Water permeability of cat corneal endothelium in vitro

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A water permeability study of the isolated cat corneal endothelium was performed with the use of a newly designed Lucite chamber and modified experimental methods. The hydraulic conductivity of the cat endothelium was 9.3 ± 1.09 × 10⁻⁷ cm sec⁻¹ atm⁻¹. The Staverman reflection coefficient to NaCl of cat endothelium was 0.6. It was determined that the resistance to the passage of either water or small solutes is negligible in the swollen stroma. The results suggest that the intercellular space of the cat endothelium is less than that of the rabbit.

Key words: cat, endothelium, water permeability, sodium chloride permeability, intercellular space.

For a better understanding of the role played by the corneal endothelium in the maintenance of corneal hydration, it is necessary to determine the rate of movement of water and small ions through this membrane.

One endothelial role which is indisputable is a passive one in which it acts as a physical barrier. To describe fully the passive permeability of a biologic membrane, it is necessary to evaluate two principal coefficients: the hydraulic conductivity (Lp), and the reflection coefficient (σ) which provides a measure of the membrane permeability.¹ The purpose of this paper is to measure these parameters in the cat endothelium. Similar studies of water permeability of the limiting membranes of the rabbit cornea have been reported by other investigators.² ³

Materials and methods

Twenty-four adult cats were killed by injection of an intraperitoneal overdose of Lethane (sodium pentobarbital).

Preparation of endothelium. An eye was

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proposed and, after removal of the epithelium, a 9 mm. button was removed. Trephination was discontinued as soon as the anterior chamber was entered to avoid damage to the endothelium. With the use of this method, little or no wrinkling of the cornea occurred. The whole stromal tissue was left adherent to the endothelium and acted as a mechanical support for the membrane. The whole tissue was mounted in the chamber and bathed in Ringer's solution for a period of 30 minutes to equilibrate with the new bathing solution.

Chambers and bathing media. The experiments were done with a newly designed Lucite chamber with an exposed area of 0.27 cm.² and volumes of 0.8 cm.³ The new chamber has several unique attributes: it affords easy manipulation and facile adjustment of both component chambers by a series of metallic threaded rings to permit a very accurate apposition (Figs. 1, 2, A and B, l) and three fixation bars (Fig. 1, m). It gives optimal visualization of any air bubbles within the chamber and permits observation of the condition of the mounted membrane through the magnifying Lucite viewers (Figs. 1 and 2, B, f).

Capillary tubes (Figs. 1 and 3, i and g) fit snugly in the stopcock outlets of each chamber. The water movement in each capillary is measured on a plastic millimeter scale rule held close to the tube by metal clips; a 1 mm. movement of the meniscus is equal to a volume change of 0.28 ml of fluid. To prevent leakage through capillaries and around the cornea, thick grease was painted around the apertures of each chamber and the base of the capillary tubes. To eliminate the influence of hydrostatic pressure, the chamber was positioned in a clamp so that both capillary tubes were in a horizontal plane. The initial bathing solutions were introduced slowly into each chamber, before inserting the capillary tubes into stopcocks, to eliminate air bubbles within the chambers. A small amount of liquid detergent was added to each capillary tube to reduce capillary effects to a minimum.

The solutions on both sides of the tissue were stirred at 400 r.p.m. with Teflon-coated magnetic stirrers (Figs. 1 and 2, A, e), which were driven by a horseshoe magnet to reduce the rate-limiting effect of an unstirred layer on water movement.² ⁵

To change the solution bathing one side of the tissue, a 20 ml. syringe containing the new solution was connected to a two-way tap, and the capillary tube connected to the opposite chamber was closed by its stopcock. The replacing solution (about 20 to 30 ml.) was introduced slowly into the chamber while stirring was continued. The normal (Krebs-bicarbonate) Ringer's, Na-free Ringer's, and Ringer's solutions with various concentrations of sucrose were used as bathing media.² No bathing medium was used for more than five days after preparation. All solutions were at pH 7.3 and a sufficient volume of solution for each experiment was brought to the required temperature (25° C.) immediately prior to the experiment.

Hydraulic conductivity (Lp). Net water flow was measured with normal Ringer's solution on each side of the tissue for one hour after the 30 minute equilibration period. Sucrose Ringer's solution at different concentrations was substituted on either side of the tissue, and the water flows resulting from different osmotic gradients were measured at 15 minute intervals following a 15 minute equilibration period after any solution change. Four or five solutions were used on each preparation with the order of presentation randomized; the total elapsed time for each experiment was five or six hours.

Reflection coefficient (α). The reflection coefficient measures the effective osmotic pressure exerted by a permeable solute (here NaCl) across a membrane relative to that of an impermeant solute (here sucrose); thus, when the water flow is zero the solutions are osmotically equivalent.

Net nonosmotic water flow determinations were made with normal Ringer's solution on each side of the tissue for one hour following a 30 minute equilibration. Water flow was then measured with normal Ringer's solution on the posterior side of the membrane and a solution...
of sucrose (as an impermeant solute) in Na-free Ringer’s solution of the anterior side, to reduce the influence on water movement of possible active ion transport from the anterior (stromal) to posterior (aqueous) surface of the endothelium.

In the results, the values are given as the mean ± S.E.M. and the numbers of experiments are given in parentheses.

Results

Stroma. Stromal tissue showed no net water flow when bathed with Ringer’s or

Fig. 2. Photographs of the Lucite chamber. Letter labels correspond to those in Fig. 1.
isotonic NaCl solution on each side, or even when one side of the swollen stroma was bathed with hypertonic solution. Thus, isolated stroma allowed almost free passage of small solutes, and results obtained in the presence of stroma plus endothelial membrane were pertinent to the presence of the membrane alone.

**Endothelium.**

*Net water flow.* Little or no net water movement was observed when the tissue was bathed on each side with normal Ringer's solution. The mean value of detectable water movement was $3.5 \pm 2.3 \times 10^{-4} \text{ cm. sec}^{-1}$. The direction of net water movement was posteriorly (stromal to aqueous surface) in most cases. In the determination of $L_p$, a nonosmotic net water flow of less than 1 mm (0.28 μl) in 15 minutes was taken as zero. Some experiments were made with normal Ringer's solution on each side of an endothelium, but with a change in the solution every hour for five hours; no net water flow greater than normal was found with the passage of time.

**Hydraulic conductivity.** To obtain $L_p$, the osmotic water flow was measured with at least four or five different concentrations of sucrose Ringer's solution (Fig. 4) over a period of five to six hours; the linearity of the slope in Fig. 4 illustrates that large and small osmotic pressure differences produce related water flows, and, therefore, that breakdown of the membrane did not occur with large-pressure differences. The relationship between the net water flow and net osmotic driving force can be expressed as:

$$ J_v = (J_v)_o + L_pRT (C_1 - C_2) $$

Thus, $L_p$ can be determined, where $J_v$ = net volume flow (cm. sec$^{-1}$); $(J_v)_o$ = water flow with no osmotic driving force; $L_p$ = hydraulic conductivity (cm. sec$^{-1}$ atm$^{-1}$); $C_1$ = concentration of sucrose in the internal bathing solution; $C_2$ = concentration of sucrose in the external bathing solution; and $R$ and $T$ have their usual meaning. The $L_p$ of the cat endothelium as calculated from the above equation is $9.3 \pm 1.09 \times 10^{-7} \text{ cm. sec}^{-1}$ atm$^{-1}$. 

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*Fig. 3.* The Lucite chamber in operating position. Letter labels correspond to those in Fig. 1. In addition: (o) horseshoe magnet connected to shielded motor; (p) versatile clamp and stand.
Staverman reflection coefficient. From the data, a graph of positive or negative water flow, depending upon the direction of flow, versus sucrose concentration was plotted. The points were joined, and from the point where the line intersected water flow equal to zero a vertical intercept was drawn to sucrose concentration. This value is the effective osmotic pressure exerted by NaCl across the membrane. Using the relationship: 
\[ \sigma \text{NaCl} = \frac{c_{\text{imp}}}{c_{\text{NaCl}}} \]
(\text{where } c_{\text{imp}} = \text{concentration of the impermeable solute and } c_{\text{NaCl}} = \text{concentration of NaCl}), and assuming sucrose to be impermeable, the reflection coefficient to NaCl (\( \sigma \)) of the endothelium can be measured. The reflection coefficient of cat endothelium (\( \sigma \)) to NaCl as experimentally determined is 0.6 ± 0.02 (12). If the cat endothelium is permeable to sucrose, it is probably of the same order of magnitude as found in the rabbit (2 x 10^{-2} cm. per hour)\(^5, 6\); this rate is sufficiently low to allow assumption of impermeability. The values of \( L_p \), however, would be slightly underestimated and the values of \( \sigma \text{NaCl} \) would be overestimated with such a small permeability.

Discussion

The function of the limiting membranes as barriers may be defined by the permeability to various solutes and water. Cogan and Kinsey\(^6, 7\) demonstrated that net water flow occurred under osmotic and hydrostatic pressure gradients across the excised cat cornea. Recently, quantitative studies on the permeability of the corneal epithelium and endothelium to nonelectrolytes\(^8, 9\) and water\(^7, 3\) have been performed. These investigators\(^2, 8\) expressed the characteristics of the membrane as a physical barrier in terms of irreversible thermodynamics.\(^10, 31\) No investigators have previously measured \( L_p \) and \( \sigma \text{NaCl} \) of the cat corneal endothelium in vitro.

The technique employed to measure water movement across the endothelium has been shown to be valid, as only small changes were found in \( L_p \) as the osmotic gradient varied from 75 to 250 mOsm. No relationship was found between \( L_p \) and the elapsed time or the gradient employed (Table I). The random application of solutions and reproducibility of \( L_p \) at different osmotic gradients assured that the characteristics of the endothelium remained

Table I. Some data on \( L_p \) of cat endothelium at the two extremes in solution presentation to the membrane. There is no obvious relationship between \( L_p \) and either time (total elapsed time for each endothelium was six hours) or osmotic gradient (irrespective of the order of presentation).

<table>
<thead>
<tr>
<th>( \Delta C_\text{c} ) (mOsm.)</th>
<th>( L_p \times 10^{-3} \text{ cm. sec.}^{-1} \text{ atm.}^{-1} )</th>
<th>( \Delta C_\text{c} ) (mOsm.)</th>
<th>( L_p \times 10^{-3} \text{ cm. sec.}^{-1} \text{ atm.}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>0.92</td>
<td>0.82</td>
<td>250</td>
</tr>
<tr>
<td>100</td>
<td>0.89</td>
<td>0.74</td>
<td>200</td>
</tr>
<tr>
<td>150</td>
<td>0.87</td>
<td>0.99</td>
<td>150</td>
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<tr>
<td>200</td>
<td>0.81</td>
<td>1.20</td>
<td>100</td>
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<tr>
<td>250</td>
<td>0.80</td>
<td>0.91</td>
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unchanged during the experimental time period used here.

A comparison of the results for the water permeability of rabbit versus cat corneal endothelium, shows the rabbit to be more permeable to water by about 25 per cent. Cat Lp is about one half that of the gall bladder. The value of $\sigma$ NaCl in cat endothelium (0.6) revealed a lower permeability to NaCl than that of rabbit (0.4) when measured with a similar technique. An average $\sigma$ NaCl value of 0.6 was found for the rabbit endothelium measured on enucleated eyes with the use of a different technique; this value was calculated from the measured Lp and not measured directly, thus a range of $\sigma$ NaCl values was found by this method. Direct measurements of $\sigma$ NaCl with the use of a technique similar to that used here, gave $\sigma$ NaCl for the rabbit endothelium as 0.4, a value at the lower end of the range found by the other methods. The direct measurement of $\sigma$ NaCl has the obvious advantage of being independent of Lp data, and thus, produces a more accurate determination of this parameter. For the discussion here, therefore, the values from direct determination will be employed. In both species, the correlation between Lp and $\sigma$ is the same, cat having lower Lp and greater $\sigma$ NaCl, while rabbit has a higher Lp and lower $\sigma$ NaCl, showing that the cat endothelium is less permeable to both NaCl and water. It is possible that the difference between the values of Lp and $\sigma$ NaCl in cat and rabbit are due to differences in the endothelial intercellular spaces, with the cat having either longer or narrower channels.

REFERENCES