Time Course of Changes in Aqueous Protein Concentration and Flow Rate After Oral Acetazolamide

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The coefficient of plasma protein entry into the aqueous humor, \( k_{in} \), was calculated in the human eyes from the aqueous protein concentration measured with a flare-cell meter and from the aqueous flow rate determined with fluorophotometry. The value of \( k_{in} \) averaged \( 3.47 \pm 0.25 \times 10^{-5} \) min\(^{-1}\) (mean \( \pm \) SEM) in 12 eyes of six normal young volunteers. The time course of changes in aqueous protein concentration after oral administration of 500 mg acetazolamide was measured with a flare-cell meter in 24 eyes of 12 subjects. Aqueous protein concentration significantly increased from 2-10 hr postadministration with a maximum increase of 41 \( \pm \) 7% (mean \( \pm \) SEM) at 6 hr postadministration. Assuming that \( k_{in} \) is not affected by the drug treatment, we calculated the time change of aqueous flow rate from that of aqueous protein concentration using the value of \( k_{in} \) above. The calculated flow rate after the administration of acetazolamide decreased between 1.25 and 8 hr, with a maximum reduction of 40 \( \pm \) 11% at 1.75 hr postadministration. These measurements obtained with the flare-cell meter corresponded well to those obtained by fluorophotometry in a separate group of volunteers given the same treatment. It was shown that oral acetazolamide increases aqueous protein concentration, and that the time change of its effect on aqueous flow rate can be monitored by measuring aqueous protein concentration. Invest Ophthalmol Vis Sci 31:527-534, 1990

Acetazolamide, a carbonic anhydrase inhibitor, is widely recognized as one of the most effective agents in reducing intraocular pressure (IOP), and has an established role in the management of glaucoma. Anjou and Dyster-Aas reported that optimal medication with this drug increased aqueous protein concentration by 27% in human eyes.\(^1\) They postulated that this increase could be attributed to two factors, namely, reduction in aqueous humor formation and a probable decrease in protein entry into the aqueous humor. The latter postulate was supported by the finding that application of acetazolamide led to vaso-constriction of the iris arteries in enucleated cat eyes.\(^2\) Later, however, an in vivo experiment in rabbit eyes demonstrated no marked effect of acetazolamide on blood flow in the anterior uvea.\(^3\) Therefore, the exact mechanisms underlying changes in aqueous protein concentration after systemic administration of acetazolamide remain to be clarified.

Since Anjou and Dyster-Aas’s pioneering work, there has been no report concerning the effect of acetazolamide on aqueous protein concentration, mostly because of the absence of a method to determine aqueous flare intensity (or aqueous protein concentration) in living eyes with reliable accuracy and sensitivity. Recently, a new instrument, the laser flare-cell meter, was developed to quantify aqueous flare intensity in vivo,\(^4\) and its sensitivity and research potential have been confirmed in several previous investigations.\(^5\) In contrast to earlier methods developed to quantify aqueous flare intensity,\(^10\)-\(^14\) measurements with this instrument can be performed with higher sensitivity and reproducibility, allowing the detection of subtle changes in aqueous flare intensity and continuous monitoring of its time course.\(^5\)

The current study was conducted to investigate the time course of changes in aqueous protein concentration after oral acetazolamide, and to determine whether the time course of the drug’s effects on aqueous flow rate can be monitored by measuring aqueous protein concentration, without applying any exogenous tracers such as fluorescein.
Materials and Methods

Subjects

A total of 24 young volunteers (21–26 yr old), who had neither systemic nor ocular disease and had only mild refractive errors, participated in the study. All studies were approved by the Ethics Committee of Tokyo University, Tokyo, Japan, and written consent was obtained from all subjects before their participation.

Instrumentation

Aqueous protein concentration was measured with a laser flare-cell meter (FC-1000®; Kowa, Tokyo, Japan), the apparatus and technique of which have been described previously.4'5 Readings with the flare-cell meter were standardized with a series of albumin solutions diluted from 0 to 50 mg/dl. The solutions were prepared with human serum albumin (A-3782; Sigma, St. Louis, MO) and commercially available artificial aqueous humor (OPE-Guard MA®; Senju Pharmaceutical, Osaka, Japan), which were contained in an artificial eye and measured with the flare-cell meter. The artificial eye was constructed with a plastic contact lens with a thickness of 0.5 mm and radius of curvature of 7.8 mm, and a black plastic sphere. The depth of the anterior chamber was approximately 4 mm.

Fluorophotometric measurements were carried out with a Topcon slit-lamp fluorophotometer (Tokyo Optical, Tokyo, Japan).

Determination of the Coefficient of Protein Entry into the Aqueous Humor (k_in)

The kinetics of protein molecule transfer from the plasma into the aqueous humor can be expressed by the following equation:15

\[ \frac{dC_a}{dt} = k_{in}(C_p - C_a) - k_{out}C_a \]  

where \( C_a \) represents aqueous protein concentration, \( C_p \) plasma protein concentration, \( k_{in} \) the coefficient of protein entry into the aqueous humor, and \( k_{out} \) the coefficient of protein loss from the aqueous humor. Since aqueous proteins are considered to leave the eye from the iridocorneal angle by bulk flow of aqueous humor,16'17 and since under normal conditions \( C_a \) is negligible in comparison to \( C_p \), the equation above can be rewritten as:

\[ k_{in} = \frac{f}{V_a} \frac{C_a}{C_p} + \frac{1}{C_p} \frac{dC_a}{dt} \]  

where \( V_a \) is anterior chamber volume, and \( f \) is aqueous flow rate, which is \( k_{out}V_a \). In six normal young adults (21–25 yr old), \( k_{in} \) was calculated from Equation (2), with \( V_a, f, C_p, \) and \( C_a \) determined as follows.

First, measurement of aqueous flow rate (f) was carried out by fluorophotometry according to method II of Jones and Maurice.18 Fourteen hours prior to the measurements, 30 µl of 10% fluorescein solution (Fluorescite®, Alcon, Fort Worth, TX) was instilled into both eyes five times at intervals of 3 min after topical anesthesia. Fluorescein concentrations in the cornea (\( F_c \)) and anterior chamber (\( F_a \)) were measured from 9:00-12:00 (time of day) at 1-hr intervals. Fluorophotometric readings were plotted on semi-log paper as a function of time. The rate of loss of fluorescein from the cornea (\( A_c \)) and the anterior chamber (\( A_a \)) were calculated from the line of best fit by the least squares method. Anterior chamber volume (\( V_a \)) was measured with the photogrammetric method of Johnson et al19 at 11:00. Average aqueous flow rate between 9:00 and 12:00 is given by

\[ f = 0.9 \left( \frac{F_c}{F_a} V_a A_c + A_v V_a \right) \]  

where \( V_c \) is the volume of the corneal stroma, which was assumed to be 70 \( \mu \)l;20 \( [F_c/F_a] \) is the mean value of \( F_c/F_a \) during the experiment; and 0.9 is the correction factor for diffusional loss of fluorescein across the iris surface.18'20'21

One week after the fluorophotometric experiment, aqueous protein concentrations (\( C_a \)) were determined with a flare-cell meter. Measurements were carried out from 9:00 through 12:00 at 1-hr intervals in the same six subjects. The flare intensity obtained was converted into albumin equivalent protein concentration by using the standard curve as described above. Values for aqueous protein concentration obtained at four measurement points were averaged for each eye, and adopted as \( C_a \) in Equation (2). The term \( dC_a/dt \) was calculated from a slope of the line fit for the 9:00, 10:00, 11:00, and 12:00 measurements by the least squares method. Venous blood samples were collected at 11 hr, and plasma protein concentrations (\( C_p \)) were determined by the biuret test.

Flare-Cell Meter Measurements After Oral Acetazolamide

Measurements were performed in 12 normal young male volunteers (22–26 yr old). Control measurements of aqueous flare intensity and IOP were taken over one 24-hr period at intervals ranging from 30 min to 4 hr, from 9:00 through 9:00 of the next day. One week later, the same measurements were carried out on the same time schedule, with oral administration of 500 mg acetazolamide given at 11 hr.
Obtained flare intensity was converted into albumin equivalent protein concentration using the standard curve described above.

The IOP was measured with an applanation tonometer (Haag-Streit, Switzerland) after flare measurements. It has been confirmed that topically applied fluorescein used in this procedure has no influence on flare-cell meter measurements.5 Of the 12 subjects, 6 agreed to undergo further tests. In these 6 subjects, venous blood samples were collected to determine plasma protein and albumin concentration at 9:00, 14:00, and 21:00 on the acetazolamide day. In these subjects, anterior chamber volume also was measured, photogrammetrically19 at 1500 hr both on the control and the acetazolamide days.

Fluorophotometric Determination of Aqueous Flow Rate After Oral Acetazolamide

The effect of oral acetazolamide on aqueous flow rate was studied by using a modified method II of Jones and Maurice.18,22 In six volunteers (22-24 yr old), measurements were performed on two different occasions separated by a 1-week interval, ie, on control day and on acetazolamide day. Fluorescein was applied in the same manner as in the first experiment mentioned above, and $F_c$ and $F_a$ were measured from 9:00-15:00 at intervals of 1 hr. On the acetazolamide day, 500 mg acetazolamide was administered orally at 11:00 hr; measurements were otherwise performed in the same manner as on the control day. Aqueous flow rate ($f$) was calculated for each hour according to the method described by Gaul and Brubaker:22

$$f = 0.9 \frac{V_a dF_c/dt + V_a dF_a/dt}{F_a}$$

(4)

$V_a$ is anterior chamber volume, which was measured photogrammetrically.19 $F_a$ is the mean concentration of tracer in the anterior chamber during 1 hr, which was determined by averaging the concentration at the beginning and the end of the hour. The terms $dF_c/dt$ and $dF_a/dt$ were approximated with difference equations, using $F_c$ and $F_a$ at the beginning and the end of the hour. The midpoint time of each period was used to designate the time of the hour.

Results

Standardization of Flare-Cell Meter Measurements

Measurement results of the diluted human serum albumin solutions from 0 to 50 mg/dl were plotted and are shown in Figure 1. Photon measurements (count/msec) with a flare-cell meter showed good linear correlation with albumin concentrations ($r = 0.99-1.00, P = 0.00$), yielding the converting equation $Y = 0.97 + 0.170X$; $Y$ denotes photon count and $X$ denotes albumin concentration. Measurement results obtained with the flare-cell meter (photon count/msec) were then converted into albumin equivalent protein concentrations (mg/dl).

Determination of $k_\text{in}$

The results are summarized in Table 1. The $k_\text{in}$ averaged $3.47 \pm 0.25 \times 10^{-5}$ min$^{-1}$ (mean $\pm$ SEM) in 12 eyes of six subjects.

Flare-Cell Meter Measurements After Oral Acetazolamide

Flare values on the control day displayed a diurnal variation (Fig. 2), ie, were high in the morning and low in the evening, which was in good agreement with the curve we reported previously.5 On the acetazolamide day, flare values before drug administration showed no significant difference from those on the control day. After the oral administration of acetazolamide at 11:00, flare values were significantly higher than those on the control day from 2 to 10 hr postadministration ($P < 0.01$, paired t-test). The maximum increase was $41 \pm 7\%$ (mean $\pm$ SEM, $n = 24$) of the values obtained on the control day at 17:00, 6 hr postadministration (Table 2).

IOP was reduced by acetazolamide from 2 to 6 hr postadministration with a maximum decrease of 1.73 $\pm$ 0.15 mmHg at 4 hr postadministration (Fig. 3).

![Fig. 1. Correlation between photon count (photon count/msec) and human serum albumin concentration (mg/dl). Vertical bars represent standard errors.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933577/)
Table 1. Determination of the coefficient of protein entry into the aqueous humor (k_in)

<table>
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<tr>
<th>Subject no.</th>
<th>Eye</th>
<th>V_a (μl)</th>
<th>C_p (10^4 mg/dl)</th>
<th>C_a (mg/dl)</th>
<th>dC_a/dt (10^{-2} mg/dl min)</th>
<th>f (μl/min)</th>
<th>k_in (10^{-5}/min)</th>
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<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>255</td>
<td>7.3</td>
<td>21.7</td>
<td>-4.90</td>
<td>4.27</td>
<td>4.29</td>
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<tr>
<td>2</td>
<td>L</td>
<td>255</td>
<td>16.9</td>
<td>0.33</td>
<td>2.62</td>
<td>2.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22.2</td>
<td>-2.94</td>
<td>2.86</td>
<td>2.52</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>238</td>
<td>26.6</td>
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<td>-3.60</td>
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<td>2.39</td>
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<td>0.33</td>
<td>2.76</td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>R</td>
<td>230</td>
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<td>-3.92</td>
<td>2.20</td>
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<td></td>
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<tr>
<td></td>
<td>L</td>
<td>218</td>
<td>18.1</td>
<td>-1.31</td>
<td>2.32</td>
<td>3.04</td>
<td></td>
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<tr>
<td>5</td>
<td>R</td>
<td>227</td>
<td>26.7</td>
<td>22.8</td>
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<td>3.97</td>
<td></td>
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<tr>
<td></td>
<td>L</td>
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<td>23.4</td>
<td>-4.25</td>
<td>3.46</td>
<td>4.01</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>R</td>
<td>195</td>
<td>21.9</td>
<td>1.31</td>
<td>3.24</td>
<td>4.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>194</td>
<td>16.7</td>
<td>0.00</td>
<td>2.65</td>
<td>2.96</td>
<td></td>
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<tr>
<td>mean</td>
<td></td>
<td>228.7</td>
<td>7.67</td>
<td>22.7</td>
<td>-1.72</td>
<td>2.84</td>
<td>3.47</td>
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<tr>
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<td></td>
<td>8.2</td>
<td>0.16</td>
<td>1.0</td>
<td>0.63</td>
<td>0.17</td>
<td>0.25</td>
</tr>
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</table>

V_a, anterior chamber volume; C_p, plasma protein concentration; C_a, aqueous protein concentration; f, aqueous flow rate; k_in, coefficient of protein entry into the aqueous humor.

Total plasma protein concentrations were 7.67 ± 0.16, 7.55 ± 0.11, and 7.65 ± 0.11 g/dl, and plasma albumin concentrations were 4.60 ± 0.08, 4.63 ± 0.07, and 4.77 ± 0.09 g/dl (mean ± SEM, n = 12) at 9:00, 14:00, and 21:00 hr, respectively. Neither plasma protein nor albumin concentration was significantly changed by drug administration. Anterior chamber volumes at 15 hr on the control and acetazolamide days were 225.6 ± 12.3 and 225.7 ± 10.4 μl, respectively, with no significant differences.

Rewriting Equation (2), aqueous flow rate at time t, f(t), is given by

\[ f(t) = V_a \left( k_in \frac{C_p}{C_a(t)} - \frac{d \ln C_a(t)}{dt} \right) \] (5)

where C_a(t) is aqueous protein concentration at time t. An equivalent equation has been given by Krakau,23 who analyzed the connection between flow and aqueous protein concentration. With the assumption that k_in is not affected by oral acetazolamide, the time course of changes in aqueous flow rate was calculated from C_a(t) by using the equation above. The averaged values for k_in, C_p, and V_a obtained in the experiment performed to determine the value of k_in were adopted in the calculation. The C_a(t) was calculated by averaging the concentration at the beginning and the end of the period, which was designated by the midpoint time, t. The term dlnC_a(t)/dt was approximated with a difference equation using the values of lnC_a(t) at the beginning and the end of the period. Calculated flow rate was averaged for 24 eyes of 12 subjects, and its time course is shown in Fig. 4. Significant differences were observed between the control and acetazolamide days from 1.25 to 8 hr postadministration (P < 0.01, paired t-test). A maximum reduction by acetazolamide was observed at 1.75 hr postadministration, resulting in a 40 ± 11% (mean ± SEM, n = 24) reduction.

Fluorophotometric Determination of Aqueous Flow Rate After Oral Acetazolamide

Flow rate calculated from Equation (4) was averaged for 12 eyes of 6 subjects (Fig. 5). Acetazolamide reduced the flow rate beginning at 1.25 hr after administration (P < 0.01, paired t-test). The maximum reduction was 49 ± 8% (mean ± SEM, n = 12), at 1.75 hr postadministration.
Table 2. Ratio of flare values between the acetazolamide and control days

<table>
<thead>
<tr>
<th>Time of day (hr)</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>12:30</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>17</th>
<th>21</th>
<th>1</th>
<th>5</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/C*</td>
<td>0.99</td>
<td>0.99</td>
<td>1.00</td>
<td>0.99</td>
<td>1.05</td>
<td>1.19</td>
<td>1.26</td>
<td>1.32</td>
<td>1.41</td>
<td>1.38</td>
<td>1.10</td>
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<tr>
<td>SEM</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* A, acetazolamide; C, control.

n = 24 eyes.

Discussion

Measurements with the flare-cell meter have been reported to show a linear correlation with bovine albumin concentrations in vitro, from 1 to 1000 mg/dl. Macromolecules, such as globulin and lipid, generate a stronger Tyndall effect than does albumin, resulting in a stronger intensity of flare. When the composition of the aqueous humor changes markedly, flare values do not linearly reflect aqueous protein concentration. Under normal conditions, however, albumin is a predominating protein in the aqueous humor, and the proportion of macromolecules is far smaller than in the plasma. This pattern does not change until aqueous protein concentration exceeds 130 mg/dl, and the concentration ratios between aqueous humor and plasma for various proteins remain inversely proportional to the molecular weight unless the blood–aqueous barrier is massively disrupted. Therefore, in the current study, the time changes in measured flare values, which were standardized by albumin solutions, can be considered to be parallel with changes in aqueous protein concentration, and so to represent actual aqueous protein levels with reasonable accuracy.

In order to study the relation between flow rate and aqueous protein concentration, it is most accurate to measure flow rate and aqueous protein concentration simultaneously. We have reported that corneal fluorescence applied with fluorescein paper as used in applanation tonometry does not affect flare-cell meter measurements. However, the fluorophotometric method which we used to determine aqueous flow rate, method II of Jones and Maurice, entails the presence of highly concentrated fluorescein in the cornea, and the effects of concentrated fluorescein on flare-cell meter measurements are not clear at the current time. Therefore, in order to avoid any possible influences on the measurements of subtle flare intensity in normal human eyes, we adapted the best alternative method and performed flare-cell meter measurements 1 week after the fluorophotometric measurements. In addition, as a result of their time-consuming procedures, it was rather difficult to perform the fluorophotometric and flare measurements as well as IOP measurements simultaneously at 30-min intervals.

Acetazolamide suppresses aqueous flow rate without significant changes in outflow facility and episcleral venous pressure. It is generally accepted that aqueous proteins exit the eye by bulk flow of the aqueous humor. Therefore, a decrease in flow rate caused by acetazolamide consequently leads to an increase in aqueous protein concentration, which implies that the time course of changes in aqueous flow rate after acetazolamide treatment can be monitored by measuring the changes in aqueous protein concentration. Anjou and Dyster-Aas first detected an increase in aqueous protein concentration after oral acetazolamide by measuring aqueous flare intensity in normal human eyes, and reported a 27% increase. However, their finding has never been confirmed until now, and their method to quantify aqueous flare intensity photographically, due to its complicated procedures, has not acquired widespread use. Moreover, in their study, flare intensity was determined before administration and at only one point after administration. Aqueous flare intensity after oral acetazolamide in the current study was deter-

![Fig. 3. Time course of intraocular pressure (mmHg) averaged in 24 eyes of 12 subjects. Open circles indicate values on the control day and filled circles indicate those on the acetazolamide day. After the oral administration of 500 mg acetazolamide at 11:00, IOP was significantly reduced between 13:00 and 17:00 (P < 0.01, paired t-test). Bars represent standard errors.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933577/ on 11/07/2018)
Flow rate (μl/min) calculated from changes in aqueous protein concentration, averaged from 24 eyes of 12 subjects. Open circles indicate values on the control day and filled circles indicate those on the acetazolamide day. Oral acetazolamide (500 mg dose) was administered at 11:00 on the acetazolamide day. Significant differences were observed between the control and acetazolamide days from 1.25 to 8 hr postadministration (P < 0.01, paired student t-test). Bars represent standard errors.

Fig. 4. Flow rate (μl/min) calculated from changes in aqueous protein concentration, averaged from 24 eyes of 12 subjects. Open circles indicate values on the control day and filled circles indicate those on the acetazolamide day. Oral acetazolamide (500 mg dose) was administered at 11:00 on the acetazolamide day. Significant differences were observed between the control and acetazolamide days from 1.25 to 8 hr postadministration (P < 0.01, paired student t-test). Bars represent standard errors.

It was demonstrated that aqueous protein concentration significantly increased from 2 to 10 hr after a single administration of 500 mg acetazolamide. The maximum increase observed was 41% at 6 hr postadministration.

Besides changes in aqueous flow rate, at least one more factor is responsible for the changes in aqueous protein concentration: the rate of protein entry into the aqueous humor (k_{in}). In fact, Anjou and Dyster-Aas postulated that acetazolamide influences the transfer of protein into the aqueous humor, since they observed a marked discrepancy between the theoretically expected increase of 100% and the actually obtained increase of 27% in aqueous protein concentration.

In the current study, assuming that k_{in} is not significantly affected by acetazolamide administration, we calculated the time course of changes in aqueous flow rate from changes in protein concentration. As shown in the results, calculated flow rate reached a maximum decrease of 40% at 1.75 hr postadministration. The validity of this assumption and the results obtained based on this assumption were verified as follows: 1) Fluorophotometric measurements were performed in another six subjects to study the effects of oral acetazolamide on aqueous flow rate. Although these measurements were not carried out over a 24-hr period as in the flare-cell meter measurements, both results showed good agreement in terms of the peak drug effect and the time course in the early postdrug period. 2) Uveoscleral outflow accounts for only a small proportion of the total amount of aqueous drainage in human eyes. The major portion of aqueous outflow, the flow across the trabecular meshwork, is then approximated by the following equation:

\[ \text{aqueous outflow} = C(IOP - P_e) \]  

where C is the outflow facility and P_e is the episcleral venous pressure, which is reported to be approximately 9.0 mmHg in normal human eyes. Since acetazolamide has been reported to have no significant influence on episcleral venous pressure and the C value, the time change of aqueous outflow is estimated from that of the IOP on the control and acetazolamide days. The flow rate thus obtained showed a maximum decrease of 37% at 3 hr after oral acetazolamide, and its time course corresponded well with that of the flow rate calculated from the time change of aqueous protein concentration.

The secretory inhibiting effect of acetazolamide, determined in the current study from the time change of aqueous protein concentration, agrees with these previous results. Furthermore, the calculated
maximum effect of 40% is also compatible with the values reported previously with fluorophotometry, 38%28 or 40%,29 after repeated administration of oral acetazolamide. These findings indicate that the effects of oral acetazolamide on $k_{in}$ were little, if any, and that changes in aqueous protein concentration caused by acetazolamide could be attributed mainly to changes in aqueous humor formation. Therefore, the time course of aqueous flow rate can be determined by monitoring aqueous protein concentration, as in the current study. However, when it is likely that certain drug treatments affect the $k_{in}$, one must first assess changes in the $k_{in}$ by using methods other than flare measurements for the estimation of changes in flow rate.

In rabbits, Yablonski et al recently measured the time course of changes in the aqueous humor flow rate after intravenous administration of acetazolamide by using a fluorophotometric method.38 They reported a biphasic change in aqueous flow, consisting of a abrupt drop during the early postdrug period and a more gradual decrease after the initial change. They attributed the latter decrease to the direct effect of the drug on aqueous humor formation, and attributed the initial reduction to the effect of base content of the injected solution.

Findings presented in the current study indicate that continuous monitoring of aqueous protein concentration also allows us to investigate the time course of ocular effects of acetazolamide in living human eyes. Advantages of the current method include the following: 1) pretreatment, eg, systemic or topical administration of fluorescein, which may cause systemic or topical side effects, is not used; 2) since measurements with this instrument are free from the influence of fluorescence applied with fluorescein paper,5 it can be performed simultaneously with applanation tonometry; and 3) methods using fluorescein suffer from limitations in continuous measurements due to the fact that administered fluorescein disappears from the eye or the blood compartment rather rapidly.18,21,22 In contrast, measurements of aqueous protein concentration as in the current study can be performed without these restrictions.

The current method does suffer from a disadvantage in that it is difficult to estimate a change in the $k_{in}$ by measuring aqueous protein concentration alone. On the other hand, in the case of the fluorescein method, concentration of the dye shortly after the intravenous injection was reported to depend largely on the $k_{in}$ but not on aqueous flow rate.21,39 That is, the fluorophotometric method can be used to assess changes in both the $k_{in}$ and flow rate in an individual.

As demonstrated by the results of this and previous studies,5-9 measurements of aqueous protein concentration with the flare-cell meter not only can serve as a method for the quantitative assessment of pathologic blood–aqueous barrier function,7-9 but also can be profitably employed in pharmacologic and physiologic investigations in living human eyes.

**Key words:** acetazolamide, aqueous protein concentration, flow rate, aqueous flare, flare-cell meter

### References

18. Johnson SB, Coakes RL, and Brubaker RF: A simple photo-