OXYGEN PERMEABILITY OF THE LIMITING LAYERS OF THE CORNEA

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ABSTRACT Using polarography and suitable mathematical analysis, the oxygen permeability of an individual layer of a bilayered tissue may be determined if the oxygen consumption and permeability of the other layer are known. Results obtained on rabbit cornea indicate that the permeability of the epithelium is about four times that of the endothelium.

INTRODUCTION

In a thin layer of living tissue, oxygen diffuses and is consumed. After the treatment by Hill (1928), the distribution of oxygen tension in the tissue is

$$D\frac{\partial^2 P}{\partial x^2} - \frac{Q}{k} = \frac{\partial P}{\partial t}, \tag{1}$$

where D is the diffusion of coefficient of oxygen in the tissue (cm^2/sec),
P is the oxygen tension (mm Hg),
Q is the oxygen consumption (ml O_2 ml tissue^{-1} sec^{-1}),
k is Henry's law solubility constant (ml O_2 ml tissue^{-1} mm Hg^{-1}),
t is time (sec), and
x is the distance perpendicular to the surface (cm).

For the steady-state case, $\partial P/\partial t = 0$, and equation 1 becomes

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

The diffusion-solubility product Dk defines the tissue oxygen permeability because it is the proportionality constant in Fick's law,

$$J = -DkA\frac{dP}{dx}, \tag{3}$$

that relates the oxygen flux J to the oxygen tension gradient dP/dx and the area A (Crank, 1956).

For a multilayered tissue, i.e. one with structurally and functionally distinct types of cells, each layer is described by equation 2 suitably subscripted to show its application to a separate component. Integration of the equations for each layer, subject to the prevailing boundary conditions, provides a description of the oxygen tension throughout the tissue.

The oxygen tension profile is particularly useful in studies of tissues such as the cornea, which is avascular except in a very small peripheral area but contains living and reproducing cells. It has been shown that the oxygen required by the cornea is obtained primarily by diffusion across its surfaces (Maurice, 1969); however, the proportionate contributions made by atmospheric and aqueous humor oxygen supplies are not established. As described above, this determination requires values of both Q and Dk for the component layers of the cornea.

Various methods have been used to determine Q (Langham, 1952; footnote 1). D has also been found experimentally for corneal tissue (Takahashi and Fatt, 1965), but corresponding values for k are not available and formidable difficulties would be encountered in its determination because gas analysis of a solid or semi-solid material would be required. In addition, direct measurements of D and k would require isolation of each structural layer, which is not practical in the case of the very thin limiting layers of the cornea.

In this paper we describe analytical and experimental procedures by which oxygen permeability may be determined for one layer of a bilayered tissue if Q and Dk of the other layer are known. We have measured Dk in rabbit stroma and with additional data on stromal Q, we have determined Dk for the other major components of the cornea, the epithelium and endothelium.

THEORY

Fig. 1A is a model cornea which includes only the metabolically active layers. They are, from anterior to posterior: the epithelium (about 10% of the total thickness), the stroma (approximately 89% of the total thickness), and the endothelium (a single layer of cells covering the posterior surface of the cornea).

Fig. 1B represents the condition where the epithelium or endothelium has been excised and subsequently replaced by an oxygen-impermeable barrier, an oxygen sensor probe, at the stromal surface. Fig. 1 C shows the same condition, but in this case the oxygen sensor probe is positioned at the epithelial or endothelial surface.

For the conditions shown in Fig. 1 (B and C), the steady-state oxygen tension distribution equations are

$$\frac{d^2P_1}{dx^2} - \frac{Q_1}{D_1k_1} = 0, \qquad (4)$$

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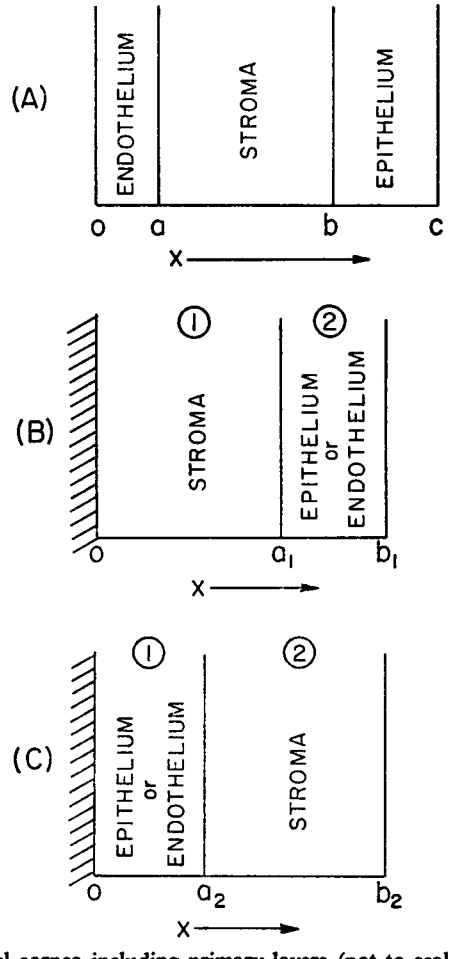

FIGURE 1 A. Model cornea including primary layers (not to scale). B. Model indicating removal of one of the limiting layers with the oxygen sensor probe at the stromal surface. C. Same as B except that the oxygen sensor probe is at the limiting layer (epithelium or endothelium).

and

$$\frac{d^2P_2}{dx^2} - \frac{Q_2}{D_2k_2} = 0, \tag{5}$$

where the subscripts 1 and 2 refer to the corresponding layers numbered in Fig. 1 (B and C).

This treatment only considers oxygen diffusion normal to the tissue surface. The basis for the assumption that longitudinal diffusion is not important is that the tissue is very thin and its area is large compared with its thickness (0.4 mm thick, 10 mm diameter). To test the assumption, we applied a cover glass to the upper tissue surface while leaving the sides exposed to the air and measuring the oxygen

tension at the lower surface. The measured tension came to equilibrium at zero thus demonstrating that only vertical diffusion is relevant in this analysis.

In equations 4 and 5, it is assumed that D, k, and Q are independent of P. This condition obtains for D and k (Goldstick and Fatt, 1970). Independence also holds for epithelial and endothelial Q, but for the stroma, Q is dependent upon P over a wide range.[2] To adjust for this, we have used stromal Q values corresponding to averaged oxygen tensions across the stroma. We compared these values with calculations of Q as linearly dependent upon P and found the differences negligible. This is to be expected because the difference in oxygen tension across the stroma in all of our measurements is small. Accordingly, our assumption of independence of Q, with the above adjustment, is reasonable and upon integration, the general solutions of equations 4 and 5 are

$$P_1(x) = Ax^2 + Bx + C, \tag{6}$$

$$P_2(x) = Ex^2 + Fx + G. \tag{7}$$

By setting equations 4 and 5 equal to the second derivatives of equations 6 and 7, we see that

$$A = \frac{Q_1}{2D_1k_1} \quad \text{and} \quad E = \frac{Q_2}{2D_2k_2}.$$

The remaining constants of equations 6 and 7 are determined from the boundary conditions as shown in the Appendix. The result is a system of linear algebraic equations. Taking into account the conditions represented in Fig. 1 (B and C), the equations may be solved for the constants A and E. With the assumption that Q and Dk are independent of whichever boundary is closed, the solutions for A and E may be equated (equations A 11 and A 15 of the Appendix). The result is

$$\frac{\frac{1}{b_1}\left(P_\alpha - a_1^2A - C_1 + \frac{2a_1^2A}{\gamma_1}\right) - \frac{2a_1A}{\gamma_1}}{b_1 - 2a_1 + \frac{a_1^2}{b_1}} = \frac{E[-(a_2 - b_2)^2] - C_2 + P_\alpha}{a_2^2 - \gamma_1 2a_2[a_2 - b_2]} \tag{8}$$

This quadratic polynomial may be solved for γ_1, the ratio of epithelial or endothelial Dk to stromal Dk, as all other values are known (see Appendix for definitions of terms). Since Dk for the stroma is known, Dk of the epithelium or endothelium is determined.

METHODS

Apparatus

The equipment is shown schematically in Fig. 2. Water-saturated gas (air or nitrogen) was passed into the tissue chamber (TC) via a stopcock gas manifold which allowed rapid change

[2] Freeman, R. D. Manuscript in preparation.

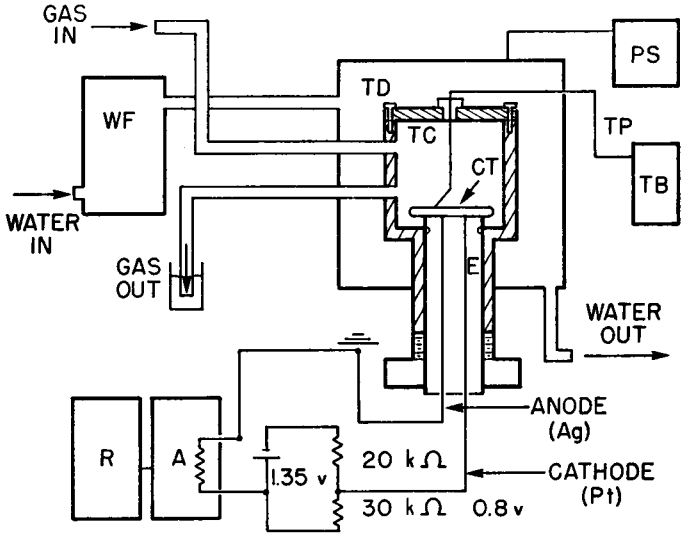

FIGURE 2 Schematic diagram of the apparatus (not to scale). WF, flowmeter; TD, thermoelectric unit; TC, tissue chamber; CT, corneal tissue; E, oxygen electrode; PS, power supply; TP, thermistor probe; TB, thermistor bridge; A, amplifier, and R, recorder.

from one gas to the next. A gas outlet from the chamber was led to a water-filled beaker to provide continual evidence of a positive pressure within the chamber.

Temperature within the chamber was controlled by a thermoelectric device (TD) operated with a water flowmeter (WF) and power supply (PS). Tissue temperature was monitored with a calibrated thermistor probe (TP) and thermometer bridge (TB). For the experiments reported here, tissue temperature was maintained at 23.5 ±0.5°C.

To measure oxygen tension at the surface of the corneal tissue (CT), an oxygen electrode (E) was used. The principles and operating characteristics of this type of oxygen sensor have been described in detail previously (Fatt, 1964; Clark and Sachs, 1968). Briefly, it consists of a platinum cathode negatively polarized with respect to a silver–silver chloride anode. The indicator and reference electrodes are both enclosed in a housing with a borate buffer electrolyte layer on the surface covered by a semipermeable polyethylene membrane. The sensor constitutes a closed boundary because oxygen consumption by the electrode is negligible. The sensor current is proportional to the oxygen tension at the outer surface of the membrane.

The applied potential to the electrode was maintained at 0.8 v, for which the oxygen sensor current is linearly proportional to oxygen tension (Halpert and Foley, 1963). We made measurements to confirm that this relation held for our apparatus. Current from the oxygen sensor was amplified by a chopper-stabilized DC amplifier, the output of which was recorded by a standard potentiometric servorecorder.

Tissue thickness was measured with a microscope fitted with a dial micrometer. A Lucite cylinder with a convex ground top was secured in the stage aperture for use as a tissue mount.

Procedure

Each corneal tissue was prepared as follows. A rabbit was killed with sodium pentobarbital injected intravenously. One eyelid was taped shut to preserve the cornea for later use, and

the other eye was proptosed with a hemostat clamped to the lids at the nasal canthus. For a sample of stroma plus endothelium, the epithelium was carefully removed from the intact eye with emery paper rotated by a small electric grinder. A perilimbal incision was then made and the cornea was excised. To obtain a sample of stroma plus epithelium, the cornea was partially excised perilimbally, the single layered endothelium was scraped off with a scalpel, and the excision was then completed. For each tissue sample, small peripheral radial incisions were made to reduce the natural curvature, and the tissue was then suspended in a moist chamber to equilibrate with air and establish the desired boundary conditions.

While the tissue was equilibrating, the oxygen sensor was calibrated with water-saturated nitrogen and air. After several minutes, the tissue was carefully positioned flat on the electrode so that there were no bubbles between the electrode membrane and the tissue. The tissue was placed with the stromal side down (see Fig. 1 B) or the epithelial or endothelial surface down (Fig. 1 C). The subsequent decrease in oxygen tension was recorded until the steady-state level was reached (about 45 sec). From the difference between the environmental P_{O_2} and the steady-state P_{O_2}, Dk was calculated from equation 8.

At the end of the experiment, the tissue was placed on the microscope stage Lucite mount attachment and thickness was determined by the difference in focus between surface particles on the tissue and fiducial marks on the Lucite. The tissue was then placed in 4% formaldehyde for histological analysis (see Discussion).

RESULTS

The oxygen permeabilities for the corneal epithelium and endothelium are given in Table I. As described above, these values are computed by evaluation of equation 8 which requires the following data: (*a*) the measured oxygen tensions at the electrode-tissue interface, (*b*) the thickness dimensions for each tissue layer, (*c*) the value of Dk in the stroma, and (*d*) the oxygen consumption of the stroma.

Experimentally determined oxygen tensions are included in Table I. The measured tissue thicknesses were quite uniform and for the total dimension, we used the mean (400 μ $\pm$10). The epithelium and endothelium are 10% and 1% respectively of the total (40 μ and 4 μ). These values agree with those reported in other studies

TABLE I

OXYGEN PERMEABILITY (Dk) OF EPITHELIUM AND ENDOTHELIUM

Measured layer	S*	N‡	P§	Stromal Q‖	Dk¶
			mm Hg		
Epithelium	Stroma	6	119.4 ±6.3	1.11	1.9
	Epithelium	8	81.3 ±4.8	0.94	
Endothelium	Stroma	5	122.8 ±4.6	1.13	0.54
	Endothelium	4	99.6 ±7.6	1.03	

* Surface of bilayered tissue at the electrode.

‡ No. of experiments.

§ Measured oxygen tension, SD (C_1 and C_2 in equation 19).

‖ Units: 10^{-5} ml O_2 ml tissue^{-1} sec^{-1}.

¶ Units: 10^{-10} ml O_2 cm^2 ml tissue^{-1} sec^{-1} mm Hg^{-1}.

TABLE II

EFFECT OF MEASURED THICKNESS RANGE ON THE CALCULATED OXYGEN PERMEABILITY (*Dk*) FOR EPITHELIUM AND ENDOTHELIUM

T*	$Dk_{\text{Epithelium}}$‡	$Dk_{\text{Endothelium}}$‡
390	1.6	0.43
400	1.9	0.54
410	2.2	0.84

* Total tissue thickness (microns).

‡ Units: 10^{-10} ml O_2 cm^2 ml $tissue^{-1}$ sec^{-1} mm Hg^{-1}.

(von Bahr, 1956; Maurice, 1969). The effect of the measured thickness range on the calculated *Dk* values is given in Table II.

We measured stromal *Dk* by a technique suitable for single-layer tissues (Aiba et al., 1968). The value obtained is 3.0×10^{-10} ml O_2 cm^2 ml $tissue^{-1}$ sec^{-1} mm Hg^{-1}. The remaining quantity required, stromal oxygen consumption, was determined from the measured oxygen tension at the electrode-stroma interface, stromal *Dk*, and stromal thickness. Details of the procedure are described elsewhere.[1] The stromal Q_{O_2} values are included in Table I.

DISCUSSION

Analysis

The analytical procedure depends upon adequate surgical isolation of the relevant tissue layers. For the tissue preparations described here, it was of prime interest to confirm that both layers were intact and no portion of a third layer was present. This was done by preserving the tissue samples and preparing them for histological examination. Standard techniques were employed with hematoxylin and eosin Y on 5-μ tissue sections. The resulting slides showed that the desired conditions held in all cases.

In deriving equation 8, we have assumed that Q of the cellular layer (epithelium or endothelium) is invariant, whether the stromal or cellular surface is closed at the electrode boundary. This must be the case functionally, because the physical condition of the tissue is unaltered; however, Q is determined from the measured oxygen tension at the tissue surface, and when the stroma is positioned down on the sensor, the remaining layer (epithelium or endothelium) is a relatively considerable distance from the electrode. The analytical consequence of this is a "lever" effect where small changes in measured oxygen tensions result in large differences in computed *Dk* values. Although this is a general limitation to the method, it is only a problem when there is a large thickness difference between the two layers of the tissue.

To assess the effect, calculations have been made using the measured oxygen

TABLE III

EFFECT OF MEASURED OXYGEN TENSION RANGE ON CALCULATED OXYGEN PERMEABILITIES

Oxygen tension			$Dk_{Epithelium}$*	$Dk_{Endothelium}$*
Stroma	Epithelium	Endothelium		
mm Hg‡	*mm Hg*‡	*mm Hg*‡		
119.4	76.5		1.94	
	86.1		1.82	
113.1	81.3		1.23	
125.7			2.81	
122.8		92.0		0.56
		107.2		0.51
118.2		99.6		0.25
127.4				2.65

* Units: 10^{-10} ml O_2 cm^2 ml $tissue^{-1}$ sec^{-1} mm Hg^{-1}.

‡ Mean values are combined with standard deviation ranges (see Table I for values and text for explanation).

tension standard deviations (Table I) in two cases. In the first, mean stromal values are used with the maximum and minimum values of the epithelium and endothelium. In the second, the means for the limiting layers are combined with the maximum and minimum values of the stroma. The results, given in Table III, show that the *Dk* range is very narrow for the first case and has a greater spread, as expected, for the second case.

For the analysis, *Dk* of one of the two layers of the tissue must be known; however, it is possible to adapt our basic methods so that *Dk* for any single layer tissue can be determined. This could be accomplished by adding to the surface of the tissue a layer with a known oxygen permeability such as an aqueous-agar gel. The material could be adjusted in thickness to approximately match that of the single-layer tissue so that the mismatched thickness effects described above would be eliminated. Subsequently, the experimental and analytical procedures would be identical with those given herein.

Oxygen Permeability Values

We have not found any reports of previous determinations of *Dk* in corneal tissue. The classical studies of Krogh (1919) provide data for comparison with other tissues. Upon conversion of his results to our units, the Krogh values for muscle and connective tissue are 3.08×10^{-10} and 2.48×10^{-10} ml O_2 ml $tissue^{-1}$ sec^{-1} mm Hg^{-1}, respectively. These are similar to the values we report here for corneal epithelium and endothelium.

Table I shows that the oxygen permeability for epithelium is approximately four times that for endothelium. Anatomical factors might account for this difference since studies on the fine structure of the rabbit cornea have shown numerous differ-

ences between these cellular layers (Kaye and Pappas, 1962; Teng, 1961). On the other hand, there are apparently few chemical differences between the epithelium and endothelium. It is well known that dissolved oxygen, because of its fat solubility, readily passes across cell membranes, and there is no obvious reason why this should not apply equally to both epithelium and endothelium.

Functionally, the oxygen permeabilities and consumptions of each corneal layer, together with the oxygen partial pressure difference between the atmosphere and aqueous humor, account for the oxygen fluxes at the anterior and posterior corneal surfaces.

APPENDIX

Fig. 1 (B and C) shows that $x = 0$ at the closed boundary, the oxygen electrode–tissue interface. At the open boundary, $x = b$ and the oxygen tension is fixed at P_α (P_{O_2} of air). In addition, continuity of oxygen tension and flux is required at a, the interface between the tissue layers. Therefore, the boundary conditions for equations 4 and 5 of the text are

$$x = 0, \qquad \frac{dP_1}{dx} = 0 \tag{A 1}$$

(no flux at the closed boundary),

$$x = a,\ P_1(a) = P_2(a) \tag{A 2}$$

(continuity of $P(x)$ at the layer interface),

$$\left.\frac{dP_1}{dx}\right|_{x=a} = \gamma \left.\frac{dP_2}{dx}\right|_{x=a} \quad \text{where} \quad \gamma = \frac{D_2 k_2}{D_1 k_1} \tag{A 3}$$

(continuity of gradient-permeability product which follows from Fick's law [equation 3 of text]),

$$x = b,\ P_2 = P_\alpha \tag{A 4}$$

(at the open boundary, oxygen tension is fixed at P_α [P_{O_2} of air]).

The first boundary condition applied to equation 6 of the text shows directly that $B = 0$. To determine the remaining constants C, F, and G, the boundary conditions are applied to equations 6 and 7 and the result is

$$a^2A + C = a^2E + aF + G, \tag{A 5}$$

$$2aA = 2a\gamma E + \gamma F, \tag{A 6}$$

$$P_\alpha = b^2E + bF + G. \tag{A 7}$$

We consider first the condition represented in Fig. 1 B, where the stroma surface is closed. In this case a, b, C, and γ are denoted a_1, b_1, C_1, and γ_1 respectively. a_1 and b_1 are known distances. Q and Dk for the stroma are known and therefore A is known. P_α is also known because it represents atmospheric oxygen tension. In addition, from equation 6, at $x = 0$,

$P_1 = C_1$ which is the measured oxygen tension at the oxygen electrode closed boundary. Equations A 5, A 6, and A 7, with the unknowns on the left and insertion of the appropriate subscripts, become

$$a_1^2E + a_1F + G = a_1^2A + C_1, \tag{A 8}$$

$$2a_1E\gamma_1 + \gamma_1F = 2a_1A, \tag{A 9}$$

$$b_1^2E + b_1F + G = P_\alpha . \tag{A 10}$$

Solving for E, we have

$$E = \frac{\frac{1}{b_1}\left(P_\alpha - a_1^2A - C_1 + \frac{2a_1^2A}{\gamma_1}\right) - \frac{2a_1A}{\gamma_1}}{b_1 - 2a_1 + \frac{a_1^2}{b_1}} . \tag{A 11}$$

For the other case, shown in Fig. 1 B, the epithelial or endothelial surface is the closed boundary. a, b, C, and γ are denoted a_2, b_2, C_2, and γ_2 respectively. Once again, Q and Dk for the stroma are known, but here they specify E. With the proper subscripts, and the unknowns placed on the left, equations A 5, A 6, and A 7 for this condition are

$$a_2^2A - a_2F - G = a_2^2E - C_2, \tag{A 12}$$

$$\frac{2a_2A}{\gamma_2} - F = 2a_2E, \tag{A 13}$$

$$b_2F + G = P_\alpha - b_2^2E. \tag{A 14}$$

Solving for A, we obtain

$$A = \frac{E[-(a_2 - b_2)^2] - C_2 + P_\alpha}{a_2^2 - \frac{2a_2}{\gamma_2}[a_2 - b_2]} . \tag{A 15}$$

From Fig. 1 (B and C) and the definition of γ, we note that γ_1 and γ_2 are reciprocals, so we can express γ_2 in equation A 15 as γ_1^{-1}. With the assumption that Q and Dk are independent of whichever boundary is closed, i.e. A and E have the same value under the conditions of Fig. 1 B and C respectively, we equate equations A 11 and A 15, and the result is equation 8 of the text.

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