Measurement of oxygen consumption in the rat lens with a flow respirometer

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The oxygen uptakes of individual rat lenses are measured in a new recording respirometer based on the oxygen electrode. An automatically controlled double flow system permits the electrode to be calibrated in the course of a determination without disturbing the steady state at the lens. The rate of oxygen consumption of a lens is flow-independent above a critical volume flow rate at which the oxygen utilization is 17 to 18 per cent. That the apparatus can measure, through at least 50 per cent utilization, uptake proportional to the amount of tissue is shown on varied numbers of amphibian embryos. Rat lenses averaging 23.9 mg. fresh weight have under standard conditions (bicarbonate-buffered TC199 medium, pH 7.5, 20 per cent O₂, 37°C) an oxygen uptake of $0.75 \pm 0.08 \mu l O_2$ per lens-hour. The rate is not changed in phosphate, Tris buffer, or by substitution of Tyrode’s solution for TC199. Respiration would appear to account for less than 4 per cent of glucose utilization in vitro at this oxygen tension.

Formation of lactic acid accounts for all but 15 to 30 per cent of the glucose utilized by the rabbit lens in vitro. The remainder is commonly assumed to be completely oxidized. Estimates based on direct measurement of oxygen consumptions indicate that the fraction respired may be even smaller. That this low respiratory activity is at least partly enzymatic in nature and capable of supplying energy is no longer questioned. Whether it is necessary to the economy of the lens remains debatable; recent studies on the effects of anaerobiosis suggest that it is not. However, up to half of the respiratory CO₂ produced by the lens appears to be derived from the hexose monophosphate shunt. A growing body of evidence shows that interference with this pathway is involved in development of the galactose cataract in rats. The possible importance of respiratory metabolism implied by this relationship warrants further study of the extent and character of lens respiration, particularly in the rat, which is being used to an increasing extent for investigations on the biochemistry of cataract formation.

No prior attempt has been made to measure respiration in the rat lenses except for one early study on prenatal stages. The great variability in results obtained later on larger species suggests the serious difficulties encountered in attempting to measure lens respiration by ordinary gasometric means. Probably the best estimates available are those obtained by Hans and co-workers who used a polarographic (dropping-mercury electrode) method. It is clear, however, from the extensive study by Schwartz that the use of open or flow systems holds the greatest promise for metabolic analysis of the lens under optimal

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conditions. Bakker and others indeed successfully cultured lenses for several days in flowing medium; unfortunately, the accuracy of his measurements of respiration was probably limited by the complicated method of oxygen analysis employed. The most suitable method would be the combination of flow culture with the direct analysis of dissolved oxygen by polarographic means or by one of its modern adaptations, the oxygen cathode or oxygen electrode. This, in effect, is the basis of a respirometer that was designed specifically to measure rat lens respiration and combines a flow system with the oxygen electrode as described in the next section.

**Principle**

If saline solution containing a known amount of dissolved oxygen is flowed at a constant rate past a lens, the oxygen taken up by the lens produces a difference between the inflow and outflow concentrations. The decrease \((C_i - C_o)\) is related directly to the rate of oxygen uptake \((U)\), and inversely to the volume flow rate \((V/t)\); \(U = (C_i - C_o) \cdot V/t\). The concentration of oxygen in the outflow may be measured by an oxygen electrode calibrated against the inflow solution. Since the respective electrode readings are directly proportional to the oxygen concentrations, \(E_i = kC_i\) and \(E_o = kC_o\), the rate of uptake may be calculated from the relative difference between electrode readings when the inflow concentration and the volume flow rate are known: \(U = (E_i - E_o)/E_i \cdot C_i/V/t\). The first term of the product in the equation simply represents the fraction of arriving oxygen consumed by the lens, whereas the second term expresses the rate of arrival.

The theoretical basis for the method is given by Carlson and associates who developed an apparatus in which the oxygen cathode was used for measurement of nerve respiration. Their cathode could be calibrated only in the absence of tissue, however, at the beginning and end of each determination. Larrabee, in elaborating the technique, provided for calibration of the cathode through occasional acceleration of the flow rate in the course of a determination. The respirometer to be described uses a form of Clark's oxygen electrode, essentially the oxygen cathode plus an anode behind a common gas-permeable membrane. Although the electrode has the advantage of being insensitive to any known constituent of physiologic media except oxygen, it is markedly responsive to flow rate. A double flow system has therefore been devised to maintain a constant flow rate. Since this also maintains the steady state in the tissue, the lag period in alternating between measurement and calibration has been reduced to a minimum. The frequent and rapid calibration thus permitted provides a high degree of precision in the measurements even with occasionally considerable drift in the electrode system.

**Materials and methods**

1. **Flow system.** The glassware of the flow system (Fig. 1) is constructed entirely of Pyrex and consists of four units connected by ball-and-socket joints. Saline solution is equilibrated with the gas phase in the reservoir assembly \((A)\). Chambers for the lens and electrode are contained in the stopcock assembly \((B)\) with which the flow pattern is controlled. A pump \((D, not shown)\) comprised of two oil-filled syringes draws two streams through the stopcock assembly at identical rates. Last, connecting units \((C)\) join each syringe to the stopcock assembly; each contains a tube for the oil-aqueous interface and a three-way stopcock through which the system may be drained. This stopcock happens to be glass but could as well have a Teflon plug as do the others. Tubing carrying aqueous solution is 1 mm. bore capillary with 12/1 joints; oil is conveyed in 3 mm. inside diameter (ID) standard wall tubing with 12/3 joints, viz., between each syringe and its interface tube. All components of the flow system except the pump are contained in a 37.0° C. forced-air incubator with a double-wall glass door. The pump lies beneath.

The operation of the respirometer centers about the stopcock assembly, as best explained with reference to Fig. 2. Of the two streams drawn by the pump, one passes through the tissue chamber. When the stopcock is positioned as in Fig. 2, left, the outflow from the chamber is pulled past the oxygen electrode by syringe \(S\). After rotation of the stopcock through a quarter turn (Fig. 2,
right), the flow through the chamber is maintained by syringe $S_1$, whereas $S_1$ draws reservoir solution directly to the electrode for its calibration. In the automatic operation of the apparatus, the stopcock plug is rotated between its two positions by a 6 r.p.m. motor actuated by a timer to give equal (5 minute) periods of measurement and calibration. Since the only critical solution changing composition after each rotation of the stopcock lies in the short capillary segment between the stopcock and the electrode, the lag period is but the time required to flush this relatively small volume.

The stopcock assembly was built around a custom-made 1 mm. ID stopcock blank* which has four capillary side arms and a Teflon plug with two parallel bores. The details of construction are largely evident from Fig. 1, and only certain general features need be considered. The tissue chamber is just large enough to contain an adult rat lens resting in a basket of stainless steel screening. A glass plug, cut down from a standard taper joint and lapped by hand for a very tight fit, closes the chamber because a rubber stopper was found to consume a small amount of oxygen. Good mixing of the flow within the chamber speeds establishment of a steady state and is aided by placing the inlet to the chamber as high on the wall as possible, actually invading the ground area of the plug (rather than as shown for clarity in Fig. 1). The design of the electrode chamber is dictated by the nature of the electrode chosen and the need to minimize the volume between it and the stopcock. The electrode is held in the chamber snugly by two rubber O-rings (Fig. 2) in such a way that the cathode area is directly over and within 0.5 mm. of the capillary inlet. The arm of the stopcock to bear the chamber was replaced with a length of 0.5 mm. capillary tubing made as short as possible.

Each of the two syringes of the pump is an 0.18 inch length of 0.1875 inch ID precision-bore tubing† fitted with a Teflon plunger and filled with silicone oil (Dow-Corning 200 Fluid, 350 csf). The steel shafts which draw the plungers are attached to the carriage of a lead-screw mechanism‡ with a pitch of 24 threads per inch. The screw is driven by a 1/8 HP 1,750 r.p.m. motor through a variable speed transmission§ and a 200:1 speed reductor. Screw speed is determined by timing several revolutions with a stopwatch and is constant after a brief warm-up.

Although a single reservoir with a simple stopcock in its outlet suffices for most purposes, the double form shown in Fig. 1 is convenient when two solutions are to be compared. Its stopcock is controlled by a shaft extending from outside the incubator so that a solution can be selected without disturbing the temperature. The three-way stopcocks of the connecting units are likewise worked from outside the incubator.

2. Electrode system. Measurement of dissolved oxygen is carried out with the circuits shown in Fig. 3. The main components are the electrode and its polarizing circuit, a sensitive galvanometer converted to a photoamplifier, and a potentiometric recorder. The electrode output is normally connected to the galvanometer except when the switching relay is activated, whereupon the elec-
The electrode circuit is maintained separately while the calibrating circuit is connected. The latter is used in ordinary measurements simply to short the galvanometer for checking zero (Fig. 4), although occasionally standard currents are recorded to check the linearity of the amplifier and recorder. The zero offset circuit may be used to increase the sensitivity of the system to small differences in electrode current, and then standard currents are automatically introduced for calibration of the chart.

The oxygen electrode employed, except in having a 0.005 inch outside diameter (OD) platinum cathode, is fundamentally that devised by Clark₁ and has the same outside dimensions as the commercially available form. A second rubber O-ring was added to hold the electrode in its chamber. When covered with a Teflon membrane 0.0005 inch thick and polarized at 0.7 v., the electrode develops about 0.01 microampere in air-saturated unstirred water at room temperature. The residual current in the absence of oxygen (excess sodium hydrosulfitie) is only 1 per cent of this value, and the electrode current is directly proportional to the concentration of dissolved oxygen.

The photoamplifier substitutes adequately for the much more expensive D.C. amplifiers that could be used for driving the recorder. It consists of a Rubicon spotlight galvanometer of 0.0006 microampere per millimeter sensitivity in which the diaphragm of the light source has been modified to give an approximately square image. This illuminates an area slightly larger than that of two 1 by 2 cm. silicon photovoltaic cells (Hoffman 120C) mounted in place of the glass scale. The cells are connected in parallel and drive a 10 mv. potentiometric recorder* connected across a variable load resistance. The polarity of the recorder was reversed internally to put the chart scale in the direction of increasing percentage oxygen uptake. Overdamping of the galvanometer as well as running both the lamp and recorder from a constant voltage transformer reduces wavering of the trace. Recorder deflection in the range most used is linearly related to galvanometer input current within 0.5 per cent of full scale. Zero and full-scale stability are usually excellent. The 6 to 8 v. lamp (GE 82) of the galvanometer is operated at 5 v. to prolong its life.

3. Procedure. Lenses were removed by a posterior approach from eyes of female Sprague-Dawley rats (100 to 150 grams) killed by decapitation. The isolated lens was not allowed to rest on its anterior surface as it was being cleaned of adherent tissue. It was then placed with the epithelium up in a basket of stainless steel screening and transferred into the tissue chamber of the previously prepared respirometer. After a temperature of 37° C. was regained in the incubator—in about 10 minutes—the system was briefly flushed

*Yellow Springs Instrument Co., Yellow Springs, Ohio.

*C. H. Stoelting Company, Chicago, Ill.
with fresh reservoir solution and recording begun. A steady state, evidenced by subsequently constant oxygen uptake over 3 consecutive 10-minute periods, was usually established in 20 to 40 minutes. At the end of a determination the lens was quickly blotted and weighed on a Roller-Smith balance.

The standard medium for respiratory measurements was TC199 obtained in dry form without bicarbonate* and made up as needed in 1.1 percent solution in 0.032M sodium bicarbonate. An antibiotic mixture† with streptomycin substituted for dihydrostreptomycin was added in concentrated solution. The gas mixture, 95 per cent air and 5 per cent CO₂ gave a pH of 7.50 at 37° C. When bicarbonate was replaced with approximately iso-osmolar sodium phosphate or tris(hydroxymethyl)-aminomethane (i.e., “Tris”) buffer adjusted to give the same final pH, the gas phase was air. When Tyrode’s solution was used,‡ the bicarbonate concentration was raised to pH 7.50 with 5 per cent CO₂ and phenol red and antibiotics were added to the same final concentrations as in the standard medium. All solutions were made with deionized water.® Sterile precautions were not found necessary if the reservoir and stopcock assemblies were stored in 70 per cent alcohol after use each day.

The rates of oxygen uptake were calculated from the formula, \( U = \frac{(E_i - E_o)}{E_i} \cdot C_i \cdot V/t \) (see section “Principle”). \( V/t \) depends upon the bore of the syringe and pitch of the lead screw and is 1.131 ml. per hour at a screw speed of 1 r.p.m. \( C_i \) at normal temperature and pressure (NTP) is (according to the equation given by Larrabee††) 21.3 times the fraction of oxygen in the dry gas phase for a physiologic saline solution at 37° C. \((E_i - E_o)/E_i\) is calculated from the record, usually as the average of three consecutive periods. In the example shown in Fig. 4, the outflow reading is 14.5 per cent lower than the inflow reading at a screw speed of 1.09 r.p.m. with 20 per cent O₂ in the gas phase. Hence, the rate of oxygen uptake by this lens was 0.145 \times 21.3 \times 0.20 \times 1.131 \times 1.09 = 0.76 \mu l O₂ per hour.

Results

1. Performance of the respirometer. It was demonstrated by varying the volume flow rates in the course of determinations on individual lenses (Fig. 5) that the rate of oxygen consumption becomes constant above a critical minimum flow of approximately 1 ml. per hour. This supplies the lens with about 4.2 \mu l O₂ per hour, at which the corresponding maximum allowable oxygen utilization is 17 to 18 per cent. Which of these represents the primary limiting factor is not known. It lies, most likely, in the insufficient oxygenation of the lens below the critical flow rate, since, as will be shown in the subsequent paper, the rate of consumption is highly dependent upon oxygen concentration. All measurements reported below have been made at flow rates demonstrated to be adequate for flow-independent oxygen consumption.

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\*Difco Laboratories, Detroit, Mich.

†Illinois research model. Illinois Water Treatment Company, Rockford, Ill.

††Larrabee (1979) 21.3 times the fraction of oxygen in the dry gas phase for a physiologic saline solution at 37° C. 

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Fig. 4. Facsimile of typical record of \( O_2 \) utilization by a rat lens weighing 22.8 mg. \( S \), artifact due to momentary cessation of flow upon rotation of stopcock.

Fig. 5. Relationship between \( O_2 \) consumption of rat lenses and volume flow rate. Symbols represent three different lenses (average weight, 25.5 mg.).
That the percentage oxygen utilization is not limited by the action of the respirometer itself is shown in Fig. 6. Clearly, utilizations up to at least 50 per cent may be tolerated without sacrificing the proportionality of oxygen consumption to the amount of the respiring tissue. Because the tissue chamber accommodates but one lens of the size being studied, this proof was obtained with amphibian embryos, *Xenopus laevis,* which offer good replication in small packets.

2. Variability in oxygen consumption.

The mean rate of oxygen consumption for 32 lenses in the particular weight range covered is 0.747 \( \mu \)l O\(_2\) per hour in the standard bicarbonate-buffered TC199 medium (Table I). The coefficient of variation is 10.8 per cent and may be considered to arise from three sources: (1) error in measurement, (2) random variation in respiratory activity, i.e., differences among hypothetical lenses of identical weight, and (3) weight-dependent trend.

Only the first source can be evaluated from available data with any completeness. In each of two determinations made at slightly different flow rates, the percentage utilization of oxygen was followed for 18 consecutive 10 minute periods. The uptakes were reasonably constant at 16.6 and 12.5 per cent over the first 90 minutes and fell slightly thereafter. The coefficients of variation in the first 9 analyses were 2.7 and 3.6 per cent, respectively. These variations are probably largely the result of errors in the electrode readings although variations in temperature in the incubator are certainly also involved.

Differences in respiratory activity among lenses of the same weight would have both intrinsic ("biologic variability") and extrinsic causes. The dissection procedure is an obvious factor in the latter. Occasionally, small fragments of the ciliary body cannot be removed for fear of damaging the lens. Lenses on which the entire rings of ciliary processes were purposely left had oxygen uptakes 13 per cent greater (average of 4 cases) than in the same lenses subsequently cleaned. The difference, probably accentuated by the extra handling, is small enough to indicate that the fragments do not contribute appreciably to the variability in measurements. It may be added that the lenses are almost invariably crystal clear upon completion of the determinations if they are gently handled during dissection and cleaning.

Studies in progress on respiration in rat lenses of different ages show that with increasing lens weight there is a noticeable decrease in oxygen consumption per lens. Consequently, use of lenses covering a range of weights smaller than now employed (20.1 to 28.2 mg.) would be expected to decrease the variation in oxygen consumption.

3. Lens respiration in various media.

Table I shows that lens respiration is not significantly different when phosphate or Tris buffering is substituted for bicarbonate in TC199 medium, or when bicarbonate-buffered Tyrode's solution is used. The comparison was made more precise by observing the effects of each of these substitutions on individual lenses (Table II).
The double reservoir was used so that oxygen uptake could be measured on each lens first in standard medium (TC199-HCO₃⁻), then in another medium, and finally again in standard medium. The average of the two determinations in standard medium was taken as 100 per cent for each lens. The results obtained in this series are also incorporated into the data of Table I.

Discussion

The respiratory activity of the rat lens, as calculated from the present data, is equivalent to a rate of glucose utilization of 0.042 mg. per gram-hour. The total rate of glucose uptake has been reported on the basis of closed-system analyses to be 1.1 mg. per gram-hour. However, in the rabbit lens at least, glucose uptake appears to be considerably greater in open than in closed systems. Thus, respiration would account for, at most, 3.8 per cent, quite possibly less, of glucose metabolism in the rat lens. There is clearly a need to check this estimate by carrying out measurements of glucose consumption, and of lactate production as well, under conditions identical to those used here for respiratory measurements.

The overwhelming use of anaerobic glycolysis makes obvious one of the great advantages of polarographic analysis over gasometric techniques for the measurement of lens respiration: high sensitivity may be attained with large liquid volumes, precluding undue depletion of glucose or accumulation of lactic acid. A flow system adds the further refinement that all metabolites reach constant steady-state concentrations at the lens. For example, at the usual flow rate of 1.5 ml. per hour and taking respiration as 5 per cent of total glycolysis, glucose is decreased from 1 mg. per milliliter in the inflow to 0.987 mg. per milliliter in the outflow; lactic acid increases from 0 to 12.6 μg per milliliter, which would not seriously alter the pH of the buffer. Another advantage of flow respirometry is the elimination of the shaking required for gas equilibration in some gasometric procedures and generally supposed to increase the oxygen uptake of the lens due to injury. Last, use of the oxygen electrode permits measurement of respiration in the presence of CO₂.

The bicarbonate-CO₂ system is the principal buffer of the aqueous humor. It is clear from the results given above that it has no unique effect on lens respiration in vitro. Nor, in fact, is respiration in Tyrode's solution different from that in the complex TC199 medium. It does not follow, however, that lens respiration should be measured in the simplest possible medium inasmuch as the quality of metabolism cannot be judged from its level. TC199 was originally selected for these measurements on the basis of its value for the moderately prolonged culture of the lens. A corollary is that respiration measured in such a medium is both quantitatively and qualitatively normal, or at least not incompatible with the survival of the lens. Bicarbonate-buffered TC199 will thus remain the medium of choice for further studies on rat lens metabolism.

<table>
<thead>
<tr>
<th>Table I. Oxygen consumption of rat lenses in different media</th>
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<tr>
<td><strong>Medium</strong></td>
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<tr>
<td>-------------</td>
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<tr>
<td>TC199-HCO₃⁻</td>
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<tr>
<td>TC199-PO₄</td>
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<tr>
<td>TC199-Tris</td>
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<tr>
<td>Tyrode's-HCO₃</td>
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*Mean ± S.D. (range).

Table II. Oxygen consumption in different media compared on individual rat lenses

<table>
<thead>
<tr>
<th><strong>Medium</strong></th>
<th><strong>Lenses (No.)</strong></th>
<th><strong>Relative consumption</strong> (%)</th>
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<tbody>
<tr>
<td>TC199-PO₄</td>
<td>4</td>
<td>98</td>
</tr>
<tr>
<td>TC199-Tris</td>
<td>4</td>
<td>96</td>
</tr>
<tr>
<td>Tyrode's-HCO₃</td>
<td>5</td>
<td>101</td>
</tr>
</tbody>
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*Uptake in TC199-HCO₃ taken as 100 per cent.
REFERENCES


