Aqueous Humor Dynamics in Mice

Makoto Aihara, James D. Lindsey, and Robert N. Weinreb

PURPOSE. To assess aqueous humor dynamics in mouse eyes.

METHODS. Aqueous humor dynamics of NIH Swiss White mouse were assessed with an injection and aspiration system, using fine glass microneedles. Intracocular pressure (IOP) was measured by a microneedle connected to a pressure transducer. Episcleral venous pressure (EVP) was measured by gradually lowering intracameral pressure until blood reflux into Schlemm’s canal was observed. Outflow facility \( (C) \) was determined based on constant pressure perfusion measurements obtained at two different IOPs. Aqueous volume \((V_a)\) was determined by direct measurement of aspirated aqueous humor. Aqueous humor production \((F_a)\) was measured by the dilution method with rhodamine-dextran. Conventional and uveoscleral outflow \((F_c\) and \(F_u\), respectively), as well as the turnover rate of aqueous humor, were also calculated.

RESULTS. IOP and EVP were \(15.7 \pm 2.0\) and \(9.5 \pm 1.2\) mm Hg, respectively \((n = 20)\). \(F_c\) was \(0.18 \pm 0.05\) \(\mu L/\text{min}\) (mean \(\pm SD; n = 8)\). \(C\) was \(0.0051 \pm 0.0006\) \(\mu L/\text{min per mm Hg}\) \((n = 8)\). Estimated \(F_c\) and \(F_u\) were 0.032 and 0.148 \(\mu L/\text{min}\), respectively. \(F_u\) was 18\% of \(F_c\), \(F_u\) was 82\% of \(F_c\), \(V_a\) was 5.9 \(\pm 0.5\) \(\mu L\) \((n = 8)\). The calculated turnover rate of aqueous humor was 25\%.

CONCLUSIONS. The mouse eye has similar aqueous production and aqueous humor turnover rate as the human eye. The presence of both conventional and uveoscleral outflow suggests that the mouse is a useful model system for future investigations of the biology of aqueous dynamics. (Invest Ophthalmol Vis Sci. 2003;44:5168–5173) DOI:10.1167/iovs.03-0504

Study of aqueous humor dynamics can enhance our understanding of normal ocular health, can clarify the relationship between fluid flow and intraocular pressure (IOP), and can delineate the response of IOP to drug treatment. Aqueous humor dynamics have been studied previously in monkey, rabbit, cat, and rat eyes. However, a complete description of the various aqueous dynamic parameters has not been reported for each of these animals.

In general, the smaller the eye size, the more difficult it has been to obtain measurements of aqueous humor dynamics using conventional methods. Because of their small eyes, it has been particularly difficult to assess aqueous dynamics in the mouse. To the best of our knowledge, a comprehensive description of aqueous humor dynamics in the mouse has not been performed. Hence, it is still unknown whether the aqueous humor dynamics of the mouse eye are similar to the human eye.

To clarify the mechanisms of IOP regulation in the mouse, it is necessary to have a better understanding of the aqueous humor dynamics. In view of these observations, the present study was undertaken to measure aqueous humor dynamic parameters, including outflow facility, aqueous volume, and aqueous humor production, in the NIH Swiss White mouse.

MATERIALS AND METHODS

Instrument System

Two microneedles made of borosilicate glass (100-\(\mu m\) tip diameter and 1.0-mm outer diameter, World Precision Instruments [WPI], Sarasota, FL) were used to make the aqueous dynamics measurements as shown in Figure 1. Both microneedles were mounted on micromanipulators. One microneedle was connected to a pressure transducer (Model BLPR, WPI). A silicon tube (0.38-mm inner diameter) linked the transducer to a fluid reservoir filled with physiological saline (BSS Plus; Alcon, Fort Worth, TX) for the measurement of episcleral venous pressure (EVP) and outflow facility \((C)\). Alternatively, the reservoir was filled with 70 k\(\mu\) rhodamine-dextran (RD; Molecular Probes, Eugene, OR) diluted in physiological saline for the measurement of aqueous humor production. Its height could be varied to modulate intracameral

Anesthesia

The mice were anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg, Ketaset; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (9 mg/kg, TranquilVed; VEDCO Inc., St. Joseph, MO) prepared at room temperature. Animals were gently restrained (Decapicone; Braintree Scientific Inc., Braintree, MA) to avoid stress, and anesthesia was administered with a 30-gauge needle. Each mouse was monitored carefully to assess the state of anesthesia.

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Animal Husbandry and Measurement Time

All experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. NIH white Swiss mice were obtained from Harlan Sprague-Dawley (San Diego, CA). This mouse strain was chosen to facilitate comparison of the present results with previously published information regarding IOP in this strain. Mice were bred and housed in clear cages covered loosely with air filters and containing white pine shavings for bedding. The environment was kept at 21°C with a 12-hour light (6 AM to 6 PM) and 12-hour dark cycle. All mice were fed ad libitum. Animal age ranged from 8 to 12 weeks. All measurements were performed between 3 and 6 PM in consideration of the mouse’s diurnal IOP rhythm and, hence, the possible diurnal variation of mouse aqueous dynamics.

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pressure. The system pressure detected by the transducer was recorded by computer (Chart software; ADInstruments, Colorado Springs, CO), as described previously. This infusion system was used to measure IOP, EVP, and C as described later.

The second microneedle was connected by a narrow silicon tube to a 50-μL syringe (Hamilton, Reno, NV)-equipped micropump (Micro4; WPI). This micropump could aspirate or perfuse specified volumes at programmed speeds. This aspiration system was used to measure the aqueous volume \( V_a \) directly. Moreover, by using both microneedle systems together, aqueous humor production \( F_a \) was measured as described later.

**Intraocular Pressure Measurement**

IOP was measured with an infusion system described previously \((n = 20)\).14

**EVP Measurement**

Before the measurement of aqueous humor production, EVP was measured using only the infusion system \((n = 20)\). Intracameral pressure was gradually decreased until there was an observable reflux of blood within Schlemm’s canal from the episcleral vein. The intracameral pressure at which reflux began was designated EVP. To obtain this measurement, the stopcock between the pressure transducer and the infusion tube was closed, the microneedle was inserted into the anterior chamber, and the baseline IOP was measured. Next, the height of the saline reservoir was adjusted to equalize the tubing pressure with IOP. After the stopcock was opened, the height of the reservoir was lowered at a rate of 0.5 mm Hg \((0.68 \text{ cm } H_2O \text{ height})/\text{min}\). As IOP became equal to EVP, there was reflux of blood from collector channels into Schlemm’s canal. While carefully monitoring the limbus under the microscope, we recorded the pressure at which the reflux of blood was first observed and designated it the EVP. After the first measurement, the reservoir was returned to the original height \((\text{baseline IOP})\). The measurement procedure was repeated in the same eye for a total of three determinations.

**Determination of Outflow Facility**

The infusion system was used to determine the outflow facility \(C\); Fig. 1). All fluid within the infusion system was replaced with physiological saline. The measurement was based on measuring total outflow volume \(V_c\) in microliters for a specific time \((10 \text{ minutes})\) at two different levels of IOP, as described previously.10 After insertion of the infusion needle into the anterior chamber, the reservoir height was controlled to maintain IOP at 25 mm Hg. After verification of IOP stability, the initial fluid level in the silicon tube was marked. After a 10-minute interval, the new fluid level was marked. The total amount of infused fluid was noted as the infused volume at 25 mm Hg \((V_{c,25})\). Next, IOP was increased to 35 mm Hg and the 10-minute infused volume \((V_{c,35})\) was also measured in the same eye. Because total outflow is equivalent to aqueous humor production \((F_a\) in microliters per minute), \(F_a\) also can be represented as the sum of conventional outflow \((F_{c,25}\) in microliters per minute) and uveoscleral outflow \((F_u\) in microliters per minute).

\[
F_c = F_a + F_u. \tag{1}
\]

This was also calculated from \(V_{c,25}\) and \(V_{c,35}\) as follows,

\[
F_c = F_{c,25} + F_u = \frac{V_{c,25}}{10} \tag{2}
\]

and

\[
F_c = F_{c,35} + F_u = \frac{V_{c,35}}{10}. \tag{3}
\]

Subtracting equation 2 from equation 3,

\[
F_{c,35} - F_{c,25} = 0.1 \times (V_{c,35} - V_{c,25}). \tag{4}
\]

By the Goldmann equation,16

\[
F_{c,35} = C \times (\text{IOP} - \text{EVP}). \tag{5}
\]

Thus,

\[
F_{c,35} - F_{c,25} = C \times (35 - \text{EVP}) - C \times (25 - \text{EVP}) = C \times 10 \tag{6}
\]

Combining equations 4 and 6,

\[
C = 0.01 \times (V_{c,35} - V_{c,25}) \muL/min \text{ per } mm \text{ Hg}. \tag{7}
\]

Using intracameraly infused RD, we have observed the histologic presence of the uveoscleral pathway in mouse eyes.17 Although there

![Diagram of the instrument system used to measure intraocular pressure, episcleral venous pressure, outflow facility, aqueous humor volume, and aqueous flow rate.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932917/)
Aqueous Humor Production

Aqueous humor production ($F_a$ in microliters per minute) was determined by measuring dilution of an infusion solution (0.5 µg/mL 70 kDa RD in physiological saline; BSS Plus; Alcon) as it was slowly perfused through the anterior chamber ($n = 8$). $^{10-13}$ RD at 70 kDa was used as a tracer because there is minimal loss of this molecule by diffusion. $^{16}$ Pilot studies established that the optimal perfusion rate and time for the mouse eye were 3 µL/min and 20 minutes, respectively. If the perfusion rate was faster, it was difficult to detect the difference of tracer concentration between in- and outflowing perfusion fluids. In contrast, prior studies in rat eyes indicate that slower rates with longer durations can lead to alteration of aqueous humor production and disruption of the blood-aqueous barrier. $^{10}$ To conduct measurement of $F_a$, EVP was first measured as described earlier, and then the height of the reservoir was adjusted to equalize the system pressure to EVP. Next, the aspiration needle was inserted into the anterior chamber, as illustrated in Figure 1. This needle was used to collect the perfusion outflow fluid. Care was taken that neither microneedle touched the iris. The micropump connected to the aspiration needle maintained the perfusion rate at 3 µL/min. Because the perfusion pressure was maintained at EVP according to the height of the fluid surface in the reservoir, pressure-dependent outflow was reduced to zero. The tubing connecting the aspiration needle to the micropump was marked every 52.9 mm to facilitate collection of 6-µL samples (0.38 mm inner diameter × 52.9 mm = 6 µL). After 20 minutes of perfusion, the perfused fluid was retrieved from the 264.5-mm segment of the tubing closest to the microneedle. This tubing segment was cut into five equal pieces (corresponding to 6 µL for each piece and totalling 30 µL). Thus, these five collected samples contained the perfusion outflow fluid that had been collected every 2 minutes during the prior 10-minute perfusion time. This strategy avoided collecting fluid during the first 10 minutes because of the possibility that the ratio of tracer to aqueous humor was not yet stable during this period. Division of the fluid collected during the final 10 minutes of the perfusion allowed confirmation that the dilution of the perfusion fluid by physiological aqueous outflow had reached a steady state.

Measurement of RD and Calculation of $F_a$

RD concentration was determined with a spectrophotometer (Model SFM25; Kontron, Zürich, Switzerland). The excitation and emission wavelengths were 550 and 580 nm, respectively. The concentration of RD in the injection fluid ($C_i$) was measured five times and averaged. The concentration of RD in the five collected samples ($C_o$) was measured and divided by $C_i$. The mean value of $C_i/C_o$ in each eye ($n = 8$) was used to calculate $F_a$ according to the following rationale. The total amount of RD the perfusion inflow fluid and the perfusion outflow fluid in a specific time are equal. Hence, the concentration of RD in the perfusion inflow fluid ($C_i$) multiplied by the rate of the perfusion inflow ($v_i$) is equal to the concentration of RD in the perfusion outflow fluid ($C_o$) multiplied by the flow rate of perfusion outflow fluid ($v_o$).

$$C_i \times v_i = C_o \times v_o. \quad (8)$$

During the perfusion, the infused fluid was diluted by the secreted aqueous humor and the magnitude of dilution was a measure of $F_a$. Hence,

$$v_i + F_a = v_o. \quad (9)$$

$$v_i = v_o - F_a. \quad (10)$$

Combining equations 8 and 10

$$C_o \times v_o = C_i \times (v_o - F_a). \quad (11)$$

In this system, the perfusion rate ($v_o$) was controlled by the aspiration system. Hence,

$$v = v_o. \quad (12)$$

Combining equations 10 and 12

$$C_o \times v = C_i \times (v - F_a). \quad (13)$$

Hence,

$$F_a = v \times (1 - C_o/C_i). \quad (14)$$

Determination of Conventional and Uveoscleral Outflow

IOP, EVP, and $C$ were measured and averaged. Using these data, the conventional outflow was calculated as

$$F_{cv} = C \times (IOP - EVP). \quad (15)$$

Uveoscleral outflow was calculated with the Goldmann equation,

$$F_u = F_{cv} + F_u = C \times (IOP - EVP) + F_u. \quad (16)$$

Thus,

$$F_u = F_a - C \times (IOP - EVP). \quad (17)$$

Also, the percent contribution of $F_a$ and $F_u$ to $F_a$ were calculated.

Determination of Aqueous Humor Volume and Turnover Rate of Aqueous Fluid

$V_a$ was measured by the aspirated volume of aqueous fluid, using the aspiration system. In nontreated mouse eyes ($n = 8$), the saline-filled aspiration needle was inserted into the anterior chamber. Aspiration of the aqueous humor (100 nL/sec) began, with the anterior segment observed under a microscope. Apposition of the central edge of the iris to the cornea was the endpoint of the aspiration. The total aspirated volume was indicated on the micropump display. The turnover rate of aqueous fluid was calculated as

$$\text{Turnover rate} \% / \text{min} = 100 \times F_a/V_a. \quad (18)$$

Aqueous Humor Protein Concentration

Aqueous protein concentration was measured after measurement of EVP to determine whether microneedle penetration followed by alteration of IOP induced a significant breakdown in the blood-aqueous barrier. To prevent dilution of aqueous, a small volume of mineral oil was aspirated into the needle tip before insertion to separate the aqueous humor from the saline in the fluid column. After insertion of the microneedle, intracameral pressure was shifted from 16 to 8 mm Hg and then back to 16 mm Hg three times over the course of 6 minutes by raising and lowering the reservoir connected to the microneedle. Immediately after this procedure, approximately 4 µL of aqueous humor was aspirated into the microneedle. Because the mineral oil in the needle tip may alter the accuracy of EVP measurement, the data obtained during this series of experiments were not included for determination of EVP. Approximately 4 µL of aqueous humor was aspirated from the contralateral control eyes to determine baseline protein concentration. The Bradford protein assay (Bio-Rad Laboratories, Hercules, CA) was used according to the manufacturer’s guidelines. The sensitivity of this assay was 2 µg/mL and the linear dynamic range extended to 50 µg/mL. Before evaluation in the assay, 1.5 µL of
each aqueous sample was diluted to 100 µL. The assay was conducted in a microtiter plate, and absorption was measured at 595 nm with a microtiter plate reader (SpectraMax 250; Molecular Devices, Sunnyvale, CA). Aqueous protein concentration was determined based on linear regression analysis of a serial dilution of bovine serum albumin standards.

### RESULTS

Mean IOP and EVP were 15.7 ± 2.0 and 9.5 ± 1.2 mm Hg, respectively (n = 20). The C/V ratio was measured five times and averaged in each eye (Table 1). F was calculated using equation 14: $F = 3 \times (1 - C/V)$. Mean F was 0.18 ± 0.05 µL/min (n = 8). Mean C was 0.0051 ± 0.0006 µL/min per mm Hg (n = 8). Mean V was 5.9 ± 0.5 µL (n = 8).

All aqueous humor dynamics parameters are shown in Table 2. Estimated F and F were 0.032 and 0.148 µL/min, respectively. The portions of F that corresponded to F and F were 18% and 82%, respectively. The calculated turnover rate of aqueous humor was 2.5%.

### No Evidence of Induced Blood–Aqueous Barrier Disruption

Mean protein concentration in the aqueous humor of untreated control eyes (n = 12) was 0.22 ± 0.07 mg/mL (mean ± SD). Protein concentration in the aqueous humor of eyes that had received microneedle insertion followed by raising and lowering intracameral pressure was 0.25 ± 0.09 mg/mL (n = 5). This difference was not statistically significant (P = 0.31, paired t-test).

### DISCUSSION

Our data demonstrated outflow through both the conventional and uveoscleral outflow pathways of the mouse eye. Moreover, a comprehensive measurement of aqueous humor dynamics parameters was obtained. The challenge of assessing aqueous humor dynamics in mouse eyes required an adaptation of a previous technique designed for larger eyes. In this study, the use of glass microneedles, fine tubing, and a micropump that could measure fluid volume, and also control the speed of perfusion flow to an accuracy of 1 mL/min, allowed measurement of mouse aqueous humor production and outflow facility based on a previously reported methods in rat eyes. Several strengths of this experimental system are apparent. First, because all components of the experimental system used for IOP measurement were either glass or hard plastic, the influence of system compliance at physiological IOP was likely to be minimal. For the measurement of EVP and F, the tubing had an inside diameter of 0.38 mm and a total length less than 1 m. Hence, the volume of the tubing was less than 113 µL. Because the measurement system was open to the atmosphere, and system pressure in each case was regulated by the height of the water column, the effect of any tubing compliance on these measurements was eliminated. The effect of pipette resistance on the measurements was minimal, as pressure change associated with entry of our microneedle into the eye, as recorded at the transducer, appeared as a square-wave shift in pressure on the chart recording. This indicated there was rapid pressure equilibrium within the microneedles. Finally, the stability of the aqueous humor protein concentration after microneedle insertion followed by raising and lowering intracameral pressure indicates that there was minimal disruption of the blood–aqueous barrier during the course of these aqueous dynamics measurements.

A limitation of this system is that anesthesia was used during the collection of the measurements. We have observed that between 4 and 8 minutes after administration of anesthesia, blood pressure was the same in anesthetized mice as in awake mice (Aihara M, Lindsey JD, Weinreb RN, unpublished observation, 2002). IOP is stable during the 4 to 8 minutes after administration of anesthesia.14 These data support the view that IOP and EVP measured during this period closely reflect IOP in the unanesthetized mouse. Future improvements in the accuracy of noninvasive methods to measure IOP without anesthesia, as well as development of noninvasive methods to assess other aqueous dynamics parameters in the awake mouse, would clarify this issue further.

The method for measurement of facility in the mouse eye was adapted from an established procedure for facility deter-

### Table 1. Measurement of F of NIH Swiss White Mice

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Co/Ci† (mean ± SD)</th>
<th>F (µL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.94 ± 0.99</td>
<td>0.96 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>0.94 ± 0.96</td>
<td>0.94 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>0.92 ± 0.95</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>0.97 ± 0.90</td>
<td>0.92 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>0.96 ± 0.95</td>
<td>0.96 ± 0.05</td>
</tr>
<tr>
<td>6</td>
<td>0.95 ± 0.95</td>
<td>0.95 ± 0.00</td>
</tr>
<tr>
<td>7</td>
<td>0.94 ± 0.91</td>
<td>0.93 ± 0.01</td>
</tr>
<tr>
<td>8</td>
<td>0.96 ± 0.95</td>
<td>0.94 ± 0.02</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.18 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

* F was determined by equation 14.
† Co/Ci (concentration of RD in postperfusion fluid)/(concentration of RD in preperfusion fluid).

### Table 2. Aqueous Humor Dynamic Parameters of NIH Swiss white Mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measurement Data</th>
<th>n</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOP (mm Hg)</td>
<td>15.7 ± 2.0</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>EVP (mm Hg)</td>
<td>9.5 ± 1.2</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>F (µL/min)</td>
<td>0.18 ± 0.05</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>C (µL/min per mm Hg)</td>
<td>0.0051 ± 0.0006</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>F (µL/min)</td>
<td>0.032 (16)</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>F (µL/min)</td>
<td>0.148 (84)</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>V (µL)</td>
<td>5.9 ± 0.5</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Turnover rate (%)</td>
<td>2.5</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

[Data expressed as the mean ± SD. Equation indicates the number of the equation used to calculate the parameter.]
mination in rat eyes. Aqueous outflow in the mouse is difficult to measure accurately because the volume of aqueous outflow at normal IOP is small. If the pressure was increased to either 25 or 35 mm Hg and C remains constant, we reasoned that the volume of aqueous outflow would be greater; this would facilitate more accurate measurement of flow. However, it is possible that the measurement of facility in the present study was affected by the pressures used for the measurements. Grierson and Lee observed structural abnormalities in trabecular outflow tissues of Rhesus monkeys with experimental increases of intraocular pressure to 50 mm Hg that were not seen at 15, 22, and 30 mm Hg. They also observed minor changes in the lysosomal system of monkey trabecular meshwork at 30 mm Hg that were not observed at 15 and 22 mm Hg.

The calculations used for our method depend on measuring aqueous humor production ($F_o$) by a modified dilution method in which pressure-dependent outflow ($F_v$) was minimized by setting IOP at the same level as EVP. An alternate method to determine $F_o$ would be to measure the dilution of a perfusate after it has passed through the anterior chamber. The following equation rigorously describes the relationship between dye concentration in the inflow solution ($C_i$) and outflow solutions during perfusion ($C_{ou}$, $C_{ov}$, and $C_v$ corresponding to the outflow cannula fluid, fluid exiting by uveoscleral outflow, and fluid exiting by conventional outflow, respectively):

$$C_i \times V_i = C_{ou} \times V_o + C_{ov} \times F_o + C_v \times F_v$$

where $V_i$ is infusion flow rate and $V_o$ is aspiration flow rate. With the perfusion method used in previous studies in the monkey and rat, $C_{ou}$ and $C_v$ were assumed to be equal to $C_{ov}$. Thus, this formula was reduced to

$$C_i \times V_i = C_{ov} \times (V_o + F_o - F_v) + C_{ov} \times F_o + C_v \times F_v$$

However, this method is inaccurate if either $C_{ov}$ or $C_v$ is not similar to $C_{ou}$, as might occur with differential permeability of the outflow pathways to aqueous humor. In contrast, $F_o$ was assumed in the current investigation to be negligible, because it was considerably less than the perfusion rate (0.15 vs. 3.0 $\mu$L/min, respectively). Moreover, $F_v$ was also considered negligible because the perfusion pressure was equal to EVP.

Aqueous volume was measured by direct collection of aqueous humor from the anterior chamber, the posterior chamber fluid, and possibly even some vitreous. The use of this aqueous volume to calculate the turnover rate is likely to be appropriate because the intraocular humor is mainly aqueous humor secreted by the ciliary body. It is possible that the anterior chamber is not completely empty when the central iris touches the cornea. Nevertheless, this is a reproducible and reasonable end point for this measurement.

Determination of uveoscleral outflow in small eyes has typically been difficult. Direct measurement of the distributed volume of tracer in ocular tissues and plasma, as assessed in monkey eyes, may be more accurate than indirect methods. This procedure is complicated and applicable only to larger animal eyes or to human donor eyes obtained at autopsy. In smaller eyes, precise separation of ocular tissues and recovery of a tracer is more difficult. Uveoscleral outflow was indirectly calculated with the Goldmann equation, using the measured EVP in the present study. However, it is not known whether uveoscleral outflow in the mouse eye is independent of pressure. Further investigations are needed to clarify this issue.

Our data demonstrate that mouse eye has aqueous humor dynamics similar to those in human eyes with respect to IOP, EVP, and turnover rate. Moreover, direct measurement of EVP by cannulation of the episcleral veins in the rabbit eye also obtained a pressure measurement essentially the same as in the present study (9.6 mm Hg). The aqueous humor turnover rate of the mouse eye (2.5%) also was similar to that of the rat eye (2.26%). This observation roughly corroborates the theory that the turnover rate is uniform at approximately 1.5% of total aqueous volume even though the aqueous humor volume varies among species. However, in our study, the aqueous volume includes the posterior chamber volume. If the mouse aqueous volume is underestimated, however, the turnover rate may be overestimated. The physiological presence of both conventional and uveoscleral outflow pathways in mouse is consistent with morphologic demonstration of the presence of these pathways. The present results indicate that there is a larger proportion of uveoscleral outflow in the mouse than in the monkey.

The mouse is a useful model to investigate ocular diseases because of the availability of transgenic mice, their similar genetic background, relatively quick breeding, and low cost. Moreover, mouse and human eyes have similar anatomic structure, outflow pathways, and response to IOP-lowering drugs. Hence, pharmacological studies using various transgenic mice should facilitate molecular analysis of the mechanism of IOP reduction. However, these studies necessitate determination of aqueous dynamics for each tested mouse strain, as normal IOP can vary substantially among them.

In conclusion, the results of this study show that aqueous humor production and turnover in the mouse eye are similar to values reported for other species. Direct measurement of uveoscleral outflow is needed to confirm whether there is a larger proportion of uveoscleral outflow in the mouse than in the monkey. Regardless, the presence of both outflow pathways suggests that the mouse is a useful model system for further investigation of the biology of aqueous dynamics.

### Acknowledgments

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