Endothelial permeability of the living cornea to fluorescein*

Yoichi Ota, Saiichi Mishima, and David M. Maurice**

A new method was developed for the analysis of fluorescein exchange between the aqueous humor and cornea which enables calculation of the permeability coefficient of the corneal endothelium and the steady-state aqueous-cornea distribution ratio from one experiment. After intravenous fluorescein injection, aqueous and corneal fluorescence was measured at intervals, using an objective fluorophotometer. Application of the method to normal human and rabbit gave the following average values: the permeability coefficient, $3.0 \pm 0.50 \times 10^{-6}$ cm. sec.$^{-1}$ in the human and $5.1 \pm 0.8 \times 10^{-6}$ cm. sec.$^{-1}$ in the rabbit, and the distribution ratio, $0.64 \pm 0.10$ in the human and $0.61 \pm 0.12$ in the rabbit.

Key words: fluorescein, permeability, cornea, endothelium.

Maintenance of normal corneal hydration depends largely on the integrity of the barrier property and on the active pump mechanism of the corneal endothelium. The former aspect of the endothelial function has been studied through determinations of the endothelial permeabilities to various substances. A slight disturbance of the cell layer can result in a rise in the permeability. Thus, clinical determination of this quantity might give valuable information as to endothelial pathology.

Development of a sensitive fluorophotometer, permitting separate fluorescence determinations in the cornea and the aqueous humor, opened the possibility of evaluating the endothelial permeability to fluorescein in the living cornea. Previously, the cornea was stained with fluorescein, and subsequent changes of the corneal and aqueous fluorescence were determined. A mathematical analysis of the cornea-aqueous fluorescein transfer yielded the endothelial permeability values. In this technique, however, the aqueous-cornea steady-state distribution ratio of the dye required for the calculation in an individual had to be obtained by a separate experiment. The method may be simplified for studies in normal cornea by the use of an average value for the ratio, but individual determinations are still desirable in

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pathologic cases where the ratio is likely to show large variations. The requirement for a separate experiment to determine the ratio complicated the previous method, preventing its extensive clinical application to pathologic cases.

In the present investigation, an analysis of the aqueous-cornea exchange of intravenously injected fluorescein led to the development of a simple method whereby both the endothelial permeability and the distribution ratio can be calculated for an individual cornea by one experiment. The method applied to the normal human and rabbit cornea yielded endothelial permeability values that are in good agreement with those reported previously. Part of the present results were the subject of a preliminary report elsewhere.7

Materials and methods

The human experiments were carried out in nine young subjects without ocular disorders excepting slight refractive error. Eight milliliters of 10 per cent fluorescein-sodium solution were injected into the cubital vein, and the fluorescence in the aqueous humor and in the stroma at the center of the cornea was determined at intervals. Adult albino rabbits weighing 3 to 5 kilograms were anesthetized intravenously followed by intraperitoneal injection of pentobarbital sodium. One milliliter of 10 per cent fluorescein solution was injected into the auricular vein, and the fluorescence in the aqueous humor and in the cornea was determined at intervals. These determinations were carried out using a fluorophotometer constructed (Hamamatsu TV Co., Hamamatsu, Japan) according to the design of Maurice.5 The instrument enabled fluorescein concentration to be measured over the range 10^-9 to 10^-5 Gm. ml^-1. Immediately before and after determinations, the meter reading of the photometer was calibrated with known fluorescein concentration and the tissue concentrations expressed as Gm. ml^-1. The corneal autofluorescence was determined at the beginning of each experiment, corresponding to 1 to 2 x 10^-8 Gm. ml^-1 of fluorescein, and used for correction of the corneal concentrations. The tissue concentration on each occasion was measured three times and averaged.

Methods of analysis. Fluorescein exchange between the aqueous humor and the cornea may be expressed by:

\[
\frac{dF_c}{dt} = \frac{A}{V_c} K_{ac} (F_o - r_{st} F_c)
\]

where the symbols have the following meaning: \(F_c\): fluorescein concentration in the corneal stroma (Gm. ml^-1); \(F_o\): fluorescein concentration in the aqueous humor (Gm. ml^-1); \(K_{ac}\): permeability coefficient of endothelium from aqueous to cornea (cm. sec^-1); \(A\): area of the corneal endothelium (cm^2); \(V_c\): volume of the cornea in which fluorescein is distributed (cm^3); and \(r_{st}\): the steady-state distribution ratio; the value of \(F_o/F_c\) when fluorescein is in equilibrium between the tissues.

Introducing the transfer coefficient \(k_{ac}\) for \(A K_{ac}/V_c\), one obtains

\[
\frac{dF_c}{dt} = k_{ac} (F_o - r_{st} F_c)
\]

Assuming that \(k_{ac}\) and \(r_{st}\) remain constant, one can integrate the above equation from time \(t_1\) to \(t_2\) to give the following expression:

\[
\int_{t_1}^{t_2} \frac{dF_c}{F_c - (F_o)_{t_2}} = r_{st} + \frac{1}{k_{ac}} \int_{t_1}^{t_2} \frac{dF_c}{F_c - (F_o)_{t_2}}
\]

A graphic integration may be carried out for the time course of changing fluorescein concentrations in the aqueous humor and the cornea, allowing calculation of \(r_{st}\) and \(k_{ac}\). The starting time of the integration, \(t_1\), can be chosen arbitrarily to suit the experimental conditions.
Implicit in Equation 1 is the assumption that the fluorescence of fluorescein is unquenched when it enters the stroma from the aqueous humor. This appears to be true to within a few per cent, at least in the rabbit. To a first approximation, therefore, $\frac{A}{V_c}$ is equal to $1/q$, where $q$ is the thickness of the stroma. The permeability coefficient $K_{ac}$ can be obtained, then, from the value of $k_{ac}$ and the stromal thickness, i.e.,

$$K_{ac} = k_{ac} \cdot q.$$  

**Results**

Fluorescein concentration changes in the aqueous humor and the cornea are illustrated in Fig. 1 for a rabbit and in Fig. 2 for a human. The aqueous concentration reached a maximum about one hour after intravenous injection, the peak time being slightly shorter in the rabbit than in the human; and subsequently the concentrations decreased rapidly. In the initial half-hour period, the aqueous concentrations varied according to the site of measurement, giving large variations in the fluorometer readings. After one hour, however, the aqueous concentration became homogeneous, and consistent readings could be obtained. The corneal concentrations increased gradually and exceeded the aqueous concentration after two to three hours in rabbits and three to four hours in humans, the maximum concentration being reached after four to five hours in both species.

Graphic integration of the data was carried out according to Equation 3 over half-hour intervals, and the results are plotted in Fig. 3 for the rabbit and in Fig. 4 for the human. The starting time of integration $t_1$ in Equation 3 was chosen as one hour, since a homogeneous aqueous concentration was achieved at about this time. A line could be fitted to the points by eye, and $r_{ac}$ was obtained from the intercept on the abscissa and $k_{ac}$ from the slope of the line. When the integration started from zero time, the values corresponding to the first hour did not fall on the straight line, indicating that there was unequal distribution of dye within the aqueous humor and stroma during this initial period. A line could, however, be fitted to the points from two to six hours, and it gave $r_{ac}$ and $k_{ac}$ values not significantly different from those calculated by integration over the one- to six-hour period.

The values of $r_{ac}$ and $k_{ac}$ were calculated for 20 eyes of 11 rabbits (Table I) and 18 eyes of nine normal subjects (Table II). A statistical analysis indicated that the

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>OD $k_{(hr.-')}$</th>
<th>$r$</th>
<th>OS $k_{(hr.-')}$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.54</td>
<td>0.60</td>
<td>0.49</td>
<td>0.62</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td>0.80</td>
<td>0.50</td>
<td>0.82</td>
</tr>
<tr>
<td>3</td>
<td>0.53</td>
<td>0.67</td>
<td>0.57</td>
<td>0.72</td>
</tr>
<tr>
<td>4</td>
<td>0.62</td>
<td>0.62</td>
<td>0.57</td>
<td>0.64</td>
</tr>
<tr>
<td>5</td>
<td>0.50</td>
<td>0.70</td>
<td>0.46</td>
<td>0.56</td>
</tr>
<tr>
<td>6</td>
<td>0.64</td>
<td>0.50</td>
<td>0.70</td>
<td>0.50</td>
</tr>
<tr>
<td>7</td>
<td>0.38</td>
<td>0.70</td>
<td>0.40</td>
<td>0.60</td>
</tr>
<tr>
<td>8</td>
<td>0.53</td>
<td>0.62</td>
<td>0.62</td>
<td>0.82</td>
</tr>
<tr>
<td>9</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.34</td>
</tr>
<tr>
<td>10</td>
<td>0.48</td>
<td>0.64</td>
<td>0.44</td>
<td>0.68</td>
</tr>
<tr>
<td>Mean</td>
<td>0.51</td>
<td>0.63</td>
<td>0.52</td>
<td>0.60</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.081</td>
<td>0.110</td>
<td>0.088</td>
<td>0.122</td>
</tr>
</tbody>
</table>
interindividual difference is significant (P < 0.01), but the difference between both eyes of an individual is not significant. Permeability coefficients, $K_{ac}$, were calculated using representative values for the normal stromal thickness, i.e., 0.36 mm. in rabbits and 0.47 mm. in humans. The average values for these coefficients were $5.1 \times 10^{-6}$ cm. sec. $^{-1}$ and $3.0 \times 10^{-6}$ cm. sec. $^{-1}$, respectively.

**Discussion**

The value for the transfer coefficient $k_{ac}$ in man can be calculated by an independent method from the results of Jones and Maurice. Using the nomenclature of that paper, a mean value of $0.28 \pm 0.15$ (S.E.M.) hr. $^{-1}$ can be obtained. This may be compared with the value $0.23 \pm 0.01$ (S.E.M.) hr. $^{-1}$ obtained in the present work. Jones and Maurice found values for $r_{ac}$ of 0.75 to 0.9 in four subjects by a separate method. This compares with the present figure of 0.64 (range, 0.44 to 0.84). In the rabbit, values for $k_{ac}$: $0.67 \pm 0.10$ hr. $^{-1}$ (S.D., five rabbits) and $r_{ac}$: $0.38 \pm 0.07$ (S.D.) have previously been obtained by independent methods. These may be compared with the present values 0.51 hr. $^{-1}$ and 0.60. Similar values for $k_{ac}$ in both human and rabbit can be derived from the results of Waltman and Kaufman. The earlier methods are more direct and, therefore, probably more accurate, at least as far as $k_{ac}$ is concerned. The present values are in fair agreement, and the method has the advantage that both $k_{ac}$ and $r_{ac}$ may be derived from the same experiment. Because of convenience
of and the reduced risk of an anaphylactic reaction to the fluorescein, it may, therefore, be more applicable to clinical studies.

The distribution ratio was less than unity, i.e., the corneal concentration is higher than the aqueous concentration at a steady-state. In a previous investigation, this distribution ratio was studied using bovine stroma. The bovine stroma enclosed in dialysis membrane was immersed in physiologic saline solution with various fluorescein concentrations until diffusional equilibrium of fluorescein was reached. In order to prevent stromal swelling, dextran was added to the solution. After measurements of fluorescein binding to dextran, the stroma-saline distribution ratio of fluorescein was calculated to be about 0.5 at normal hydration, a value close to the present distribution ratio. It is, therefore, possible that the distribution ratio reflects fluorescein binding to stromal macromolecules. The results of the present analysis showed that the ratio remains practically constant within the concentration range used in the in vivo experiments. This is in agreement with experiments in man and rabbit, where the ratio of the fluorescein concentrations in the cornea and aqueous humor remains virtually constant as the dye concentration diminishes a hundredfold with time.

REFERENCES