Oxygen Consumption Rate of Tissue Measured by a Micropolarographic Method

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ABSTRACT A new method for measuring the oxygen consumption rate of a sheet of homogeneous tissue is described. The method measures, by a Clark-type oxygen electrode without a membrane, the time for the tissue to consume all its dissolved oxygen. The electrode is applied to one surface of the tissue sheet and the other surface is sealed from the atmosphere by a cover slip. The consumption is calculated from an estimate of the oxygen dissolved in the tissue at the moment it is covered and the time for the oxygen tension at one surface to fall to zero. The data also yield the oxygen diffusion coefficient in the oxygen-consuming tissue.

INTRODUCTION

The rate of oxygen consumption by tissue is a quantity of fundamental importance in physiology. Prompted by the need for an accurate, rapid, and convenient method of measuring the oxygen metabolism of excised tissue, particularly from the eye, we have developed a new micropolarographic technique which can measure the rate of oxygen consumption by a thin, homogeneous sheet of tissue. We have applied this technique to excised corneal stromas.

Several investigators, employing the Warburg microrespirometer, have studied the oxygen consumption of the in vitro cornea. These studies have been reviewed by Hill and Fatt (1963). The early investigators used macerated tissue. The process of maceration probably injured the cells, thereby casting doubt on the oxygen consumption determination. In later investigations the whole cornea or its components were placed in the respirometer chamber. This eliminated some of the problems encountered by earlier workers but had the disadvantage of a large chamber volume in which the oxygen concentration was analyzed, leading to excessive time for a measurable change in oxygen content. The long time required for each measurement could introduce uncertainty in the observed oxygen consumption rate because of tissue ageing.

Recently, the polarographic oxygen electrode has been suggested as a replacement for the Warburg apparatus in studies of tissue oxygen consumption. Matsumoto and Kudo (1960) measured the rate of oxygen consumption of the component parts of the rabbit cornea by a polarographic technique. They used a mercury polarographic electrode to measure the oxygen concentration in a 10 ml chamber filled with Ringer's solution in which the oxygen-consuming tissue was suspended. Their method, however, required a long time for each measurement and therefore was only a slight improvement on the Warburg method. Hill and Fatt (1963) placed a platinum polarographic electrode in a saline-filled chamber fitted to a contact lens. With this apparatus they measured the oxygen flux across the anterior surface of the in vivo human cornea and thereby determined the amount of atmospheric oxygen used by the cornea.

This paper describes a new polarographic technique for measuring the total oxygen consumption rate of a sheet of tissue in vitro, with a particular application to the excised corneal stroma. The method consists of sealing off the tissue from the atmosphere and then determining the time required for it to consume all of its dissolved oxygen. The oxygen tension is measured with a Clark-type oxygen electrode (Clark, 1956), but without the membrane.

A bare electrode was used because it is only for this system that an exact mathematical analysis could be made. In exploratory experiments, not included in this paper, identical results were obtained when using the electrode bare or covered with a 12 μ polyethylene sheet, which gave a rapidly responding sensor (Goldstick, 1966). In fact, there are several advantages in the use of the membrane-covered electrode such as lower noise level and easier calibration. Because of the advantages of the covered electrode and because the membrane appears not to affect the mathematical analysis given here in any significant way, the authors recommend use of a membrane-covered electrode.

The record of oxygen tension as a function of time after sealing the tissue from the atmosphere also gives the oxygen diffusion coefficient in the tissue.

METHODS AND MATERIALS

Preparation of Tissue

Albino rabbits, weighing approximately 2 kg, were killed with sodium pentobarbital. Their eyes were immediately enucleated and the corneas excised. The epithelium and endothelium, including Descemet's membrane, were then scraped off with a sharp razor blade to leave only the stroma. At the four quadrants of each stroma, 2 mm radial incisions were made with a razor blade to reduce the natural curvature of the tissue.

Apparatus

The experimental equipment, with the tissue in place, is shown schematically in Fig. 1. The electrode currents were amplified and recorded by a Beckman Model 160 physio-

logical gas analyzer and a Model 93500 potentiometric recorder. The electrode fitted into a Lucite cell equipped with a micrometer for measuring tissue thickness. Temperature in the cell was measured by a calibrated thermistor connected to an Industrial Instruments Inc. (Cedar Grove, N.J.), Model RC-1, conductivity bridge. For the experiments reported here the gas inside the cell was always water-saturated air.

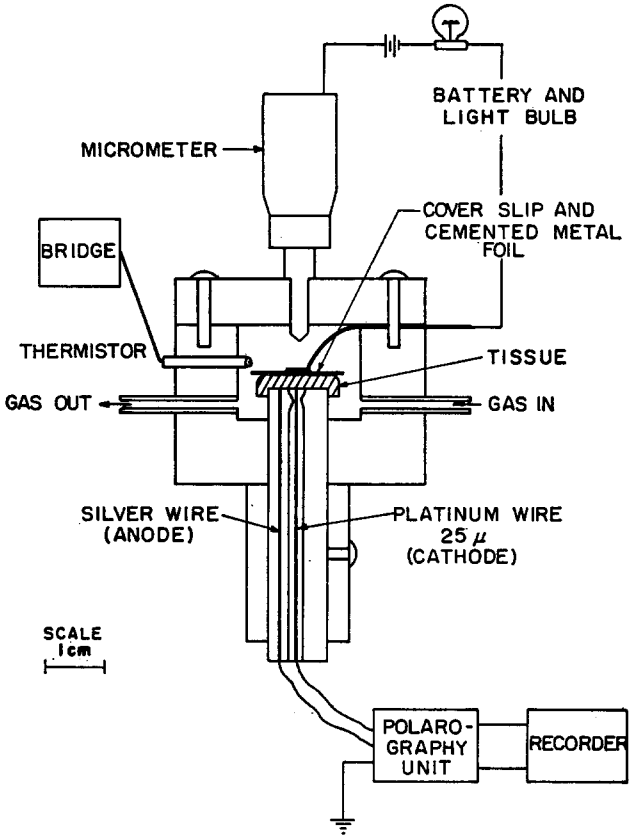

FIGURE 1. Apparatus used for measuring oxygen consumption rate of tissue.

Procedure

Prior to a measurement both surfaces of the tissue sample were allowed to equilibrate in water-saturated air for several minutes. During the same period the electrode was covered with air-saturated aqueous humor from a rabbit eye. The sample was then draped over the electrode assembly, taking care to squeeze out any air bubbles trapped under the tissue. When the electrode current became constant a cover slip was gently placed on the sample, again taking care to avoid trapped air bubbles between the sample and the cover slip. The current was then recorded until zero oxygen tension was reached. The zero line was recorded for a few minutes to establish the base line on the recording. A sample record is shown in Fig. 2.

Thickness measurements were made after each oxygen consumption test. Fig. 1 also shows the thickness-measuring circuit. The battery and bulb are connected in series with a micrometer and a thin piece of metal foil cemented onto the cover slip. A reading on the micrometer was made when the micrometer tip just touched the metal foil when the cover slip was on the bare electrode assembly. A reading was again made when the tissue was under the cover slip. The difference was the tissue thickness. Such thickness readings were probably accurate to ± 10 μ.

All experiments were performed at a temperature maintained within one degree of 25°C.

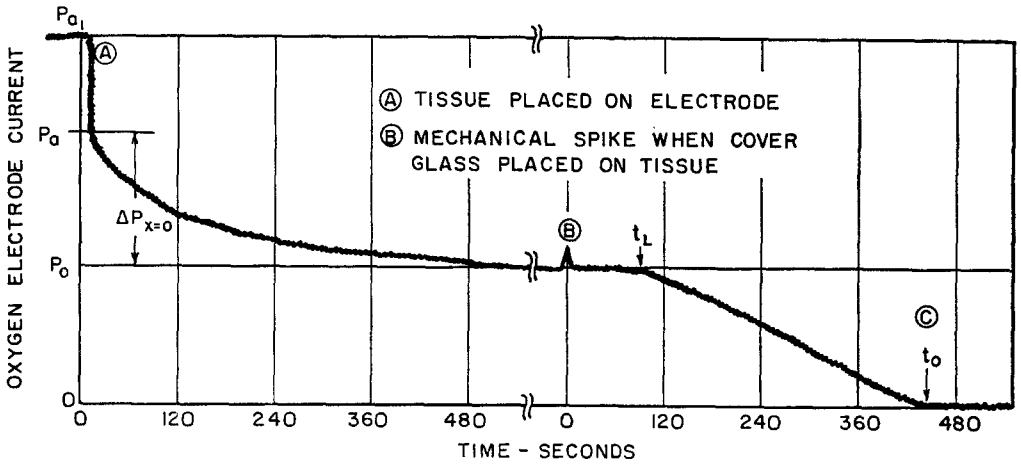

FIGURE 2. Sample record of electrode current when measuring oxygen consumption rate of tissue.

RESULTS

Fig. 2 shows a sample recording of electrode current as a function of time. The point marked P_{a_1} is the equilibrium current for the electrode when it is covered with a thin layer of air-saturated aqueous humor. At point A the tissue was draped over the electrode assembly. The rapid drop to the point marked P_a is a result of the change of electrode environment from aqueous humor to tissue. At constant oxygen tension, the electrode current is proportional to the product of the diffusion coefficient and solubility of oxygen in the medium covering the electrode. As would be expected (Goldstick, 1966), this product for tissue is somewhat lower than it is for aqueous humor because of the higher solids content of the tissue. There is, therefore, a drop in electrode current from P_{a_1} to P_a even though both media are air-saturated next to the electrode.

Fig. 3 shows a plot of the logarithm of the distance that selected points from Fig. 2 are above the horizontal line P_o, as a function of time, between points A and B. Beyond 100 sec this procedure gives a straight line on the semi-

logarithmic plot. The time, t_1, for the line shown in Fig. 3 to fall one logarithmic cycle is 440 sec. Substituting this value of t_1, and the measured thickness, l, of 0.0375 cm into equation 1 below,* the oxygen diffusion coefficient, D, is calculated to be 0.30×10^{-5} cm²/sec.

$$D = \frac{0.9332l^2}{t_1} \tag{1}$$

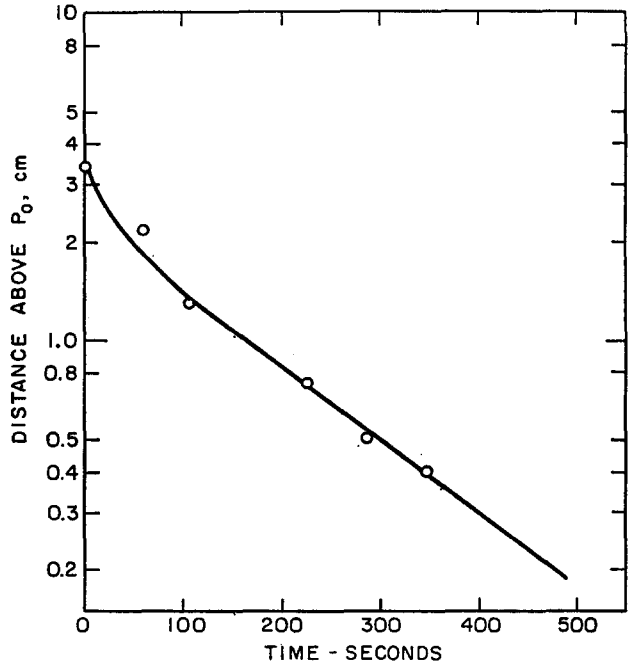

FIGURE 3. Semilogarithmic plot of oxygen tension at the closed surface of the tissue as a function of time after placing the air-equilibrated tissue on the electrode.

If P_a is taken to be 155 mm Hg, the normal oxygen tension in air, then Fig. 2 shows that the drop in oxygen tension caused by closing one surface of the tissue, $\Delta P_{x=0}$, is 75 mm Hg. For normal corneal stroma of 0.0375 cm thickness, the hydration, H, is 3.50 mg water per mg dry tissue material, the solubility of oxygen in pure water, k_w, is 3.55×10^{-5} ml O_2 (STP)‡/ml water × mm Hg, and ρ_w/ρ_t, the ratio of water density to dry tissue density is 0.73 as measured by Maurice (1957). Equation 2 below then gives the oxygen solubility, k, as 2.80×10^{-5} ml O_2 (STP)/ml tissue × mm Hg.

* All equations used here are derived either in the section on Theory or in the Appendix.

‡ All gas volumes quoted have been reduced to conditions of standard temperature and pressure, 0°C and 760 mm Hg.

$$k = \frac{0.955 k_w H}{H + \rho_w/\rho_t} \tag{2}$$

The use of these data in equation 3 below gives the oxygen consumption rate, Q, as 0.89×10^{-5} ml O_2 (STP)/ml tissue $\times$ sec.

$$Q = \frac{1.866 k \Delta P_{x=0}}{t_1} \tag{3}$$

One difficulty in using the experimental data from point A to B in Fig. 2 stems from the uncertainty of the exact location of P_a, the point on the vertical scale of the record at which the oxygen tension begins to fall slowly with time. Another difficulty arises in the determination of the exact time corresponding to point A, the time when the tissue is satisfactorily placed on the electrode. Although, in theory, to obtain t_1 it is only necessary to find the time interval for a fall of the linear portion by one logarithmic cycle, in practice this interval must be near the early portion of the curve. At later times the distance from the horizontal line P_o to the curve becomes small and the uncertainty in this distance increases.

Because of the difficulties mentioned above in obtaining P_a and t_1, the use of the record from A to B for measuring Q and D is believed to give only approximate data. Because the record from B to C must be obtained in any case for calibration purposes, it is recommended that Q and D be calculated from the data between points B and C as described below.

When the cover slip is placed over the tissue, at point B in Fig. 2, there is a spike which is probably caused by unavoidable mechanical pressure on the tissue and electrode. The spike is followed by a period of constant current during the time when the effect of covering the top surface has not yet reached the surface in contact with the electrode. There is then a linear fall in current until almost zero current. The slight rounding off near zero current may be caused by a decrease in oxygen consumption rate at very low oxygen tension.

The oxygen consumption rate is calculated from equation 4 below where t_L and t_o are the times (after point B) when the straight line portion of curve BC cuts the lines at P_o and zero, respectively.

$$Q = \frac{P_a k}{t_o + 2t_L} \tag{4}$$

The diffusion coefficient of oxygen in the tissue is calculated from

$$D = \frac{l^2}{6t_L} \tag{5}$$

The data from 10 separate runs on rabbit corneal stroma at 25°C are shown in Table I. It is clear that the samples were of varying thickness despite every

precaution taken to prevent imbibition of aqueous humor or water by the excised tissue. This variation may be only an apparent one because of error in the thickness measurement or may be real and reflect a constant and normal hydration but a variation in dry tissue material content. Duane (1949) found, on 25 eyes, that freshly enucleated and excised rabbit corneas had an average hydration of 3.50 with a standard deviation of 0.05. We have chosen to accept Duane's result and calculate oxygen solubility in all samples from equation 2 using $H = 3.50$. The oxygen solubility calculated in this way is then used in equation 4 to calculate oxygen consumption rate in terms of volumes of oxygen used per unit volume tissue per unit time. These rates are given in column 5 of Table I.

TABLE I

OXYGEN CONSUMPTION BY RABBIT CORNEAL STROMA AT 25°C

1 Sample No	2 l	3 t_0	4 t_L	5 Q	6 q	7 D
	mm	*sec*	*sec*	*ml O_2(STP)/ml tissue × sec*	*ml O_2(STP)/cm² × sec*	*cm²/sec*
1	0.370	870	58	0.444×10^{-5}	1.64×10^{-7}	0.40×10^{-5}
2	0.405	600	50	0.622	2.53	0.55
3	0.375	660	48	0.578	2.16	0.49
4	0.395	555	43	0.679	2.68	0.61
5	0.405	750	50	0.513	2.07	0.55
6	0.340	495	58	0.712	2.42	0.32
7	0.352	720	42	0.543	1.91	0.49
8	0.502	915	44	0.435	2.18	0.95
9	0.337	780	60	0.484	1.63	0.30
10	0.355	510	54	0.708	2.51	0.39
Average = 0.384				0.57×10^{-5}	2.17×10^{-7}	0.50×10^{-5}
SD =				0.09×10^{-5}	0.35×10^{-7}	0.18×10^{-5}
SEM =				0.03×10^{-5}	0.11×10^{-7}	0.057×10^{-5}

The oxygen consumption rate, q, in terms of volumes of oxygen consumed per unit area of material per unit time, as used by some authors (Maurice, 1962), is given by

$$q = \frac{P_a k l}{t_o + 2t_L} \tag{6}$$

or

$$q = Ql \tag{7}$$

q from equation 7 and the data of columns 2 and 5 in Table I are given in column 6.

Since we have chosen above to ignore the thickness variation in calculating

k we can choose to use the average thickness, 0.384 mm, in equation 7 to calculate q. This gives $q_{av} = Q_{av}l_{av}$. The data in Table I lead to $q_{av} = 2.19 \times 10^{-7}$ ml O_2 (STP)/cm^2 × sec, or 0.79 μl O_2 (STP)/cm^2 × hr. Table I shows that about the same q_{av} is obtained if the variations in tissue thickness are considered to be real, and each value of q is calculated from the individual values of Q and l.

The oxygen consumption rate, q^a, in terms of volumes of oxygen per unit weight of dry tissue material is given by

$$q^a = \left(\frac{P_a k}{t_o + 2t_L}\right)\left(\frac{H}{\rho_w} + \frac{1}{\rho_t}\right) \tag{8}$$

or

$$q^a = Q\left(\frac{H}{\rho_w} + \frac{1}{\rho_t}\right) \tag{9}$$

If all samples are assumed to have the same hydration of 3.50, then q^a_{av}, as calculated from equation 9 and Q_{av}, is 0.087 μl O_2 (STP)/mg dry tissue material × hr.

The average value of q^a at 25°C can be converted to its approximate value at 37°C by using the observation that the respiration rate of corneal stroma doubles for every 10°C increase in temperature (Langham, 1960). On this basis the average q^a at 37°C is 0.205 (δ = 0.03, SEM = 0.01)* μl O_2 (STP)/mg dry tissue × hr. Langham (1952) reported 0.231 (± 0.038, 6 determinations) μl O_2 (STP)/mg dry tissue × hr for rabbit corneal stroma at 37°C.

Although the procedure described here was designed to obtain oxygen consumption rates it can also be used to obtain the oxygen diffusion coefficient in oxygen-consuming tissue. In this respect the technique described here is an improvement over that reported by Takahashi and Fatt (1965) where the tissue had to be made nonconsuming before the oxygen diffusion coefficient could be measured.

Table I also shows D calculated from equation 5, for the rabbit corneal stroma. The average D, at 25°C, is 0.50 × 10^{-5} cm^2/sec. This D can be compared with D in the steer corneal stroma, obtained by Takahashi and Fatt (1965), after correcting for the different experimental temperatures. Their data were collected at 4°C where they found an average D of 0.54 × 10^{-5} cm^2/sec. Using the temperature coefficient suggested by Roughton (1959), namely that D increases 2.5%/°C, their average D becomes 0.90 × 10^{-5} cm^2/sec at 25°C. A direct comparison of this D with the present value is difficult because Takahashi and Fatt (1965) used corneas swollen to 134% of the

* δ is the standard deviation of the mean and SEM is standard error of the mean.

normal thickness. This swelling undoubtedly increased their measured D above that in corneal stromas of normal thickness.

In the Appendix it is shown mathematically that, soon after closing the second surface of the oxygen-consuming tissue sheet, the oxygen tensions at both surfaces become approximately the same and both surfaces reach zero tension at essentially the same time. This theoretical finding was verified experimentally. For this experiment, the top surface instead of being closed with a cover slip was closed by an electrode identical to the one on the bottom. The records of electrode current vs. time, for the two electrodes, became essentially identical after about 60 sec and reached zero current at essentially the same time.

THEORY

Oxygen Consumption in Tissue

When both surfaces of a homogeneous sheet of tissue of thickness l, in which there are oxygen-consuming cells, are exposed to the atmosphere, the steady-state oxygen tension, as a function of distance from the surface of the tissue, is given by

$$P = \frac{Qx^2}{2\,Dk} - \frac{Qlx}{2\,Dk} + P_a \tag{10}$$

The derivation of this equation and definition of terms are given in the Appendix. A plot of equation 10 in terms of the dimensionless parameters $[1 + 2\,(P - P_a)Dk/Ql^2]$ and x/l is shown in Fig. 4 A. This is the oxygen tension profile in the tissue just before it is placed on the electrode assembly shown in Fig. 1.

If the sheet of tissue is placed in contact with an electrode, the surface at $x = 0$ (taken to be at the electrode surface) is no longer exposed to the atmosphere. Instead this surface is closed and there is no oxygen flux across it. The steady-state oxygen tension profile, as derived in the Appendix, now becomes

$$P = P_a - \frac{Q(l^2 - x^2)}{2\,Dk} \tag{11}$$

A plot of equation 11 is shown in Fig. 4 B. The steady-state oxygen tension at the closed (electrode) surface is obtained by evaluating equation 11 at $x = 0$ to give

$$P_o = P_a - \frac{Ql^2}{2\,Dk} \tag{12}$$

The change in equilibrium oxygen tension at the surface adjacent to the elec-

trode, caused by closing this surface, is obtained by subtracting equation 12 from equation 10 evaluated at $x = 0$. The result is

$$\Delta P_{x=0} = \frac{Ql^2}{2\,Dk} \tag{13}$$

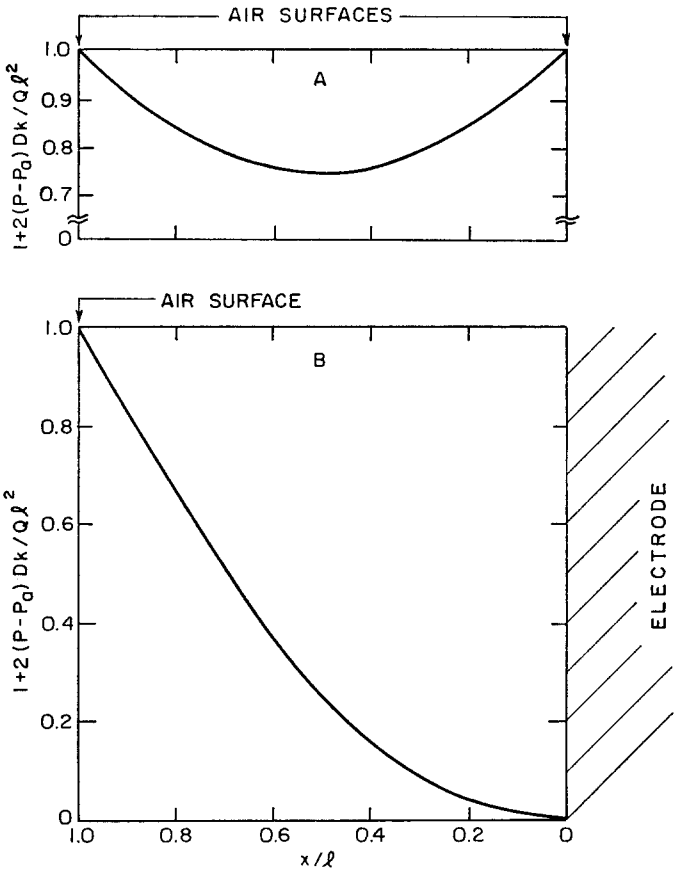

FIGURE 4 A. Steady-state oxygen tension profile in an homogeneous oxygen-consuming tissue with both surfaces exposed to air. B. Steady-state oxygen tension profile in an homogeneous oxygen-consuming tissue with one surface open to the air and the other closed by an electrode.

The time course of the fall in oxygen tension from P_a to P_o at the surface adjacent to the electrode, when plotted on a semilogarithmic graph with $\frac{P - P_o}{P_a - P_o}$ on the log scale and time on the arithmetic scale, will, after an initial short period, be linear as shown by equation 10 A of the Appendix. The time for the linear portion to fall one logarithmic cycle is given by equation

11 A of the Appendix as

$$t_1 = \frac{0.9332l^2}{D} \tag{14}$$

When equations 13 and 14 are combined and solved for the oxygen consumption the result is equation 3.

When the current from the tissue-covered electrode has become constant, indicating a steady-state oxygen tension profile in the tissue, a cover slip is gently placed over the air-exposed surface of the tissue. This is shown as point B in Fig. 2. For about 1 min, in the case of rabbit corneal stroma, the electrode is not affected by the change in boundary condition at the other surface. Then, as the tissue consumes the oxygen dissolved within it, the electrode current begins to fall until finally, when all the oxygen is consumed, it reaches the current corresponding to an oxygen tension of zero, at point C in Fig. 2.

In the Appendix it is shown, from solution of the nonsteady-state equation governing oxygen tension in the closed tissue, that, for a tissue of 0.384 mm thickness and for an oxygen diffusion coefficient of 0.50×10^{-5} cm²/sec, both closed surfaces will reach zero oxygen tension at essentially the same time if the observed time is about 120 sec or greater. This condition was met for all the corneal stromas tested here as shown by the data in Table I.

Equation 21 A of the Appendix shows that the time to reach zero oxygen tension at the electrode surface is given by

$$t_o = \frac{P_a k}{Q} - \frac{l^2}{3D} \tag{15}$$

It is also shown in the Appendix that when the linear portion of the curve in Fig. 2 is extrapolated to P_o it intersects this line at a time given by

$$t_L = \frac{l^2}{6D} \tag{16}$$

Combining equations 15 and 16 and solving for Q gives

$$Q = \frac{P_a k}{t_o + 2t_L} \tag{17}$$

Equation 17 is the basis used in this paper for measuring oxygen consumption rate. If the rate is to be reported in ml O_2 (STP)/ml tissue $\times$ unit time, then no thickness measurement is needed other than to ascertain that the tissue meets the requirement for $Dt/l^2 \geqq 0.4$. If the rate is to be reported in ml O_2 (STP)/unit area $\times$ unit time, as is done by some authors (Maurice,

1962), then the thickness can be measured by the micrometer shown in Fig. 1 and this thickness used in equations 6 and 7.

If the oxygen consumption is desired in ml O_2 (STP)/unit dry weight of tissue × unit time, then equation 8 or 9 is used. Equations 8 and 9 are based on the assumption that the total volume of the tissue is the sum of its water volume and dry tissue volume.

Oxygen Solubility in Tissue

The only term in equation 17 not easily measurable is k, the oxygen solubility in the tissue.

Since a direct measurement of oxygen solubility in corneal stroma could not be found in the literature it was estimated from published solubilities in protein solutions. For this estimate, the rabbit corneal stroma was considered to dissolve the same amount of oxygen as a lipid-free solution of protein in isotonic saline with the same dry weight solids content. This assumption appears justified because Stoddard (1927) has shown that dissolved proteins are inert to atmospheric gases and simply reduce the volume available for gas in solution. The corneal stroma is reported to be essentially free of lipid (Krause, 1934, states that the bovine stroma has 2.07 g lipid/1000 g wet weight or 11.12 g lipid/1000 g dry weight).

To estimate the oxygen solubility in an isotonic saline solution with the same protein content as the solids of the stroma, the equation first proposed by Bohr (1905) was used. It states that

$$\frac{k^o}{k_w^o} = c \tag{18}$$

where k^o is the oxygen solubility in the water portion of an isotonic protein solution, in ml O_2 (STP)/g dissolved water × mm Hg, k_w^o is the oxygen solubility in pure water, in ml O_2 (STP)/g water × mm Hg, and c is a constant at all temperatures and protein concentrations. Sendroy et al. (1934) found that equation 18 was valid for oxygen in blood plasma and that c had a value of 0.955. This value is used here.

Using this numerical value of c in equation 18 and the definition of hydration, the oxygen solubility in corneal stroma can be expressed as given in equation 2.

Oxygen Electrode

The microelectrode, of a type originated by Clark (1956), was constructed by one of the authors (Fatt, 1964). As shown in Fig. 1, it consists of a 25 μ diameter polarized platinum cathode and a 500 μ diameter silver-silver chloride reversible reference anode. By polarizing the cathode at −0.6 v with respect to the anode, oxygen can be chemically reduced on the platinum, pro-

ducing an electrical current. Fatt (1964) has shown that the current produced by this electrode varies linearly with the oxygen tension in the medium above the electrode. Takahashi and Fatt (1965) have shown that the electrode consumes a negligible amount of oxygen compared to the corneal tissue.

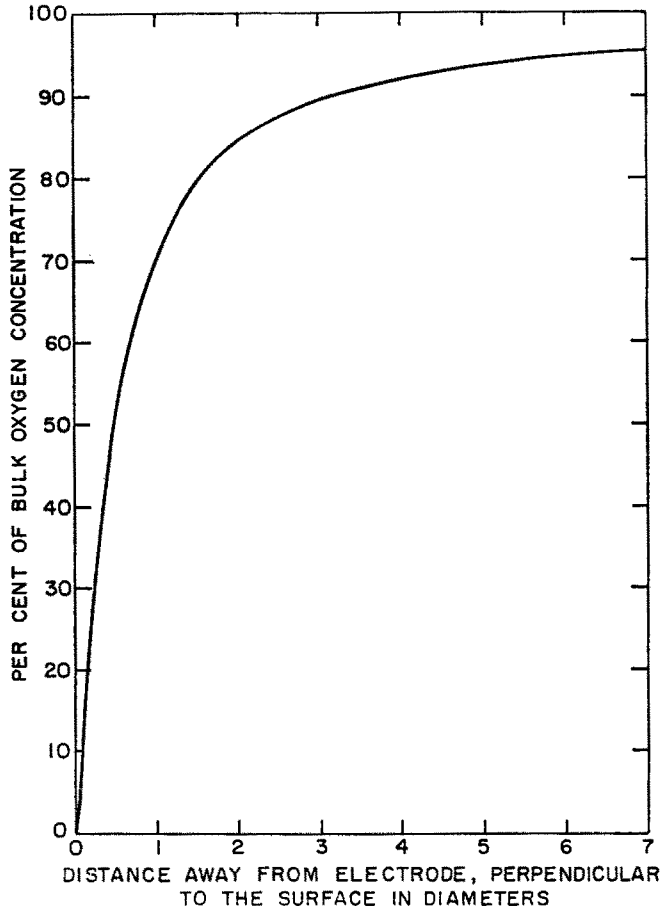

FIGURE 5. Oxygen tension along the perpendicular from the center point of a disc electrode at the surface of a semi-infinite medium. The surface of the disc is maintained at zero oxygen tension.

It is now necessary to discuss the point in the medium at which the electrode is measuring oxygen tension. To supply itself with oxygen the electrode must establish an oxygen tension gradient from the electrode surface into the medium being measured. It is generally believed that, in an electrolytic cell whose current is diffusion-controlled, the concentration of the electrolyzed species is zero at the electrode surface. Moving away from this electrode into the medium the concentration rapidly rises. Carslaw and Jaeger (1959 *a*) give

the concentration profile caused by a disc maintained at zero concentration on the surface of a homogeneous, semi-infinite medium with a constant bulk concentration. Fig. 5 shows the oxygen tension along the perpendicular from the center point of a disc electrode at the surface of a semi-infinite medium. This profile is independent of the physical, chemical, or electrical properties of the medium. Although an exact solution of the analogous situation in a consuming medium is unavailable, it is reasonable to conclude that the profile is essentially the same in Fig. 5. Fig. 5 shows that one-half the tension drop occurs within one radius of the electrode, 13 μ for the microelectrode used here. Because all the tissues measured in this study were about 400 μ in thickness, and the oxygen tension profile was essentially flat over most of the measuring time, the effect of depth of penetration of the electrode was considered to be negligible. The oxygen microelectrode was, therefore, considered to be measuring the oxygen tension at a point on the surface of the tissue.

SUMMARY

A method for measuring the oxygen consumption rate of a sheet of tissue by a polarographic electrode has been described. This method when applied to rabbit corneal stromas at 25°C gave an oxygen consumption rate of 0.087 μl O_2 (STP)/mg dry tissue material × hr. This rate at 37°C becomes 0.21 μl O_2 (STP)/mg dry tissue material × hr, according to Langham's observation of the doubling of the oxygen consumption rate for a 10°C rise in temperature.

The data obtained for calculating consumption can also be used to calculate the oxygen diffusion coefficient in the tissue. At the normal stromal hydration of 3.5 mg water/mg dry tissue material, and 25°C, the measured oxygen diffusion coefficient is 0.50 × 10^{-5} cm^2/sec. This can be corrected to 37°C to give 0.67 × 10^{-5} cm^2/sec, using Roughton's temperature coefficient for diffusion of 2.5%/°C.

APPENDIX

Oxygen Tension in a Homogeneous Sheet of Tissue That Is Consuming Oxygen

The differential equation which gives the oxygen tension as a function of time and distance from the surface, in a sheet of tissue in which there is oxygen consumption, is

$$k\frac{\partial P}{\partial t} = Dk\frac{\partial^2 P}{\partial x^2} - Q \qquad (1\text{ A})$$

where

D = diffusion coefficient of oxygen in the tissue, cm^2/sec.
k = solubility of oxygen in tissue, ml O_2(STP)/ml tissue × mm Hg.
P = oxygen tension, mm Hg.
Q = oxygen consumption rate, ml O_2(STP)/ml tissue × sec.
t = time, sec.
x = distance, cm.

We assume here and in all subsequent derivations that the sheet is large in area compared to its thickness so that only oxygen diffusion normal to the surface is important. We also assume that Q and D are independent of P, x, and t.

To obtain the oxygen tension distribution in an oxygen-consuming sheet we assume that an open surface will be at the oxygen tension of the surrounding gaseous atmosphere, symbolized here as P_a.

At the steady state there is no change in oxygen tension with time at any point so that

$$\frac{\partial P}{\partial t} = 0 \tag{2 A}$$

For the steady state, therefore, equation 1 A becomes the ordinary differential equation

$$\frac{d^2P}{dx^2} - \frac{Q}{Dk} = 0 \tag{3 A}$$

For a tissue exposed on both surfaces to air with oxygen tension P_a the required boundary conditions are $P = P_a$ at $x = 0$ and $x = l$. For physical reality an implicit condition is that $P > 0$. The solution of equation 3 A with these conditions is,

$$P = \frac{Qx^2}{2\,Dk} - \frac{Qlx}{2\,Dk} + P_a \tag{4 A}$$

Equation 4 A gives the oxygen tension distribution in the sheet just before it is placed on the electrode. If the tissue is placed on the electrode at $t = 0$, then this equation becomes the initial condition. When the sheet is on the electrode the boundary conditions become $P = P_a$ at $x = l$, and $dP/dx = 0$ at $x = 0$ since the upper surface, $x = l$, is exposed to the air and the bottom surface, $x = 0$, is closed and there can be no oxygen flux across this surface. Using the mathematical methods described by Carslaw and Jaeger (1959 *b*) we can now derive an equation for the change in oxygen tension with time after the tissue is placed on the electrode surface.

We define a variable p such that

$$p = P - P_a - \frac{Qx^2}{2\,Dk} + \frac{Qlx}{2\,Dk} \tag{5 A}$$

Since P is given by equation 4 A at $t = 0$ we see that $p = 0$ for $t = 0$. If equation 5 A is differentiated once with respect to t and twice with respect to x the results can be combined with equation 1 A to obtain

$$\frac{\partial p}{\partial t} = D\frac{\partial^2 p}{\partial x^2} \tag{6 A}$$

This equation is much simpler than equation 1 A and solutions for it are available in standard reference works, such as those ot Carslaw and Jaeger (1959).

We now need only the boundary conditions for equation 6 A when one surface is exposed to the air and the other surface is closed. We have already shown that the initial condition is $p = 0$ for $t = 0$.

We differentiate equation 5 A with respect to x to obtain,

$$\frac{dp}{dx} = \frac{dP}{dx} - \frac{Qx}{Dk} + \frac{Ql}{2\,Dk} \tag{7 A}$$

At $x = 0$, $dP/dx = 0$, therefore one boundary condition is $x = 0$, $dp/dx = Ql/2\,Dk$. We also note that at $x = l$, $P = P_a$, therefore equation 5 A gives the second boundary condition for equation 6 A as $x = l$, $p = 0$.

The solution of equation 6 A under these initial and boundary conditions is given by Carslaw and Jaeger (1959 *c*). Using the symbols of this paper their solution is

$$p = -\frac{Ql(l-x)}{2\,Dk} - \frac{Ql^2}{2\,Dk}\frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{(-1)^n}{(2n+1)^2} \cdot \exp\left[-\frac{(2n+1)^2 D\pi^2 t}{4l^2}\right]\sin\frac{(2n+1)(l-x)\pi}{2l} \tag{8 A}$$

When equations 5 A and 8 A are combined the result is

$$P = P_a + \frac{Qx^2}{2\,Dk} - \frac{Ql^2}{2\,Dk} + \frac{Ql^2}{2\,Dk}\frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{(-1)^n}{(2n+1)^2} \cdot \exp\left[-\frac{(2n+1)^2 D\pi^2 t}{4l^2}\right]\sin\frac{(2n+1)(l-x)\pi}{2l} \tag{9 A}$$

At $x = 0$ the equation for P is

$$P_{x=0} = P_a - \frac{Ql^2}{2\,Dk} + \frac{Ql^2}{2\,Dk}\frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-\frac{(2n+1)^2 D\pi^2 t}{4l^2}\right] \tag{10 A}$$

Equation 10 A shows that the oxygen tension at the electrode will fall from P_a at $t = 0$ to $P_a - Ql^2/2\,Dk$ at infinite time. It can be demonstrated that higher terms in the exponential series of equation 10 A can be neglected if $Dt/l^2 > 0.2$. At these longer times $[P_{x=0} - P_a + Ql^2/2\,Dk]$ is exponential with t.

The term $P_a - Ql^2/2\,Dk$ is P_o of Fig. 2, and $P_a - P_o$ is $\Delta P_{x=0}$ by definition. $\Delta P_{x=0}$ depends only upon time-invariant properties of the sample on the electrode. $\Delta P_{x=0}$ is the difference in oxygen tension, at infinite time, between the surface exposed to air and the surface next to the electrode. It is also the total change in oxygen tension, at $x = 0$, from $t = 0$ to $t = \infty$. Therefore, the quantity $(P_{x=0} - P_o)/\Delta P_{x=0}$ will fall, in time t_1, by a factor of 10 (that is, one logarithmic cycle). When the logarithm of this quantity is plotted vs. time, and t_1 is given by,

$$t_1 = \frac{0.9332l^2}{D} \tag{11 A}$$

then

$$D = \frac{0.9332l^2}{t_1} \tag{12 A}$$

The total drop in oxygen tension at the electrode, $\Delta P_{x=0}$, after infinite time is given from equation 10 A as

$$\Delta P_{x=0} = \frac{Ql^2}{2\,Dk} \tag{13 A}$$

Combining equations 12 A and 13 A and solving for Q gives,

$$Q = \frac{1.866k\Delta P_{x=0}}{t_1} \tag{14 A}$$

Equation 1 A can also be solved to give the oxygen tension as a function of time and position after the tissue on the electrode has been covered. The initial condition is that the surface at $x = l$ has $P = P_a$ and at $x = 0$, $dP/dx = 0$. If equation 3 A is solved for these conditions the result is,

$$P = P_a - \frac{Q(l^2 - x^2)}{2\,Dk} \tag{15 A}$$

Equation 15 A is now the initial condition. At a later time both surfaces are covered so that the boundary conditions become $dP/dx = 0$ for both $x = 0$ and $x = l$. The method used to solve equation 1 A for these conditions is similar to the one used above. A new variable p is defined as,

$$p = P - P_a + \frac{Q(l^2 - x^2)}{2\,Dk} \tag{16 A}$$

If equation 16 A is differentiated once with respect to t and twice with respect to x the results can be combined with equation 1 A to yield equation 6 A. This means we need only find the initial and boundary conditions in terms of p to use the well known solutions of equation 6 A.

The initial condition is that equation 15 A hold. Then from equation 16 A it is clear that the initial condition on p is $p = 0$.

A single differentiation of equation 16 A with respect to x gives,

$$\frac{dp}{dx} = \frac{dP}{dx} - \frac{Qx}{Dk} \tag{17 A}$$

Since both surfaces are closed $dP/dx = 0$ at both $x = 0$ and $x = l$. From equation 17 A we see that under these conditions $dp/dx = 0$ at $x = 0$ and $dp/dx = -Ql/Dk$ at $x = l$. These are now the boundary conditions for equation 6 A. The solution is given by Carslaw and Jaeger (1959 *d*) and in terms of the symbols used here is

$$p = -\frac{Qt}{k} - \frac{Ql^2}{Dk}\left\{\frac{3x^2 - l^2}{6l^2} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}e^{-\frac{n^2D\pi^2t}{l^2}}\cos\frac{n\pi x}{l}\right\} \tag{18 A}$$

Equating equations 16 A and 18 A and solving for P gives the solution for the oxygen tension in a covered tissue on the electrode, namely,

$$P = P_a - \frac{Q(l^2 - x^2)}{2\,Dk} - \frac{Qt}{k} - \frac{Ql^2}{Dk}\left\{\frac{3x^2 - l^2}{6l^2} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\,e^{-\frac{n^2D\pi^2t}{l^2}}\cos\frac{n\pi x}{l}\right\} \tag{19 A}$$

The electrode is measuring P at $x = 0$, therefore we evaluate equation 19 A at $x = 0$ to give,

$$P_{x=0} = P_a - \frac{Qt}{k} - \frac{Ql^2}{Dk}\left\{\frac{1}{3} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\,e^{-\frac{n^2D\pi^2t}{l^2}}\right\} \tag{20 A}$$

From equation 20 A it can be shown that the time t_o for $P_{x=0} = 0$ is given by,

$$t_0 = \frac{P_ak}{Q} - \frac{l^2}{3D} \tag{21 A}$$

provided that t_o is large enough so that the exponential terms are negligible.

Equation 20 A also shows that for t large enough to make the exponential term negligible, the oxygen tension at $x = 0$ will be a linear function of t, namely,

$$P_{x=0} = P_a - \frac{Ql^2}{3\,Dk} - \frac{Qt}{k} \tag{22 A}$$

The line given by this equation will intersect the horizontal line labeled P_o in Fig. 2 at a time t_L given by,

$$t_L = \frac{l^2}{6D} \tag{23 A}$$

P_o is given by equation 13 A as $P_o = P_a - Ql^2/2\,Dk$. D can be eliminated from equations 21 A and 23 A to give,

$$Q = \frac{P_ak}{t_0 + 2t_L} \tag{24 A}$$

If equation 19 A is evaluated at $x = l$ and the result compared with equation 20 A it can be seen that when the exponential term approaches zero, the oxygen tension at both surfaces is the same. Therefore if the experimental conditions are such that the exponential term is negligible, a measurement of the time for the oxygen tension to fall to zero at $x = 0$ gives the time for oxygen tension to be zero at all points in the tissue. It can be demonstrated that, after Dt/l^2 has increased to 0.4, the exponential term never contributes more than 2% to P. Therefore, for an average tissue, 0.0384 cm thick and with $D = 0.50 \times 10^{-5}$ cm²/sec, the time for the oxygen tension at the top and bottom surfaces to become less than 2% apart is 120 sec. This means

that if the time for the oxygen tension to fall to zero is 120 sec or more, then essentially all of the oxygen will be consumed when the oxygen tension at the electrode just reaches zero.

We wish to thank Dr. D. M. Maurice for pointing out the possibility of calculating the diffusion coefficient from the experimental data and for other helpful suggestions in preparing this paper.
This investigation was supported in part by National Heart Institute grant HE-06796.
Received for publication 22 February 1965; revised manuscript received 8 March 1966.

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