A Respirometer for Organ Cultures

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1. A differential respirometer that can measure the oxygen uptake of organ cultures for periods of several days is described. 2. Diethanolamine is used as an external carbon dioxide buffer so that oxygen consumption can be measured in the presence of physiological concentrations of carbon dioxide and bicarbonate. 3. The efficiency of carbon dioxide retention with 5% carbon dioxide as gas phase is estimated to be 75% and the accuracy to be $\pm 5\%$ with a measured rate of oxygen uptake in excess of $40 \mu\text{l./hr.}$ 4. Some experiments with guinea-pig retina are reported.

This paper describes a respirometer that can follow for several days the oxygen uptake of organ cultures maintained in the usual bicarbonate-buffered media with carbon dioxide present in the gas phase.

A differential respirometer described by Cruickshank (1954) designed to measure the oxygen uptake of small fragments of skin formed the starting point for the present apparatus. The Cruickshank respirometer was based on a design by Stern & Kirk (1947-48) and the major improvement was the invention of the rotating gas valve that opened both chambers simultaneously, thus avoiding fragmentation of the indicator droplet in the horizontal connecting capillary. The principal limitation of the method used was that the pH of the culture medium could not be controlled. Though a medium containing 50% of serum was used, so as to benefit from the buffering power of the serum proteins, the initial working pH was 8.5. However, no ill effects attributable to the high pH were observed and during experiments the pH fell as lactic acid was produced.

Bermann (1964) designed a respirometer adapted to the organ-culture method of Wolff & Haffen (1952). In this method, some bicarbonate was retained in the medium and oxygen consumption was calculated by comparing the pressure changes occurring during consecutive periods of incubation with and without a carbon dioxide absorber in the system. The principal disadvantage of the method was that an observation period of about 5 hr. gave only a single estimate of the rate of consumption on a particular day.

Numerous other respirometers have been described (Umbreit, Burris & Stauffer, 1957), mostly depending on the complete absorption of carbon dioxide and intended for short-term experiments.

Pardee (1949) showed that by using carbon dioxide buffers such as diethanolamine it was feasible to measure the oxygen uptake of tissue incubated in bicarbonate-buffered media with carbon dioxide in the gas phase. Krebs (1951) explored the possibilities and limitations of these buffers in considerable detail and concluded that, under carefully controlled conditions, 95% of the carbon dioxide evolved could be absorbed. Whalen (1957) made use of the principle to devise a respirometer for measuring the oxygen uptake of skeletal muscle for periods up to 12 hr.

The technique devised by Trowell (1959) for maintaining mammalian organ fragments is now well-established, and when cultured by this method many organs retain their histological structure for several days. It seemed a logical step therefore to design a respirometer that would exploit this technique in conjunction with the carbon dioxide buffer. The present apparatus is the outcome.

DESCRIPTION OF APPARATUS

The main features of the apparatus are illustrated in Figs. 1 and 2. The base, which measures $6\frac{1}{2}\text{in.} \times 3\frac{1}{2}\text{in.} \times \frac{3}{4}\text{in.}$, and lid are both made of Perspex (Lucite). They are bolted together by means of six 2BA stainless-steel screws (a) the nuts of which are pressed into counterbores. A lever (b) with a lug turned in over a recess cut in the upper surface of the base facilitates parting lid and base after they have been sealed together with grease.

The principal components of the respirometer are a reaction chamber (RC) and compensation chamber (CC), which are connected by a capillary manometer (CM). The chambers may also be connected together by a transfer valve (TV) or to a gas line by a gassing valve (GV). A microsyringe (MS) is attached to the lid by three 6BA stainless-steel screws (c). The whole assembly is operated on a simple stand.

Chambers. The chambers are wells 2.4 in. in diameter and 0.5 in. deep machined out of a block of Perspex $6\frac{1}{2}$ in. $\times$ $3\frac{1}{2}$ in. $\times$ $\frac{1}{2}$ in. Two culture dishes (d) made from polytetrafluoroethylene lodge in a slight depression in the floor of each chamber. They are 1.6 in. in internal diameter and accept standard (Trowell, 1959) culture grids. The wall of the culture dishes is 0.1 in. thick so that there is a moat 0.3 in.

wide around them. Access to the culture medium is possible by removing a 2BA stainless-steel screw (e) that is not fully countersunk and thus seals efficiently.

Manometer. The manometer is made from 0.5 mm.-bore borosilicate-glass tubing. The ends of the capillary are fitted to the lid by stainless-steel unions consisting of a flanged barrel (f) threaded so that it can be fixed in the lid by a knurled ring-nut and washer (g). A gas-tight seal to the glass capillary is effected by screwing down the knurled screw-cap (h) on a silicone rubber or Perbunan 'O' ring (i). The 'O' ring and the joint between the upper surface of the manometer union flange and the lid are both smeared with Apiezon M Grease or lanolin to effect a seal. For clarity, the union has been illustrated on one side only in Fig. 1.

The manometer loop is about 6 in. in height and is fitted with a graticule (j) constructed from two squares of Perspex 1 mm. in thickness that are lightly screwed together with two 6BA screws provided with spring washers. Thin lines scratched across the Perspex are used as a zero point for the manometer fluid.

The manometer fluid is 1% (v/v) Stergene in water coloured with 0.05% of Trypan Blue. This fluid is less sluggish than that of Krebs (1951) in the 0.5 mm.-bore capillary. The concentration of Stergene is such that the braking effects of the trailing meniscus and viscosity are both minimal. Evans blue, as used in Krebs fluid, is unsatisfactory because it is precipitated out at 37° in 1% (v/v) Stergene. Before this fluid was chosen, many organic solvents such as trimethylpentane (Whalen, 1957; Bermann, 1964), light petroleum (b.p. 100–120°), chlorobenzene, xylene, acetonylacetone and Cellosolve were considered, but in all cases the vapour was highly toxic for lymph-node cultures. Though the concentration of vapour under the conditions of the tests probably far exceeded what might be expected under working conditions, it seems inadvisable to use potentially toxic materials.

Valves. The valves (GV and TV) are made from similarly shaped pieces of Perspex $\frac{1}{2}$ in. in thickness. They rotate about a 2BA stainless-steel bolt (k) fixed in the lid and fitted with a spring washer padded with several plain washers (l) to discourage the nut from unscrewing. Rotation is

Fig. 1. Diagrams to show construction of the respirometer. A, Plan showing general arrangement. B, Sectional diagram showing construction of manometer union and transfer valve (spring-loaded clamping bolt omitted). C, Sectional diagram to show construction of gassing valve and microsyringe. Principal components: CC, compensation chamber; RC, reaction chamber; CM, capillary manometer; TV, transfer valve; GV, gassing valve; MS, microsyringe. Individual items: a, lid fixing screws; b, lid opening lever; c, microsyringe fixing screws; d, culture vessel; e, access screw for culture vessel; f, manometer union cylinder; g, knurled ring-nut; h, knurled screw-cap; i, 'O' ring; j, graticule; k, spring-loaded valve clamping bolts (omitted in diagram B); l, valve bolt washers; m, stop-pins (omitted in diagrams B and C); n, gas port exits in lid; o, gassing tubes; p, transfer port; q, transfer valve access screws; r, microsyringe barrel; s, microsyringe cap; t, microsyringe nozzle; u, microsyringe piston; v, 'O' ring; w, coil spring; x, driving screw; y, zero marking screw; z, revolution marker.

Fig. 2. Photograph of respirometer showing layout of lid in detail.

limited in each direction by stainless-steel stop-pins (m) in the lid. We have found the best lubricants are Edwards High Vacuum Stopcock Grease (hard) or Apiezon N Grease.

The gas port exits (n) in the lid overlying the chambers are all the same. They consist of a short length of stainless-steel tubing ($\frac{3}{8}$ in. external diam., $\frac{1}{8}$ in. internal diam.). The lower end of the tube protrudes 0.1 in. into the chamber so that any grease squeezed out when the lid is tightened down does not enter the port. The upper end of the tube is surrounded by a moat formed by a $\frac{1}{32}$ in. counterbore of depth $\frac{3}{32}$ in. In the open position, these register with a similar tube and moat in the valve. The purpose of the moats is to form grease traps.

In the gassing valve (GV) the stainless-steel tubes (o) simply extend upwards to a length of 3 in. or so to allow operation under water.

In the transfer valve (TV) the ports are connected by a $\frac{1}{8}$ in. transfer port (p). This is drilled from the outside and the entry hole then sealed with a Perspex plug and cement. Two further vertical holes are drilled to enter each end of the transfer port and tapped to take 6 BA stainless-steel screws (q). These screws are not fully countersunk so that they seal efficiently. They enable the ports to be cleared (without dismantling the apparatus) should they become blocked.

Syringe. The syringe is made of stainless steel. The barrel (r) is threaded to take a screw cap (s), and a nozzle (t) is pressed into the lower end. The piston (u) is fitted near the lower end with a silicone rubber or Perbunan 'O' ring (v) and loaded with a coil spring (w). It is operated by a brass screw (x) that has 112 threads/in. The bore of the syringe is nominally 0.300 in. and each turn of the driving screw effects a change in volume of about 10 μ l. (to save time precision is not aimed at and an exact factor is calculated for each syringe when the piston has been fitted). The driving screw has a knurled head marked off in ten equal divisions. Opposite one of these is a notched screwhead (y) that acts as a zero point. A triangular brass rod (z) soldered to a flat spring that is screwed to the syringe cap acts as a revolution indicator. The spring is adjusted so that a click is felt at each full turn and these can thus be counted by the operator while watching the manometer. The lower of the two screws fixing the revolution indicator locks the cap on the barrel. Fractions of a turn are read off when the manometer has been levelled off. Each division corresponds to about 1 μ l. The best lubricant found for the syringe piston is Apiezon Oil K. Sufficient should be added to form a pool round the piston as it enters the bore.

Volumetric constants. The total volume of each chamber (dry) is 30 ml. The apparatus is designed to work with 5–7 ml. of buffer and 5 ml. of medium so that the working gas volume is 20 ml. The syringe has a working capacity of 0.5 ml.

Carbon dioxide buffer. Following the method of Krebs (1951), a stock solution (approx. 5M) is prepared by mixing equal volumes of diethanolamine and distilled water and adding 0.1% of thiourea as an antioxidant. A few drops of phenolphthalein are added and the solution is gassed with 50% CO₂ in a gas-washing bottle with a fritted-glass plate until the phenolphthalein is decolorized. The stock solution keeps well in a glass-stoppered bottle.

Pardee (1949) and Krebs (1951) used the buffer at this strength, but in long-term experiments this is unsatisfactory because it has a much higher osmolarity than the culture medium and water distils over. It was found that the

solution still provided adequate CO₂ buffering when diluted tenfold without causing volumetric changes in the medium. As a further check, the Na⁺ concentration of the medium was estimated by flame photometry after incubation for 7 days in the presence of the diluted buffer and found to be unchanged.

To save time on the day when cultures are set up, it is convenient to equilibrate the diluted buffer the previous day. The stock solution is diluted, more phenolphthalein added and the solution gassed with 50% CO₂ in air until decolorized again. It is then gassed briskly for at least 30 min. with 5% CO₂ at 37° and stored at this temperature in a large syringe with the nozzle capped off (the caps were made by removing the shaft of a hypodermic needle from the hub and filling the resulting hole with Araldite). During the gassing with 5% CO₂, a tinge of colour rapidly returns, but it requires several hours to equilibrate with 5% CO₂ if the preliminary saturation is omitted. The buffer is not sterilized before use.

The efficiency of the buffer was tested in a stainless-steel prototype with a Perspex lid. It was set up first with 7 ml. of water in the moat and then with 7 ml. of buffer. In place of medium, 4 mg. of dry BaCO₃ was placed in the culture dish. The central compartment of the compensation chamber was left dry. After equilibration with 5% CO₂, an excess of 0.1N-HCl was added through the access screw aperture to the reaction chamber. By tilting the respirometer, the reaction could be postponed until the screw was replaced. The pressure difference between the two chambers was then followed on a scale attached to the manometer and the volume of gas liberated finally measured by bringing the levels to zero by adjusting the syringe screw. The time sequence of the pressure difference with buffer and water is shown in Fig. 3. With water in the moat, the volumes recovered were 370 and 380 μ l. of dry gas at S.T.P., and, with buffer, 87 and 94 μ l. respectively. The efficiency of CO₂ absorption is therefore greater than 75% and may well be better under normal working conditions when the rate of liberation of CO₂ is very much lower. The calculated volume of CO₂ that should have been liberated from 4 mg.

Fig. 3. Diagram showing the result of liberating a calculated 455 μ l. of CO₂ (dry gas at S.T.P.) with buffer (●) or water (O) in the moat. After 30 min. with water in the moat, the driving screw of the microsyringe had to be given 20 turns to prevent the manometer fluid being blown into the compensation chamber (shown by the broken line).

of BaCO_3 is 455 μl . and the discrepancy is no doubt accounted for by loss through some dissolving in the water. It is estimated that the water in the moat would contain 0.20 ml. of CO_2 and the amount liberated from the BaCO_3 would increase the partial pressure by about 50%.

TECHNIQUE

Preparations. The respirometer was originally designed to work under water, but it was found that satisfactory results could be obtained in a warm room maintained at $37 \pm 0.5^\circ$ if an aluminium cover is provided. Conditions are further improved by enclosing several respirometers in a long Perspex case to shield them from draughts when the door is opened. The top of the case is made in sections that can be removed to adjust the several syringe screws. Under my working conditions the temperature fluctuation in the Perspex case is less than $\pm 0.25^\circ$ over several hours. The gas cylinders, flowmeters etc. are also kept in the warm room.

It is, of course, much more convenient to take readings, change media etc. under dry conditions and only the technique for working in a warm room is described below.

The respirometers are assembled the day before an experiment and left in the warm room overnight. It is unnecessary to sterilize any part other than the polytetrafluoroethylene dishes. During assembly, excess of grease from the manometer unions is wiped from the inside of the lid. This leaves behind a thin film to which microbial contaminants presumably adhere.

The polytetrafluoroethylene culture dishes are cleaned with a soap solution [1g. of Na_3PO_4 (dried), 1g. of NaHCO_3 and 5g. of sodium oleate/l. of hot water], soaked in conc. HNO_3 , rinsed in distilled water followed by glass-distilled water and then sterilized in a suitable container by dry heat at 110° .

Culture medium. I have used the chemically defined medium T8 (Trowell, 1959), the working pH of which is 7.6 when equilibrated with 5% CO_2 .

Setting up the cultures. The cultures are prepared exactly as described by Trowell (1959) and placed on standard grids (covered by a sheet of lens paper or agar) in the culture vessels. It is important to keep the fragments as uniform in size as possible and pieces larger than 1.5 mm. in diameter are avoided. Central necrosis will occur if the fragments are too large and it is advisable to examine sample cultures histologically before embarking on any respirometry experiments.

While the cultures are being prepared, the dishes are kept in a shallow Perspex box with a sliding lid. It is convenient to have the lid in two halves. The box is provided with a gas inlet tube (stainless steel like the respirometer gassing ports) opening in the centre of the floor. A brisk stream of 5% CO_2 is passed continuously through this tube to avoid loss of CO_2 from the medium.

Equilibration. The culture dish and a dish containing medium and grid covered by a sheet of lens paper or agar, but no cultures, are covered with a sterile glass plate (see below) and transferred respectively to the reaction and compensation chambers of the respirometer. To avoid cooling the latter, this should be done in the warm room.

Apiezon M Grease or lanolin having been applied to the top face of the base of the respirometer, the lid is screwed down. The two centre screws should be tightened first.

The respirometer is then flushed through (100 ml./min.) for several minutes with 5% CO_2 (in air or O_2 according to working gas phase). The outlet valve (GV) is closed but the transfer valve (TV) is left open.

When all the respirometers have been closed, the already equilibrated buffer is squirted carefully into the moats round the culture dishes. This is done by means of a special cannula that reaches through the gas inlet tubes to the floor of the moat. The cannula is made by replacing the shaft of a no. 1 stainless-steel hypodermic needle with a length of stainless-steel tubing (0.047 in. outside diam.). It is convenient to make a right-angle bend near the hub because it is much easier to work with the buffer syringe in a horizontal position. The straight part must be 5 in. in length.

When all the respirometers are ready, the outlets are opened and connected in line with silicone rubber tubing. The respirometers having thus been connected in series, the working gas mixture is passed through the whole assembly for 20–30 min. at 100 ml./min. The gas line is then disconnected, GV is closed and the respirometers are left to equilibrate with TV open.

About 1–2 hr. is required for equilibration and it is advisable to test for leaks during this period (see below).

Readings. To start measuring O_2 consumption, GV is opened momentarily to adjust to atmospheric pressure and TV then closed. At convenient intervals (30–60 min.), the level of the manometer fluid is restored by adjusting the operating screw of the syringe and keeping a record of the total number of turns. No calculation is necessary other than conversion into μl . by the appropriate factor for the syringe and of the volume measured to S.T.P.

Maintenance. At the end of the day, the transfer valve is opened and the operating screw of the syringe unscrewed to the starting position. If it is intended to continue the following day without changing the medium, it is advisable to re-gas for 20–30 min. The following morning it is then necessary only to bring to atmospheric pressure and close the transfer port before starting to take readings.

If it is desired to change the medium or remove a sample, the access screw (e) is removed. The medium is sucked off through a hypodermic needle (bevel removed) bent near the hub to enable the syringe to be used horizontally. This needle must be sterilized before use and the tip must not be touched against the side of the access hole, which is not sterile. The medium sucked off is squirted into a 5 ml. measuring cylinder and replaced by an equal volume of fresh medium. This medium, which must have been warmed and equilibrated, can conveniently be kept until required in a capped-off all-glass syringe.

When the medium is changed it is also advisable to change the existing buffer with freshly equilibrated buffer, otherwise more prolonged equilibration is required because the existing buffer will have absorbed some respiratory CO_2 and this is given off rather slowly. The special cannula described above makes changing the buffer quick and easy.

After medium and buffer have been changed, the respirometers are gassed and equilibrated as described above.

If the respirometers are used for periods of longer than 3–4 days, it may be necessary to regrease the valves. To do this they have to be disassembled, but it is not necessary to remove the lid of the respirometer.

At the end of the experiment, the Perspex parts, manometer unions, screws etc. are washed in light petroleum

(technical grade). The 'O' rings, however, should not be exposed to light petroleum. Once set up, the syringe rarely needs attention but it is advisable to unscrew the operating screw so that the spring is relaxed when not in use.

Difficulties. Leaks are the principal hazard to successful operation because very slight leaks can cause serious drifting. Most of the grease seals are under Perspex surfaces and defects in them can therefore be seen. Damaged or defective 'O' rings on the capillary occasionally give trouble and it is advisable to change these often.

The best way to test the apparatus for leaks is under slight pressure under water, but this is impracticable with dry operation.

A fairly sensitive method of testing is to occlude one of the gassing outlets, connect a 1ml. syringe to the other and close TV. GV is then opened and the manometer fluid sucked into one limb of the capillary. GV is now closed and the fluid watched to see if the pressure difference is maintained. The advantage of this method is that it can be done at any time without disturbing equilibration. Leaks sufficiently large to be detected in this way usually give rise to a drift of at least 4–5 μ l./hr. Under normal conditions, the drift rate should be less than 2 μ l./hr. and rates of less than 1 μ l./hr. have been obtained over many hours.

Blockage of the valve ports rarely occurs if Edwards High Vacuum Stopcock Grease (hard) is used. If it does occur the gassing outlets can be cleared with a long nylon rod. To clear the transfer ports, it is necessary to remove the access screws (q). Apiezon N Grease is reasonably satisfactory for the valves. It has the advantage of being easier to spread than the Edwards Grease recommended, but is slightly more prone to block the ports. Silicone greases should not be used for the valves because they have poor lubricating properties, are much more liable to block the ports and leave a white deposit when cleaned off with light petroleum.

Another problem is condensation of water on the ends of the manometer capillary. When this occurs it indicates a slight temperature gradient from the base to the top of the respirometer and measures, which obviously depend on individual circumstances, must be taken to abolish this.

Unless condensation is very serious, the blockage of the capillary is usually prevented by placing a glass plate 1.25 in. $\times$ 1.75 in. (cut from a histological slide) across the top of each culture dish (including control dishes). These plates are used as a routine because they have the added advantage that, if there is an accident with the manometer fluid, it is not expelled into the culture medium, thus ruining the experiment. There is sufficient space between the edge of the plate and the side of the culture vessel to insert a needle to change the culture medium.

EXPERIMENTAL RESULTS

Design of experiments. To demonstrate the performance of the apparatus, the results of two experiments, each with four respirometers, are presented.

The oxygen consumption of adult guinea-pig retina was measured on 3 or 4 successive days. Air or oxygen (containing 5% of carbon dioxide) was used as gas phase and the technique of dissecting out the retina and setting up cultures was as

described by Lucas (1962). Retinal cultures become necrotic after 2 days in oxygen, and it was decided to see how this was related to the oxygen consumption. The retina from one eye was placed in each respirometer.

The medium and buffer were changed each day and, at the end of the experiments, the dry weight of the tissue was determined as described by Lucas (1965). The consumption of oxygen has been calculated in terms of μ moles/mg. dry wt.

Results. The results of the experiments are set out in Fig. 4 and Table 1.

With air as gas phase, oxygen consumption on the first day ranged from 4.96 to 8.39 μ moles/mg./min. and, with oxygen as gas phase, from 7.88 to 8.28 μ moles/mg./min. The results are more consistent with oxygen as gas phase than with air. Though this may simply be a matter of variation among eyes, it is not altogether surprising because some infolding of the edges of the cultures is inevitable and the infolded parts are likely to be anoxic with air as gas phase. Variation between cultures might therefore reflect variation in the amount of infolding.

At first sight, the relatively small difference between the oxygen consumption with air or oxygen as gas phase may seem surprising. It is considerably less than was found with the rat retina (Lucas, 1965). However, the guinea-pig retina is avascular and most of its respiratory activity probably takes place in the visual cells, which, in life, are separated from the choriocapillaris only by the pigmented epithelium. In organ culture, the retina is normally placed so that visual cells are uppermost, and, in the absence of folding, this would constitute the best oxygenated region of the culture.

Fig. 4. Diagram showing results from an experiment with four respirometers in which the O₂ consumption of guinea-pig retina was measured on 4 consecutive days with air as gas phase (●) and with O₂ (○). Readings were at 30min. intervals (for clarity the plots have been spaced out along the abscissa). There is a fall in O₂ consumption on day 2 with O₂ as gas phase; on day 3 it was not measurable. To save space, O₂ consumption on day 3 with air as gas phase has been omitted.

Table 1 *Oxygen consumption by retina cultures, and their terminal dry weights*

The mean rate of O_2 consumption $\pm$ S.E.M. on consecutive days was calculated by fitting regression to readings taken at 30 or 45 min. intervals. The values were derived from two experiments each with four respirometers, but no mean rate of consumption could be calculated with O_2 as gas phase after day 1 because consumption declined during day 2 and was nil thereafter. Expt. II was not continued beyond day 3. Each respirometer contained the retina from one eye and the two eyes from the same animal were allocated to the same gas phase. The terminal dry weights of the retina cultures are also given.

	O ₂ consumption (μ moles/mg. dry wt./min.)			
	Expt. I		Expt. II	
	O ₂	Air	O ₂	Air
Day 1	8.2 $\pm$ 0.42	8.4 $\pm$ 0.10	8.3 $\pm$ 0.32	5.0 $\pm$ 0.13
	8.0 $\pm$ 0.17	7.4 $\pm$ 0.06	7.9 $\pm$ 0.16	6.4 $\pm$ 0.11
Day 2		8.0 $\pm$ 0.16		5.7 $\pm$ 0.07
		7.4 $\pm$ 0.05		6.7 $\pm$ 0.10
Day 3		7.9 $\pm$ 0.08		4.8 $\pm$ 0.16
		7.4 $\pm$ 0.05		5.8 $\pm$ 0.05
Day 4		7.5 $\pm$ 0.10		
		6.3 $\pm$ 0.10		
	Terminal dry wt. (mg.)			
	Expt. I		Expt. II	
	O ₂	Air	O ₂	Air
	2.39	3.38	2.81	3.45
	2.46	3.41	2.54	2.92

Particularly striking is the decline in oxygen consumption on the second day with oxygen as gas phase, and, by contrast, the constancy with which consumption is maintained with air as gas phase. Similar results have been reported for the rat retina (Lucas, 1965). In both cases the decline in oxygen consumption preceded the onset of overt necrosis.

No previous estimate of oxygen consumption by the guinea-pig retina has been traced, but Cohen & Noell (1960) give Q_{O_2} 10 (7.44 μ moles/mg./min.) and Illing & Gray (1951) Q_{O_2} 10.9 (8.11 μ moles/mg./min.) for the rabbit retina, which has a similar structure. Both these results were obtained in a simple phosphate-buffered medium with glucose (180 mg./100 ml.) as substrate and oxygen as gas phase.

DISCUSSION

Material. Cruickshank (1954) constructed his respirometer out of brass, which is easily machined and has good thermal properties. However, heavy metals are highly toxic and there must be some

risk of contamination of the glass culture dishes through ionic migration in surface films. Since I wished to use a chemically defined medium and would not benefit therefore from the ability of serum proteins to fix heavy-metal ions, I constructed a prototype with integral culture dishes from solid stainless steel. This worked well, but had two disadvantages: (1) Stainless steel is not easy to machine and the block became badly distorted when the wells were cut out; prolonged lapping was then necessary before the lid would seal to the base. (2) The cultures cannot be inspected without removing the lid, so that the working pH is unknown and the medium cannot easily be changed.

In an effort to overcome these drawbacks, a Perspex lid was tried on the stainless-steel base. This arrangement worked, but the condensation problems were considerable, especially during the warming up period. A base of similar design was therefore made of Perspex but the inner wells were made as separate polytetrafluoroethylene dishes. Both these materials are easy to machine. Polytetrafluoroethylene is non-toxic and easy to sterilize. A theoretical disadvantage of these materials is their poor thermal conductivity, but in practice this was not a serious difficulty. C. N. D. Cruickshank (personal communication) found Perspex was unsatisfactory for small respirometers. This is perhaps because the inherent drift does not increase proportionately with size and can be tolerated if the capacity of the respirometer is increased sufficiently.

Design. To enable experiments to be run for several days with a minimum of manipulation, the present respirometer has been designed so that the medium can be changed and the valves serviced without having to remove the lid, which would entail complete loss of gaseous equilibrium and risk of infection.

Efficiency. In the Trowell (1959) technique of organ culture, the cultures are supported at the surface of the culture medium so that the principal gaseous exchanges take place between the upper surface of the cultures and the gas phase. It is this that enables the system to work without agitation.

Because of incomplete carbon dioxide absorption, oxygen consumption is to this extent underestimated, but, as Krebs (1951) pointed out, this is frequently of little consequence. The composition of the incubation medium, on the other hand, is of the utmost importance (Krebs, 1950). I am presently more interested in measuring trends in oxygen consumption over periods of several days rather than in establishing absolute values.

It has not been established that efficiency of carbon dioxide absorption is constant irrespective of the rate at which it is liberated. If the efficiency decreased as the rate increased, as seems possible,

differences in the rate of uptake of oxygen might be, to some extent, masked. It seems unlikely, however, that error from this source could be very serious.

If greater efficiency of carbon dioxide absorption were required, the percentage of carbon dioxide in the gas phase could be decreased and the concentration of bicarbonate in the medium decreased accordingly. Though it might be necessary to increase the buffering capacity of the medium by other means, it should, according to Krebs's (1951) observations, be possible to reach an efficiency of 95%.

It may be observed here that a bicarbonate-carbonate mixture containing carbonic anhydrase has been used as an external carbon dioxide buffer (Warburg & Krippahl, 1960; Warburg, Geissler & Lorenz, 1961; Warburg & Gawehn, 1962). However, even with equal volumes of buffer and cell suspension, the working strength of the buffer was 2 or 3 M. This would be unsatisfactory for long-term experiments because, as mentioned above, for these it is essential that the buffer is isosmolar with the culture medium.

Accuracy. Since the drift rate may be as much as 2 μ l./hr., a consumption of 40 μ l./hr. should be aimed at so as to achieve an accuracy of $\pm 5\%$. In practice this normally means working with 4–6 mg. dry wt. of tissue.

In the experiments cited, the amount of tissue was actually less than this (Table 1). With the retina, it is difficult to attain a high enough oxygen consumption without having the medium become acid rather rapidly as a result of the high rate of lactic acid production.

Since individual retinæ were placed in the respirometers, possible differences between retinæ have to be taken into consideration in comparing

results for different respirometers. However, the results do give an indication of the day-to-day reproducibility which can be expected from a single respirometer.

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