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# OXYGEN CONSUMPTION BY THE COMPONENT LAYERS OF THE CORNEA

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## SUMMARY

1. The structural integrity of the cornea is maintained by an active fluid transport system which depends on metabolism. Experiments were designed to establish the respiratory activity of each corneal layer.

2. A rapid micropolarographic technique was used to determine the oxygen consumptions of the individual layers of the rabbit cornea.

3. Inherent problems of the determinations have been made minimal by the use of both denuded and whole corneal tissue preparations. Four independent measures were obtained for each limiting layer (epithelium and endothelium).

4. Results show that the endothelium, epithelium and stroma use 21, 40 and 39 % respectively of the total oxygen consumption of the cornea. On the basis of volumes of oxygen per unit volume tissue, epithelial oxygen utilization is about ten times that of the stroma and approximately 0·2 that of the endothelium.

5. The endothelium has a larger oxygen uptake than previously reported.

6. The present results, in conjunction with other studies, indicate that the ratio of glycolytic to oxidative activity in the rabbit cornea is 0·87 : 0·13.

## INTRODUCTION

Although the cornea is avascular except for a limited peripheral zone, the metabolic requirements of living and reproducing cells must be met. The considerable oxygen permeability and respiration of the tissue suggest a negligible oxygen penetration from the cornea's outer capillary network. Furthermore, experiments on tissue fluid flow (Maurice, 1960) and diffusional spread (Maurice & Watson, 1965) indicate that the limbal blood supply cannot provide metabolites for the entire tissue. Therefore, the cornea respires primarily across its anterior and posterior surfaces.

The nature of corneal respiration is important because the tissue's structural integrity is maintained by a metabolically dependent fluid

transport system. Corneal utilization of atmospheric oxygen has been demonstrated (de Roetth, 1950; Langham, 1952), but questions have remained regarding the amount of oxygen consumed by each layer. Previous studies are subject to two main criticisms.

First, the process of isolation of the tissue layers may cause cell damage. Some trauma is likely in the mechanical separation of layers and cell environment is necessarily altered. For example, it has been reported that the staining properties of stromal cells are modified by substances released from a damaged epithelium (Weimar, 1962). Another effect of trauma is illustrated by the finding that homogenization of the epithelium considerably decreases its oxygen uptake (de Roetth, 1950).

Secondly, the different corneal layers may interact metabolically. This could account for the observation that higher lactate levels are found in isolated stroma than in the stromal portion of intact cornea (Hermann & Hickman, 1948). If metabolic interaction is substantial, estimates of oxygen consumption based on differences in rate between whole and denuded cornea could be spurious.

In addition to these inherent problems, previous experimental determinations have been made with macerated as well as whole tissue, various types of tissue media, variations of the Warburg technique, and several polarographic methods. As a consequence, results of different studies are not readily comparable.

This paper describes an investigation intended to minimize the intrinsic difficulties noted above by the use of rapid micropolarography on samples of both whole cornea and denuded tissue. Measurements have been made, from which the oxygen consumptions of the component layers of the rabbit cornea have been calculated. The methods incorporate a distinct advantage in that two independent determinations may be made with both whole and denuded cornea. Thus, each layer may be obtained in four ways by an identical experimental procedure.

Results show that the oxygen utilization of both limiting layers is of the same order of magnitude. In terms of unit tissue volume, the endothelial consumption is about five times that of the epithelium and around fifty times that of the stroma. For the endothelium, oxygen uptake is significantly greater than previously reported.

## THEORY

For a thin slab of living tissue, oxygen consumption may be determined by monitoring oxygen tension at one surface under given conditions. The analytical procedures used for a single-layer may be readily extended to the case of a multilayered tissue.

In the experiments described here, measurements were made with an oxygen electrode on corneal tissue samples consisting of stroma, stroma plus endothelium, stroma plus epithelium, and whole cornea. To compute oxygen consumptions from the measured oxygen tensions, the following relationships are required. Details are given in the Appendix.

*Single layer tissue*

In accordance with the classic treatment (Hill, 1928), the non-steady state diffusion equation that gives oxygen tension as a function of time and position for a homogeneous slab of oxygen consuming 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 coefficient of oxygen in the tissue ($cm^2\ sec^{-1}$), $P$ is the oxygen tension (mm Hg), $k$ is Henry's law constant (ml. $O_2$ ml. $tissue^{-1}$ mm $Hg^{-1}$), $Q$ is the oxygen consumption rate (ml. $O_2$ ml. $tissue^{-1}\ sec^{-1}$), $x$ is the distance perpendicular to the surface (cm), $t$ is time (sec). For the steady-state case, $\partial P/\partial t = 0$ and eq. (1), with rearrangement, becomes

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

The boundary conditions for eq. (2) are established by the experimental procedure of suspending the excised tissue in a moist air chamber before placement on the oxygen electrode.

$$\left.\begin{aligned} x &= 0, \quad P = P_e, \\ x &= \lambda, \quad P = P_e, \end{aligned}\right\} \tag{3}$$

where $P_e$ is the oxygen tension of air (155 mm Hg) and $\lambda$ is the thickness of the corneal tissue (cm). Integrating eq. (2) and imposing the conditions of (3) yields

$$P(x)=\frac{Qx^2}{2Dk}-\frac{Q\lambda x}{2Dk}+P_e. \tag{4}$$

Assumptions of this solution are considered further on (see Results).

After air equilibration, the corneal tissue is placed on the surface of the oxygen sensor so that one side is closed off. Therefore, eq. (4) is the initial condition ($t = 0$) of eq. (1). There is no oxygen flux at the electrode-tissue interface and the top surface of the tissue is exposed to air. Therefore, the boundary conditions of eqn. (1) are

$$\left.\begin{aligned} x &= 0, \quad \frac{dP}{dx} = 0, \\ x &= \lambda, \quad P = P_e. \end{aligned}\right\} \tag{5}$$

The general solution of eqn. (1) with the above conditions is given in the Appendix. For the single layer tissue (the stroma in this case) the result

shows that the steady-state oxygen tension difference ($\Delta P_{x=0}$) between the top and bottom surfaces is $Q\lambda^2/2Dk$, or

$$P_1 = P_e - \frac{Q\lambda^2}{2Dk}, \quad \text{where} \quad P_1 = P_{\substack{x=0 \\ t=\infty}}. \tag{6}$$

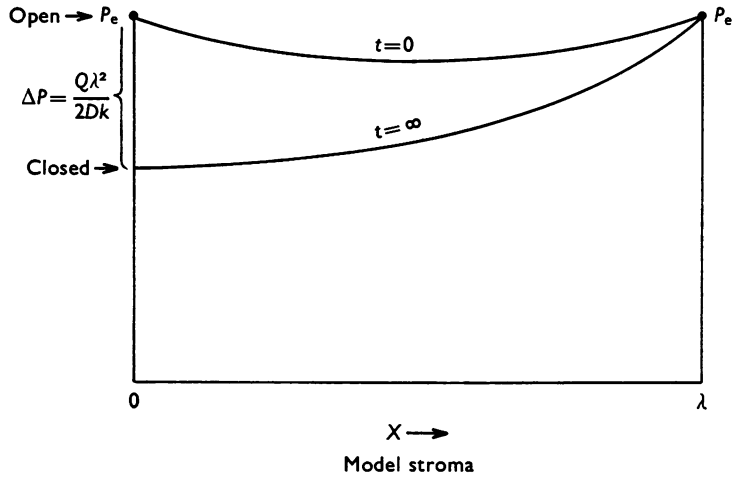


Fig. 1. Model stroma indicating the conditions for analysis. Before placement on the electrode, both surfaces are equilibrated at an oxygen tension of 155 mm Hg. The steady-state oxygen tension drop at the electrode surface is equal to $Q\lambda^2/2Dk$.

These relationships are indicated in Fig. 1, a model of the stroma. Rearrangement of eqn. (6) gives

$$Q = \frac{2Dk(P_e - P_1)}{\lambda^2}, \tag{7}$$

which is used to compute the steady-state oxygen consumption of the stroma.

*Multi-layered tissue*

Models for a two-layered tissue (stroma plus epithelium or endothelium) and a whole cornea are shown in Fig. 2. The condition for either surface closed at the electrode is illustrated. For the two-layered case, the descriptive steady-state equations are

$$\frac{d^2P_1}{dx^2} - \frac{Q_1}{D_1k_1} = 0, \tag{8}$$

$$\frac{d^2P_2}{dx^2} - \frac{Q_2}{D_2k_2} = 0. \tag{9}$$

The general solutions are

$$P_1(x) = Ax^2 + Bx + C, \tag{10}$$

$$P_2(x) = Ex^2 + Fx + G. \tag{11}$$

It is directly determined that $A = Q_1/2D_1k_1$ and $E = Q_2/2D_2k_2$. The boundary conditions of the experiment are as follows. At the closed tissue-electrode boundary, there is no flux. At the layer interface, there is

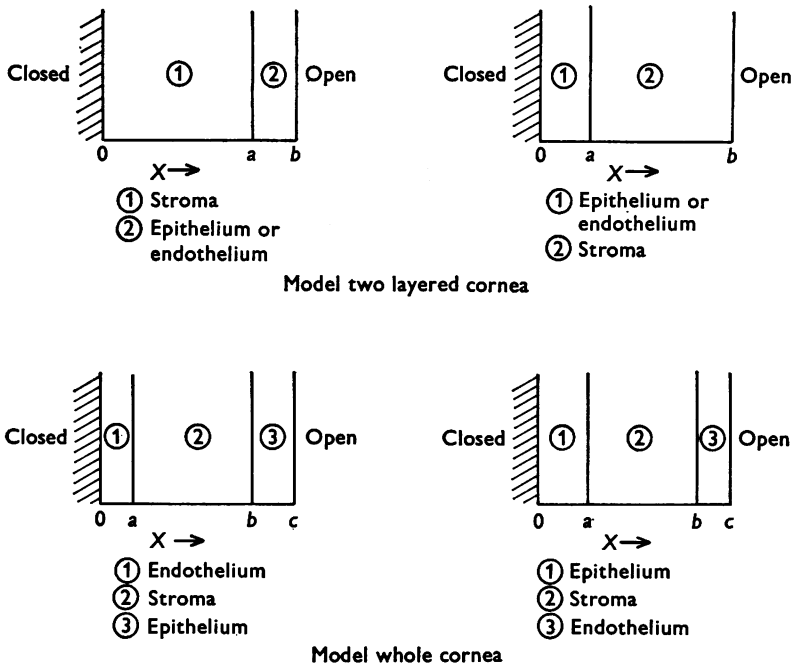


Fig. 2. Models of denuded cornea and whole cornea. In each case, the closed boundary is the surface positioned down on the oxygen electrode and the open surface is at an oxygen tension of 155 mm Hg.

continuity of $P(x)$. In addition, there must be continuity of the gradient-permeability product in accordance with Fick's law. Finally, at the open boundary, oxygen tension is fixed at a $P_{O_2}$ of air. So the boundary conditions for eqns. (8) and (9) are:

$$\left.\begin{aligned} &x = 0, \quad \frac{dP_1}{dx} = 0, \\ &x = a, \quad P_1(a) = P_2(a), \\ &\left.\frac{dP_1}{dx}\right|_{x=a} = a\left.\frac{dP_2}{dx}\right|_{x=a}, \quad \text{where} \quad \alpha = \frac{D_2k_2}{D_1k_1}, \\ &x = b, \quad P_2 = P_e. \end{aligned}\right\} \tag{12}$$

Also, $P_1(0) = C$, which is therefore the measured $P_1$. Substitution of the above conditions into eqns. (10) and (11) gives a system of linear algebraic equations that may be arranged with unknowns on the left for either surface positioned down on the oxygen sensor. For the stroma placed down, the equations are

$$\left.\begin{aligned} a^2E + aF + G &= a^2A + C, \\ 2a + F &= 2aA/\alpha_1, \\ b^2E + bF + G &= P_e, \end{aligned}\right\} \tag{13}$$

and for epithelial or endothelial placement on the electrode, the result is

$$\left.\begin{aligned} a^2A - aF - G &= a^2E - C, \\ 2aA/\alpha_2 - F &= 2aE, \\ -bF - G &= b^2E - P_e. \end{aligned}\right\} \tag{14}$$

In these systems of eqns., (13) and (14), the left-hand upper case letters are constants, the lower-case letters denote the distances indicated in Fig. 2, $\alpha_1$ is the ratio of epithelial or endothelial $Dk$ to stromal $Dk$, and $\alpha_2$ is the inverse of $\alpha_1$. $A$ and $E$ in systems (13) and (14) respectively, represent the known ratio of stromal $Q$ to stromal oxygen permeability ($Dk$) (see Results for values).

For the experiments using whole cornea, modelled as a three-layered system in Fig. 2, the analysis is just an extension of that described above. The result is a system of five equations and five unknowns (see Appendix).

All the systems of equations were expressed in matrix notation and the solutions were obtained using a digital computer (Control Data Corp. 6400).

## METHODS

### *Apparatus*

A schematic representation of the main components of the equipment is shown in Fig. 3. Pumped air or nitrogen was passed through gas-washing bottles into a gas manifold which fed into a Lucite tissue chamber. Temperature within the chamber was controlled by a thermoelectric device driven by a low-ripple d.c. power supply. Tissue temperature, maintained at $23{\cdot}5 \pm 0{\cdot}5$ °C, was monitored with a calibrated thermistor probe and connecting bridge.

Tissue oxygen tensions were measured by a micropolarographic technique. The electrode is a modification of the membrane-covered Clark type (Clark, 1956) and its theory, construction and operation have been fully described previously (Fatt, 1968). In brief, the electrode is comprised of a silver–silver chloride anode and a platinum cathode enclosed in a Lucite cylinder. A very thin buffer layer is placed on the surface of the sensor which is covered by a semipermeable polyethylene membrane. Oxygen diffuses through the membrane, dissolves in the buffer and is reduced at the cathode in a system isolated from the corneal tissue. The oxygen concentration gradient does not extend outside the membrane and the flux to the cathode and the sensor current are proportional to the oxygen concentration at the membrane's outer

surface. Oxygen consumption by the electrode is negligible, so the sensor surface comprises a closed boundary.

By means of a simple Hg cell and voltage divider the electrode received an applied potential of 0·8 V. Measurements confirmed the previous finding (Halpert & Foley, 1963) using this voltage, that oxygen tension bears a linear relation to electrode current. The sensor current output was amplified by a chopper-stabilized d.c. amplifier and recorded by a standard servo-recorder.

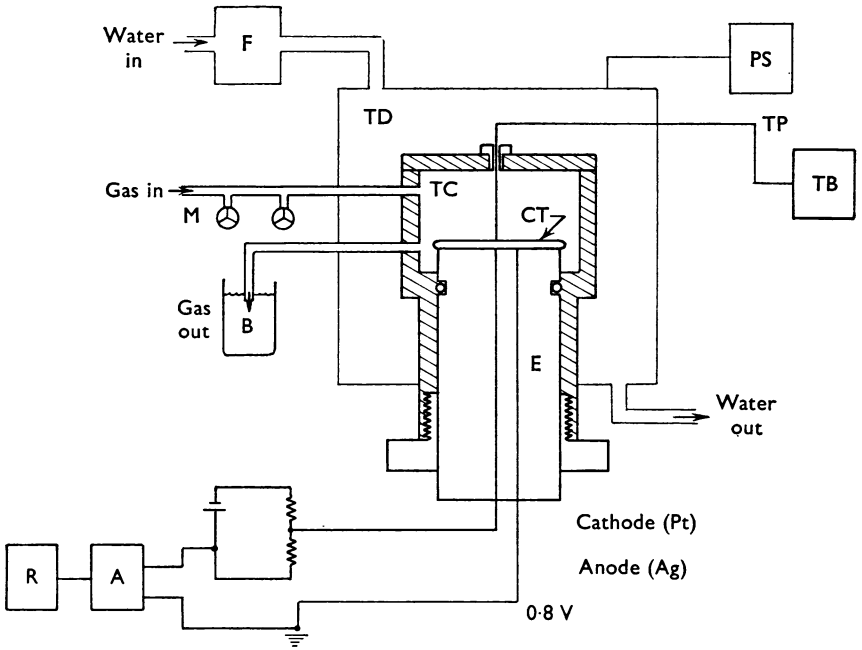


Fig. 3. Schematic diagram of the apparatus (not to scale). A, amplifier; B, water beaker; CT, corneal tissue; E, oxygen electrode; F, water flowmeter; M, gas manifold; PS, power supply; R, recorder; TB, thermistor bridge; TC, tissue chamber; TD, thermoelectric unit; TP, thermistor probe.

A microscope fitted with a dial micrometer was used to measure tissue thickness. A specially constructed Lucite tissue mount was positioned in the centre of the microscope stage.

*Procedure*

Following a lethal dose of sodium pentobarbital, one eye of the rabbit was closed with tape and the other was proptosed in preparation for corneal excision. A stromal sample was prepared as follows. The epithelium was carefully removed from the *in situ* cornea with a small electrically rotated emery disk. A nearly complete perilimbal excision was made, the endothelium was removed with a scalpel, and the remaining stroma was cut free. Small radial edge incisions were made in the tissue and it was then suspended in a moist chamber to obtain boundary equilibration with air.

For tissue samples consisting of whole cornea, stroma plus epithelium, or stroma plus endothelium, the procedure was similar to the above with the relevant layers left intact.

Before each measurement the oxygen electrode was calibrated using water-saturated nitrogen for the zero current level and air for the initial condition of tissue placement. The sample was then gently placed flat on the electrode and an optical check was made to ensure that no air bubbles remained between the tissue surface and electrode membrane. The subsequent decrease in oxygen tension was recorded for about 45 sec, the time required to reach the steady-state level at which $P_i$ of eqn. (6) and $C$ of eqn. (10) are defined. Total preparation and measuring time was such that no significant diminution of glucose level could have occurred (Reim & Lichte, 1965). Fig. 4 represents a typical experimental run and indicates quantities involved in stromal tissue analysis.

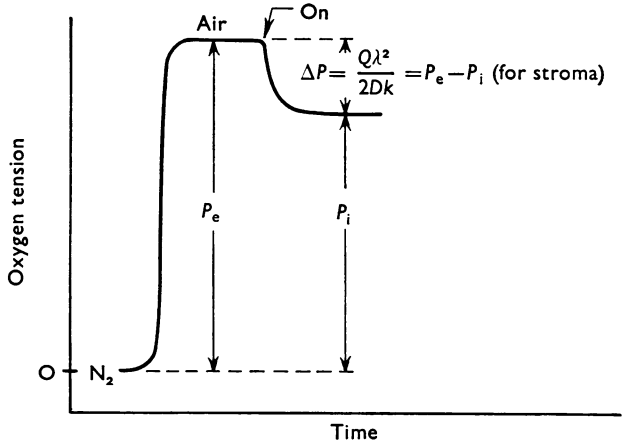


Fig. 4. A typical experimental run. The arrow (On) indicates placement of the tissue on the electrode surface. The time from tissue placement to equilibrium is about 45 sec.

At the conclusion of each run, the tissue was positioned on the microscope stage and thickness was measured by noting the difference in focus between tissue surface granules and the Lucite mount. The tissue was then placed in a formaldehyde solution to preserve it for histological examination.

## RESULTS

### *Data analysis*

The solution of eqn. (2) requires $Q$, $D$, and $k$ to be independent of $P$. This condition holds for $D$ and $k$ (Goldstick & Fatt, 1970), but for the stroma, $Q$ is an approximately linear function of $P$ over a considerable range (R. D. Freeman, in preparation). To take this into account, eqn. (2) would be

$$\frac{d^2P}{dx^2} - \frac{Q(P)}{Dk} = 0, \tag{15}$$

which has the general solution

$$P(x) = A' \sinh \gamma x + B' \cosh \gamma x, \tag{16}$$

where

$$\gamma = \sqrt{\frac{[Q(P)]}{Dk}} \quad \text{and} \quad Q(P) = \frac{Q_{P_e}}{P_e}.$$

For the boundary conditions of the experiment, eqn. (16) becomes

$$P_{x=0} = \frac{P_e}{\cosh \sqrt{\frac{[Q(P)]}{Dk}}\lambda}, \tag{17}$$

from which $Q$ can be found for any $P$, where $Q$ is a linear function of $P$ with origin at zero.

For tissue samples of stroma there is only a small drop in $P_{O_2}$ across the tissue, i.e. $P_i$ and $P_e$ are both high. Therefore there should not be a significant difference in stromal $Q_{O_2}$ results between eqns. (15) and (2). But for the multi-layered samples, the variation in $P_{O_2}$ across the stroma is large and the dependence of stromal $Q_{O_2}$ on $P_{O_2}$ must be taken into account. This may be done by either using eqn. (15) or by making an adjustment to permit use of eqn. (2). The latter would, of course, be preferable and it is worth examining the possibility. A simple way of allowing for the dependence of $Q_{O_2}$ upon $P_{O_2}$ is to use an average oxygen tension from which $Q_{O_2}$ is obtained, i.e.

$$\bar{P} = \frac{\int_0^\lambda \left[\frac{Qx^2}{2Dk} - \frac{Q\lambda x}{2Dk} + P_e\right] dx}{\int_0^\lambda dx}, \tag{18}$$

where the term in brackets is given by eqn. (4). To demonstrate the accuracy of this adjustment, Fig. 5 shows a linear regression fit of experimentally determined stromal $Q_{O_2}$ *vs.* $\bar{P}$ data compared with $Q_{O_2}$ as a function of $P_{O_2}$ obtained by eqn. (17). It is evident by the close agreement that the dependence of $Q_{O_2}$ upon $P_{O_2}$ is adequately accounted for by use of $\bar{P}$. So $\bar{P}$ is used for the single and multilayered computations.

To evaluate eqn. (7) and the various systems of equations [see (13) and (14)], data are required for $P_i$, $P_e$, $\lambda$, $D$, and $k$. $P_i$ is the measured oxygen tension at the electrode–tissue interface. $P_e$ is set at the oxygen tension of air (155 mm Hg). $\lambda$, the over-all tissue thickness, varied little between samples and the mean (403 $\mu \pm 14$ S.D.) was used in calculations, as it was found that tissue thickness variability of $\pm 25\,\mu$ did not greatly affect $Q_{O_2}$ results. Epithelium and endothelium comprise 40 and 4 $\mu$ respectively of the total. These values are in accord with other studies (von Bahr, 1956; Maurice, 1969). Finally, values for $D$, the diffusion coefficient, and $k$, the solubility constant, are required. The product $Dk$ defines the tissue oxygen permeability because it is the proportionality constant in Fick's law relating oxygen flux to the oxygen tension gradient. A separate

procedure, described elsewhere, was devised to determine *Dk* of each corneal layer (Freeman & Fatt, 1972). The values for the stroma, epithelium, and endothelium are respectively, 3·0, 1·9, and 0·54 ($10^{-10}$ ml. $O_2$ $cm^2$ ml. $tissue^{-1}$ $sec^{-1}$ $mm\ Hg^{-1}$).

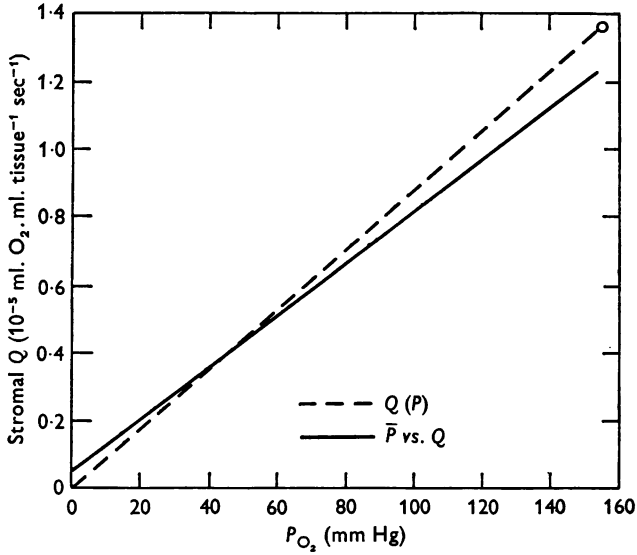


Fig. 5. Stromal oxygen consumption as a function of oxygen tension. Equations for $Q(P)$ and $\bar{P}$ are given in the text.

### *Histological analysis*

A great deal of care was given to tissue preparation. But it was of obvious importance to confirm that what was intended surgically was in fact achieved. To do this, samples of each type of tissue used were halved, and 5 $\mu$ sections were sliced at about 0·5 mm and 1·0 mm from the centres. Harris's haematoxylin was applied regressively followed by Eosin Y staining. Mallory's heidenhain was used for some stromal samples.

Examination of mounted sections showed that the tissues conformed to what was intended. Epithelial and/or endothelial removal was complete when desired or one or both layers were intact with the stroma.

The endothelium was given special attention because it is thought to exhibit weak adhesion and cohesion (Maurice, 1969). On the other hand, it has been reported that persistent rubbing of the posterior corneal surface with a blunt instrument fails to completely remove the endothelium (Harris, 1957). The structural question is complicated by the very rapid post-mortem development of cytoplasmic vacuoles in the endothelium which can only be prevented by special fixation techniques (Hodson, 1968). With regard to the present study, some endothelial alteration may

have occurred in the tissue samples used. But the histological findings suggest that the layer was adequately intact for the measurements.

TABLE 1. Oxygen consumption of individual corneal layers

| Layer* | Tissue sample† | Surface on electrode‡ | $P$ (mm Hg)§ | $Q_{O_2}$‖ |
|---|---|---|---|---|
| Stroma | Stroma | — | 131·1 ± 3·1 (9) | 1·13 ± 0·15 |
| Epithelium | Epithelium plus stroma (endothelium removed) | Epithelium | 81·3 ± 4·2 (8) | 10·5 ± 1·4 |
| | | Stroma | 120·7 ± 4·4 (4) | 11·7 ± 1·6 |
| | Whole cornea | Epithelium | 70·9 ± 6·4 (5) | 9·8 ± 1·9 |
| | | Endothelium | 97·5 ± 5·1 (3) | 15·3 ± 2·2 |
| Endothelium | Endothelium plus stroma (epithelium removed) | Endothelium | 100·2 ± 4·1 (6) | 55·6 ± 3·1 |
| | | Stroma | 126·3 ± 4·5 (3) | 69·1 ± 4·8 |
| | Whole cornea | Endothelium | 98·1 ± 4·2 (4) | 49·8 ± 3·8 |
| | | Epithelium | 77·5 ± 5·0 (3) | 59·3 ± 4·1 |

* The layer for which $Q_{O_2}$ is determined.

† Composition of the experimental tissue used.

‡ The tissue surface in contact with the electrode.

§ Measured oxygen tension at the electrode-tissue interface, S.D., and the number of samples.

‖ Oxygen consumption and S.D. in units of $10^{-5}$ m $1{\cdot}0_2$ ml. tissue$^{-1}$ sec$^{-1}$ at 23·5 ± 0·5° C.

### *Oxygen consumption values*

Results of individual layer oxygen consumptions are given in Table 1. Each column contains the following information. The first indicates the layer of interest. Composition of the tissue used in the experiments is given in the second. The third indicates which tissue surface is positioned down on the electrode. Measured oxygen tensions ($P_i$) are given in the fourth along with the S.D. and number of experiments. The last column contains computed $Q_{O_2}$ values with S.D.

As shown in Table 1, the bi-layered and whole cornea samples are each measured in two surface positions. This means that the oxygen consumptions of the limiting layers of the cornea (epithelium and endothelium) are determined in four separate types of measurement. Thus, there is an opportunity to check the internal consistency of the results. For the type of experiment, the agreement indicated in column 5 of Table 1 is quite good. This constitutes firm support for the validity of the methods used and the results obtained.

The mean $Q_{O_2}$ findings are given in column 1 of Table 2. For the epithelium and endothelium, selected mean values are used (most experiments, least variability). $Q_{O_2}$ for the whole cornea (Table 2, column 1, row 4) was determined from the sum of the component consumptions weighed by

the proportionate volume of each layer. For the model whole cornea of Fig. 2,

$$Q_T = \frac{(Q_1\lambda_1 + Q_2\lambda_2 + Q_3\lambda_3)\,A}{(\lambda_1 + \lambda_2 + \lambda_3)\,A}, \tag{19}$$

where $Q_T$ is total corneal oxygen consumption (ml. $O_2$ ml. tissue $^{-1}$ sec$^{-1}$),

TABLE 2. Principal oxygen utilization values

| | Tissue | | | |
|---|---|---|---|---|
| | Epithelium | Stroma | Endothelium | Whole cornea |
| $\frac{10^{-5}\text{ ml. } O_2}{\text{ml. fresh tissue} \times \text{sec}}$ (23° C) | 10·5 | 1·13 | 55·6 | 2·61 |
| $\frac{\mu\text{l. } O_2}{\text{mg dry wt} \times \text{hr}}$ (23° C) | 1·22 | 0·17 | 6·47 | 0·40 |
| $\frac{\mu\text{l. } O_2}{\text{mg. dry wt} \times \text{hr}}$ (37° C) | 3·09 | 0·44 | 16·4 | 1·01 |
| $\frac{10^{-7}\text{ ml. } O_2}{\text{cm}^2 \times \text{sec}}$ (23° C) | 4·19 | 4·04 | 2·22 | 10·4 |
| $\frac{\mu\text{l. } O_2}{\text{cm}^2 \times \text{hr}}$ (37° C) | 3·83 | 3·68 | 2·03 | 9·54 |

$A$ is area (cm$^2$), $\lambda$ is thickness (cm). The other data in Table 2 are conversions of column 1 to other units for comparison purposes. Values expressed at 37° C have been calculated using the finding that $Q_{10} \cong 2$ for the rabbit cornea (R. D. Freeman, in preparation). Dry tissue weights are determined from the ratio of water density to dry tissue density (Maurice, 1957) and the mean stromal and limiting layer hydration values found by Duane (1949) and Otori (1967) respectively. Dry tissue weight is obtained from fresh tissue volume by the relation

$$Q_d = \frac{HQ_f}{\rho_w} + \frac{Q_f}{\rho_t}, \tag{20}$$

where $Q_d$ is oxygen consumption of dry tissue ($\mu$l. $O_2$ mg dry wt.$^{-1}$ hr$^{-1}$), $Q_f$ is oxygen consumption of fresh tissue (ml. $O_2$ ml. tissue$^{-1}$ sec$^{-1}$), $H$ is hydration (g $H_2O$ g dry tissue$^{-1}$), $\rho_w$ is density of water (g cm$^{-3}$), $\rho_t$ is density of tissue (g dry tissue cm$^{-3}$).

Table 2 contains the main experimental findings. On the basis of volumes of oxygen per unit volume tissue (column 1), epithelial oxygen utilization is about ten times that of the stroma and 0·2 that of the endothelium. In

terms of volumes consumed per unit surface area (columns 4 and 5), endothelium, epithelium, and stroma consume 21, 40 and 39% respectively of the total corneal $Q_{O_2}$. The $O_2$ uptake across a unit area of stromal or epithelial tissue is nearly twice that for endothelium. Assuming the ratio of epithelial to endothelial cells is about 7:1, the per cell consumption of the endothelium is approximately three times that of the epithelium.

## DISCUSSION

### *Theoretical considerations*

The inherent difficulties in corneal $Q_{O_2}$ determinations, mentioned at the outset, are concerned primarily with interactions between component layers. For example, with selected layer removal, there is the possibility that damaged epithelial or endothelial cells might cause metabolic or other changes in the stroma. In one study, injury to the rat cornea appeared to activate fibrocytes in the stromal cells to take up the vital dye neutral red (Weimar, 1962). The reaction did not occur in normal stromal cells, nor was it observed with atraumatic removal of the epithelium. It was thought that the mechanism depended on actively functioning cell metabolism because the dye uptake was prevented by metabolic inhibitors. However, it took a few hours after injury for the stromal cells to become activated. Even after severe injury caused by removing the epithelial cells by crushing, it took around an hour for the effect to occur. Because of the very short measuring time required in the present experiments, it is rather unlikely that the $Q_{O_2}$ results have been influenced by this type of phenomenon.

But other metabolic interactions between corneal layers could affect $Q_{O_2}$ determinations. Removal of one or both limiting layers might alter the respiratory activity of the remaining layer of interest resulting in spurious $Q_{O_2}$ values. Evidence supporting this possibility was reported by Hermann & Hickman (1948) who found a high lactate level in the isolated stroma and concluded that the rate of glycolysis in that layer was influenced by the presence of the epithelium. They further suggested that the epithelium consumed the lactate produced by the stroma. However, in their experiments, the epithelium was removed from the stroma a few hours after enucleation and the lactate concentration would have significantly increased (Langham, 1954). Furthermore, their conclusions did not allow for the possible removal of lactate via the aqueous humour.

The present $Q_{O_2}$ results provide relevant information regarding corneal inter-layer metabolic reactions. If interactions do occur, the oxidative component is apparently not significantly affected since $Q_{O_2}$ values for denuded tissue are concordant with those for whole cornea.

*Implications of $Q_{O_2}$ values*

Table 3 summarizes the results of previous determinations of $Q_{O_2}$ on the rabbit cornea.

In spite of the variations in experimental conditions, most of the stromal and epithelial values are in fair agreement. The present result for endothelial $Q_{O_2}$ appears significantly higher than previous findings with the possible exception of Ridge's (1956). He did not report experimental temperature. If his study was done at room temperature, our values would agree (see Table 2, column 2). Also, the dry weights given by Matsumoto & Kudo (1960) are high, indicating that some stromal tissue may have been included in their samples which would cause artificially low endothelial $Q_{O_2}$ values.

TABLE 3. Previous determinations of the oxygen consumption of the rabbit cornea ($\mu$l. $O_2$ mg dry wt.$^{-1}$ hr$^{-1}$ at 37° C)

| Stroma | Epithelium | Endothelium | Whole cornea | Method |
|---|---|---|---|---|
| 0·8 | 4·8 | 6·0 | 1·6 | Warburg (Ringer)[a] |
| 0·15 | 7·0 | — | 0·46 | Warburg (Ringer)[b] |
| 0·23 | 6·2 | 5·7 | 0·86 | Warburg (dry)[c] |
| — | — | 4·8* | — | Semi-microrespirometer[d] |
| 0·7 | 3·2 | 6·8 | 1·1 | Polarography (Ringer)[g] |
| 0·2 | — | — | — | Polarography (dry)[f] |
| — | — | — | 1·1 | Polarography (perfusion)[g] |
| 0·44 | 3·1 | 16·4 | 1·01 | Polarography (buffer)[h] |

*a*, Kohra (1935); *b*, Robbie, *et al.* (1947); *c*, Langham (1952); *d*, Ridge (1956); *e*, Matsumoto & Kudo (1960); *f*, Takahashi *et al.* (1966); *g*, Riley (1969); *h*, present experiments.

* Probably at room temperature; not converted to 37° C.

Since only about 2·5% of the stroma is cellular (Otori, 1967), stromal keratocytes have a high metabolic activity. It has been suggested that this could be a factor in the mechanism of corneal deturgescence, but calculations indicate that the energy required to maintain normal hydration is only a fraction of that available in any corneal layer (Maurice, 1969).

As already noted, the present results indicate that endothelial per cell consumption is around three times that of the epithelium. Evidence relevant to this finding is somewhat conflicting. One of the dissimilarities found in the fine structure of these layers is that endothelial cells contain a substantially greater number of ovoid mitochondria than epithelial cells (Kaye & Pappas, 1962; Jakus, 1962). This indicates a high endothelial oxidative rate. On the other hand, many endothelial inner mitochondrial membrane invaginations, the cristae, are in parallel alignment to the mitochondrion axis (Iwamoto & Smelser, 1965). And it is thought that this

arrangement indicates a low oxidative capacity (Maurice & Riley, 1969). The question of limiting layer $Q_{O_2}$ difference is confounded by the fact that the epithelium replaces itself every few days. Therefore, at any given time, it must contain a number of inactive cells and a number of very active cells involved in synthetic functions.

For the whole cornea, Table 3 shows that the recent $Q_{O_2}$ findings are in rather close accord. One recent study is of particular interest because the experiments were done using whole cornea mounted in a chamber which enabled posterior perfusion with synthetic aqueous, thus simulating *in situ* conditions (Riley, 1969).

In addition to oxygen utilization, Riley determined usage of glucose, lactate and glycogen. He found that the total substrate used was 92·5 $\mu$g glucose (plus 6·7 $\mu$g endogenous glucose) plus 4·7 $\mu$g glycogen, or an equivalent total of 104 $\mu$g glucose $cm^{-2}\,hr^{-1}$. Of this amount, 91·2 $\mu$g was converted to lactate and therefore 13·2 $\mu$g had to be oxidized. This amount of glucose requires an oxygen uptake of 9·5 $\mu$l. $O_2$ $cm^{-2}\,hr^{-1}$, which is exactly that found in the present experiments (Table 2, column 5).

The above results show that 87 % of the glucose and glycogen is converted to lactate and 13 % is oxidized. This is the ratio of glycolytic to oxidative activity in the rabbit cornea.

## APPENDIX

### *Single layer tissue*

For very thin layers, diffusive flow can be considered unidirectional, i.e. perpendicular to the plane of the layer. In this case, the layer is a slab of oxygen consuming corneal tissue which for convenience may be taken to be a parallelepiped with area $A$, thickness $dx$, and volume $A\,dx$. By considering a mass balance, one obtains

$$-\frac{\partial Jx}{\partial x}-Q=\frac{\partial C}{\partial t}, \tag{1A}$$

where $J_x$ is the flux dissolved oxygen perpendicular to the surface (ml. $O_2$ $cm^{-2}\,sec^{-1}$), $C$ is the concentration of oxygen in the tissue (ml. $O_2$ ml. $tissue^{-1}$) and the other terms are defined in the text. Fick's first law and Henry's law state that

$$J = -D\frac{\partial C}{\partial x}, \tag{2A}$$

and

$$C = kP. \tag{3A}$$

Substituting eqns. (2A) and (3A) into (1A) and rearranging gives eqn. (1) of the text. Its solution, with the conditions stated, is obtained as follows.

The sink parameter, $Q/k$, is eliminated by using a transformation variable to obtain an equation of a form which has a solution available in a

standard reference. From Carslaw & Jaeger (1959) the general result, expressed in the present terms, for the oxygen sensor at $x = 0$ is

$$P(x = 0) = P_e - \frac{Q\lambda^2}{2Dk} + \frac{4Q\lambda^2}{2Dk\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left[\frac{(2n+1)^2 D\pi^2 t}{4\lambda^2}\right]. \quad (4A)$$

This equation shows that the oxygen tension at the electrode falls from $P_e$ at $t = 0$ to $P_e - (Q\lambda^2/2Dk)$ at infinite time. In the experiments reported here, equilibrium is reached in about 45 sec. Therefore, the steady-state oxygen tension difference between the air and electrode surface is $Q\lambda^2/2Dk$, which is expressed as eqns. (6) and (7) in the text.

*Multi-layered tissue*

Fig. 2 shows model 2 and 3 layered corneas for which either surface may be closed at the electrode. The analytical steps required for the whole cornea model are given in what follows. The descriptive steady-state equations are

$$\frac{d^2P_1}{dx^2} - \frac{Q_1}{D_1 k_1} = 0, \quad (5A)$$

$$\frac{d^2P_2}{dx^2} - \frac{Q_2}{D_2 k_2} = 0, \quad (6A)$$

$$\frac{d^2P_3}{dx^2} - \frac{Q_3}{D_3 k_3} = 0. \quad (7A)$$

The general solutions are

$$P_1(x) = Ax^2 + Bx + C, \quad (8A)$$

$$P_2(x) = Ex^2 + Fx + G, \quad (9A)$$

$$P_3(x) = Hx^2 + Ix + K. \quad (10A)$$

It is directly determined that

$$A = \frac{Q_1}{2D_1 k_1}, \quad E = \frac{Q_2}{2D_2 k_2} \quad \text{and} \quad H = \frac{Q_3}{2D_3 k_3}.$$

The boundary conditions of the experiment are

$$\left.\begin{aligned}
&x = 0, \quad \frac{dP_1}{dx} = 0, \\
&x = a, \quad P_1(a) = P_2(a), \\
&\left.\frac{dP_1}{dx}\right|_{x=a} = \alpha_1 \left.\frac{dP_2}{dx}\right|_{x=a}, \quad \text{where } \alpha_1 = \frac{D_2 k_2}{D_1 k_1}, \\
&x = b, \quad P_2(b) = P_3(b), \\
&\left.\frac{dP_2}{dx}\right|_{x=b} = \alpha_2 \left.\frac{dP_3}{dx}\right|_{x=b}, \quad \text{where } \alpha_2 = \frac{D_3 k_3}{D_2 k_2}, \\
&x = c, \quad P_3 = P_e.
\end{aligned}\right\} \quad (11A)$$

Also, $P_1(0) = C$ which is therefore the measured $P_1$. Substituting the boundary conditions into eqns. (8A) to (10A) gives a system of linear algebraic equations which may be arranged with unknowns on the left for either surface positioned down on the oxygen sensor. For endothelial $Q_{O_2}$ determination, when the epithelium is placed down on the electrode, the equations are

$$\left.\begin{aligned} Fa+G &= a^2(A-E)+C, \\ -Fb-G+Hb^2+Ib+K &= 2bE, \\ \alpha_1 F &= 2a(A-\alpha_1 E), \\ -F+2\alpha_2 Hb+\alpha_2 I &= 2Eb, \\ Hc^2+Ic+K &= P_e, \end{aligned}\right\} \tag{12A}$$

and for the endothelium positioned down, the result is

$$\left.\begin{aligned} -Aa^2+Fa+G &= C-Ea^2, \\ -Fb-G+Ib+K &= b^2(E-H), \\ -2Aa+\alpha_1 F &= -2\alpha_1 Ea, \\ -F+\alpha_2 I &= 2b(E-\alpha_2 H), \\ Ic+K &= P_e-Hc^2. \end{aligned}\right\} \tag{13A}$$

For epithelial $Q_{O_2}$ the equations are similar to the above and take into account the reverse direction. These equations are expressed in matrix notation for computer solution.

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