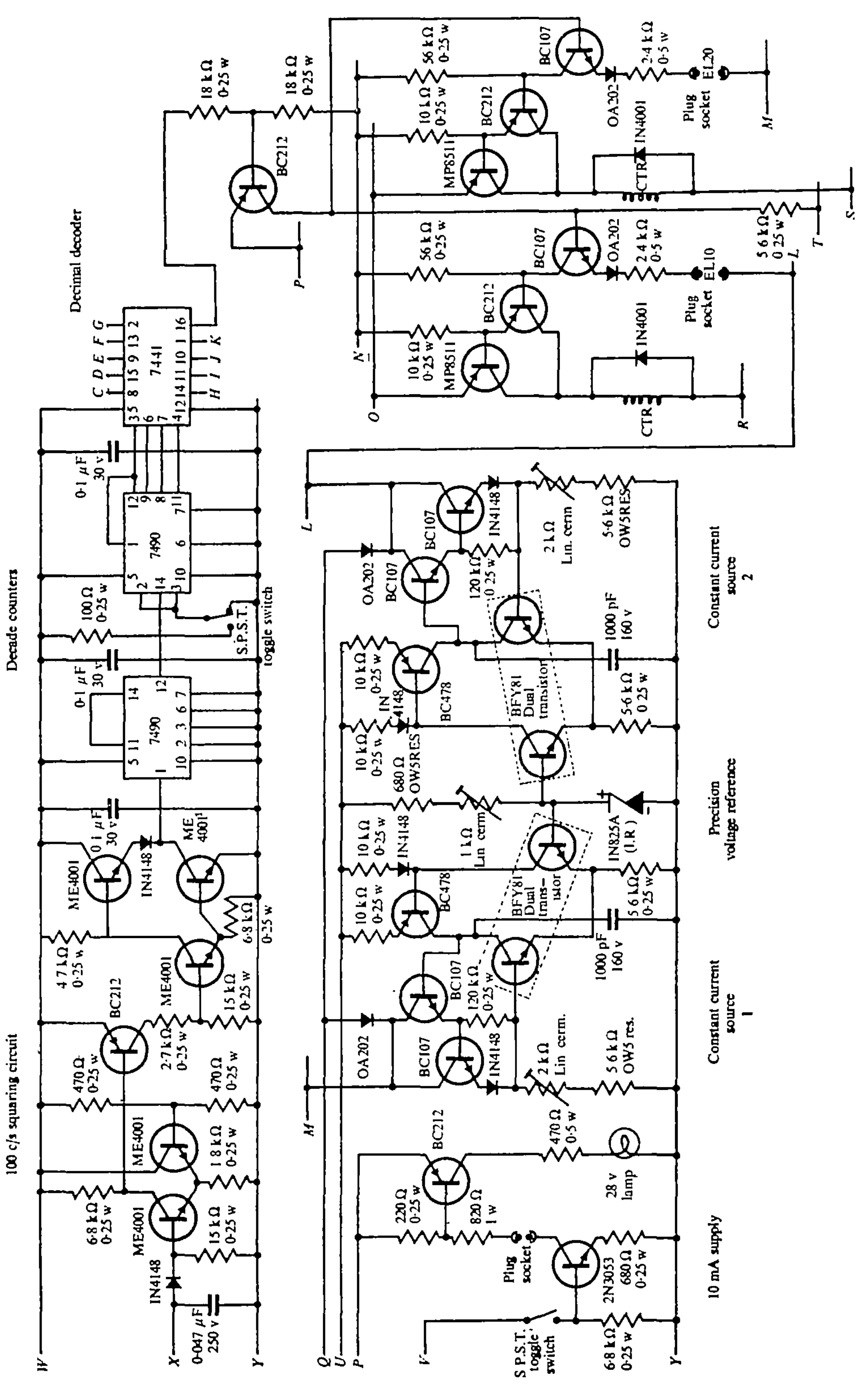

Fig. 4. Full circuit diagram of the 100 c/s squaring, counting and decoding unit and of the constant current sources. The 10 mA supply is for use in rapidly lowering the level of the electrolyte in the capillary tube. Only one pair of the electrode circuits is shown (EL 10 and EL 20). CTR, counters, ex G.P.O., 24 V, 500 Ω.