

Measuring the Outflow of Aqueous Humor

These assessments have helped elucidate the pathophysiology of glaucoma and the mechanism of action of IOP-lowering treatments, but difficulties are inherent in each method of measurement.

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The production, circulation, and drainage of aqueous humor into and out of the anterior chamber of the eye maintain the IOP at a relatively constant level (aqueous humor dynamics) (Figure 1). When the pressure is higher than normal, the problem usually resides in the tissues of the drainage pathways, an area targeted by many IOP-lowering drugs, surgical procedures, and drainage devices. Aqueous humor drainage is measured by several methods, each with advantages and inherent weaknesses. Understanding the limitations of each method ensures a proper interpretation of the results of clinical trials and animal studies.

TRABECULAR OUTFLOW FACILITY

Overview

The trabecular meshwork offers a certain resistance to the outflow of aqueous humor that is needed to maintain a steady-state IOP. The inverse of this resistance is trabecular outflow facility, a measure of the compliance of the trabecular meshwork.

Techniques for Measuring Outflow Facility

Tonography

Researchers use a Schiotz tonometer (Figure 2) or the tonography setting on a pneumotonometer to determine outflow facility in a noninvasive manner. Tonography was developed originally in the 1940s¹ to assess outflow facility in patients and assist in the diagnosis of glaucoma. A value of less than 0.2 $\mu\text{L}/\text{min}$ per mm Hg generally was considered to be in the glaucomatous range. Although employed throughout the 1960s and 1970s, the method is rarely used today in routine clinical practice because of its poor accuracy in identifying cases of glaucoma. Tonography shows wide variation among healthy subjects and in the same patient over

the course of several visits. Nevertheless, it continues to be a valuable research tool in studies of aqueous humor dynamics in human and animal eyes.

The tonography procedure involves placing a tonometer's probe with a calibrated weight on the anesthetized cornea of the supine subject for 2 or 4 minutes. The weight causes the IOP to rise initially, but, over time, the pressure slowly decreases, because aqueous humor drains at an increased rate from the anterior chamber into the drainage pathways. The drop in IOP during the measurement is assumed to be caused solely by increased drainage of aqueous humor from the trabecular meshwork, the pressure-dependent pathway. The rate of fluid outflow from the eye during the time of the test is determined from reference tables.¹ Outflow facility is the ratio of the flow rate (from tables) to the change in pressure (determined by tonometry). If the IOP decreases little during the test, the fluid flow rate from the tables would be small, and trabecular outflow facility would be calculated to be low. This is expected in eyes with ocular hypertension with or without glaucoma.

An important factor affecting the tonography measurement is ocular rigidity. This factor is a measure of the resistance that the eye exerts to distending forces. The stiffer the eye, the greater the ocular rigidity, with more force required to indent the cornea. Ocular rigidity increases by 25% in older versus younger people.^{2,3} Because elderly eyes are therefore less compliant than younger eyes, measurements of outflow facility assessed by tonometry are lower in older individuals on the basis of increased ocular rigidity rather than a true reduction in outflow facility. Tonography performed with an indentation tonometer (Schiotz) (Figure 2) assumes that the change in pressure, as a function of time, is based on the accuracy of the ocular-rigidity coefficient during

the measurement. Indentation tonography makes no compensation for individual variations in ocular rigidity. Tonography assessed with a pneumatic tonography unit is less affected by ocular rigidity than the Schiøtz unit, because the probe that is placed on the eye creates a relatively smaller corneal indentation. Both instruments derive a change in flow from standard tables.

Outflow facility measured by tonography (Cton) includes pseudofacility (Cps) and uveoscleral outflow facility (Cfu) in addition to trabecular outflow facility (Ctrab), as in Equation No. 1:

$$C_{\text{ton}} = C_{\text{trab}} + C_{\text{fu}} + C_{\text{ps}}$$

Cfu is the facility of fluid flow through the ciliary muscle. This facility is about 10-fold less than trabecular outflow facility. Pseudofacility is the facility of the flow of aqueous humor from the posterior chamber into the anterior chamber resulting from the probe-induced increase in IOP. An assumption in tonography is that the rate of aqueous humor's inflow into the anterior chamber during the measurement remains unchanged by the applied pressure (ie, pseudofacility is zero). If pseudofacility and/or uveoscleral outflow facility are disturbed during the measurement, a change in tonographic outflow facility may not indicate a change in true trabecular outflow facility.

Fluorophotometry

Fluorophotometry provides another way to assess outflow facility.⁴ Aqueous flow (F) is determined by measuring the disappearance rate of a tracer from the anterior chamber. Next, an aqueous flow suppressant such as acetazolamide, dorzolamide, or timolol is given to reduce the IOP and aqueous flow. Brimonidine and apraclonidine are not appropriate for this purpose, because these drugs affect outflow as well as aqueous flow. The drug-induced change in IOP ($IOP_2 - IOP_1$) is measured by tonometry, and the change in aqueous flow ($F_2 - F_1$) is measured by fluorophotometry. Outflow facility is calculated by Equation No. 2:

$$C = (F_2 - F_1) / (IOP_2 - IOP_1)$$

C by fluorophotometry usually is labeled Cfl.

The main advantage of fluorophotometry over tonography is that fluo-

rophotometry directly measures changes in aqueous flow instead of referencing standard tables. Additionally, ocular rigidity and pseudofacility are not part of the measurement, because a weight is not applied to the eye. Researchers have found different results and come to different conclusions when using tonography versus fluorophotometry to assess outflow facility. For example, 1 week of twice-daily treatment with apraclonidine did not change outflow facility when measured by tonography, but it increased outflow facility when measured by fluorophotometry.⁵ The reason is because apraclonidine was thought to reduce pseudofacility, an effect that hid the increase in trabecular outflow facility when measured by tonometry but not fluorophotometry (see Equation No. 1). In another example, there is an age-related decrease in outflow facility when measured by tonography^{3,6} but not fluoro-

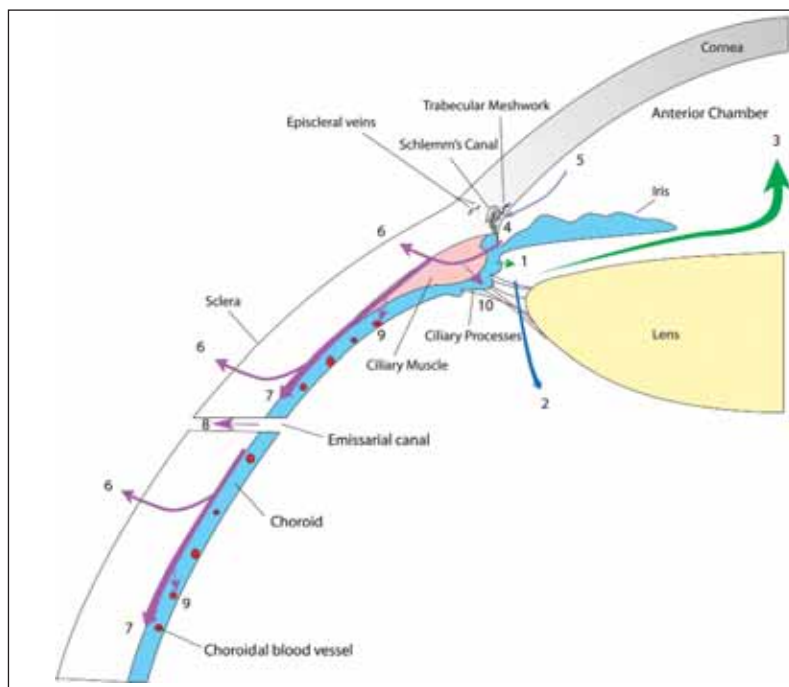


Figure 1. Aqueous humor secreted into the posterior chamber (1) flows across the vitreous cavity (2) or through the pupil into the anterior chamber (3). Fluid circulates around the anterior chamber and eventually drains into the anterior chamber angle (4). Aqueous humor drains from the anterior chamber angle via two routes: the trabecular meshwork, Schlemm's canal, and the episcleral veins (5) or the uveoscleral outflow route. The latter route starts with the ciliary muscle. From there, fluid flows in many directions, including across the sclera (6), within the supraciliary and suprachoroidal spaces (7), through emissarial canals (8), into uveal vessels (9) and vortex veins (not shown), and possibly into the ciliary processes (10), where it could be secreted again. (This image was redrawn with permission from Figures 1 and 3 in Toris CB. Aqueous humor dynamics I, measurement methods and animal studies. The eye's aqueous humor. In: Civan MM, ed. *Current Topics in Membranes*. San Diego, CA: Elsevier, Inc.; in press.)

tometry.⁷ This discrepancy may be caused by the increased rigidity in older versus younger subjects. Ocular rigidity is part of the tonography but not the fluorophotometry measurement.

A few problems are associated with the fluorophotometric method. First, it is assumed that uveoscleral outflow facility is very small and affected little by the measurement. If an experimental manipulation were to increase uveoscleral outflow facility, it could be interpreted erroneously as an increase in trabecular outflow facility. This problem is also inherent in the tonography measurement. Second, the method does not work well in normotensive eyes in which a change in IOP by the aqueous flow suppressant is not effective. Similarly, tonography does not work well in normotensive eyes in which the IOP changes little by the weight of the probe. Third, fluorophotometry requires several hours for a complete determination versus 4 minutes for tonography.

Invasive Methods

The two-level, constant-pressure perfusion technique⁸ (Figure 3A) is an invasive procedure that is used to measure outflow facility in research animals. A needle is attached, via tubing, to a reservoir of mock aqueous humor. The investigator inserts the needle into the anterior chamber and sets the IOP by the level of the reservoir above the eye. Next, one measures the rate of fluid flow into the anterior chamber (F1) that is needed to maintain a constant IOP (IOP1). A variety of techniques may be used. The distance that the fluid moves in the tubing over a specific period of time can be measured, and the volume of fluid can be calculated from the diameter and length of the tubing. The volume is divided by the time to yield flow rate. Alternatively, an investigator collects the fluid in the tubing during a specific period of time and weighs it. The fluid weight is converted to fluid volume and then divided by the time to obtain a flow rate (F1). One measures the flow rate (F2) needed to maintain a new IOP (IOP2) in a similar manner. Equation No. 2 is used to calculate outflow facility. These methods are frequently employed for enucleated human eyes but cannot be used in clinical studies.

The flow-to-blood method is arguably the most accurate technique to assess trabecular outflow facility. A radioactive isotope is infused into the anterior chamber at a set pressure (IOP1) for a set period of time. One collects a blood sample at a specific time interval and measures it for radioactivity. Any radioactivity in the blood is thought to have drained solely through the trabecular meshwork, and the rate of its accumulation in the blood is assumed to be trabecular

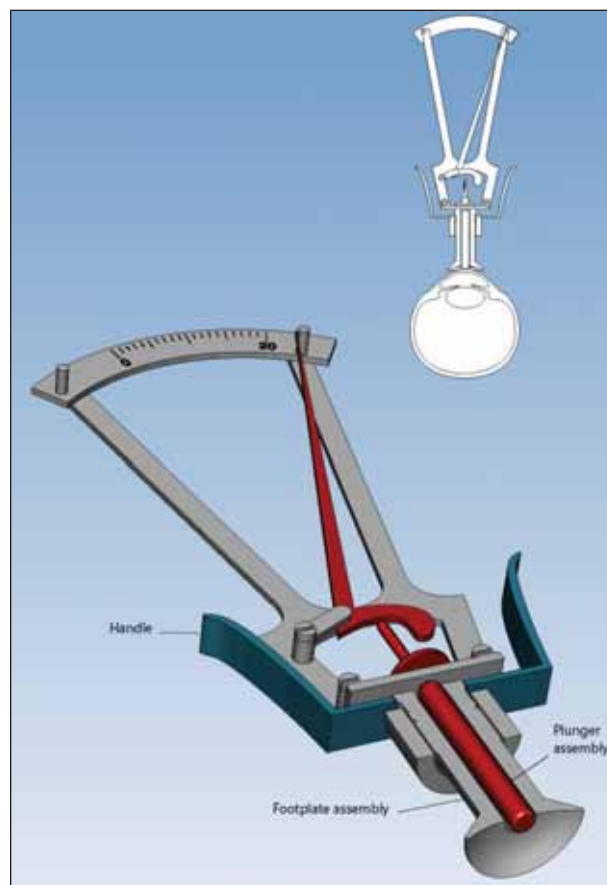


Figure 2. A Schiotz tonometer of known weight is placed on the eye of a supine subject for 4 minutes. The rate at which the IOP decreases during this time is an indication of tonographic outflow facility. (This image was redrawn with permission from Figure 14 in Toris CB, Yablonski ME, Tamesis R. Aqueous humor dynamics. In: Choplin N, Lundy C, eds. *Atlas of Glaucoma*. 2nd ed. Colchester, Essex, UK: Informa UK Ltd.; 2007:13-27.)

outflow (F1). Next, the isotope is infused at a different pressure (IOP2), and the new rate of accumulation of radioactivity in the blood is assumed to be a new trabecular outflow (F2). Equation No. 2 is used to calculate trabecular outflow facility. If done carefully, this method is repeatable and can be used to evaluate outflow facility over time.

The major problems with all invasive techniques are the direct and indirect effects of anesthesia on the IOP and the trauma of the needle's insertion into the eye. Additionally, ocular rigidity, pseudofacility, and uveoscleral outflow facility confound the measurement. An important assumption with the flow-to-blood method is that any tracer in the blood enters solely through the trabecular meshwork. In reality, some tracer may enter

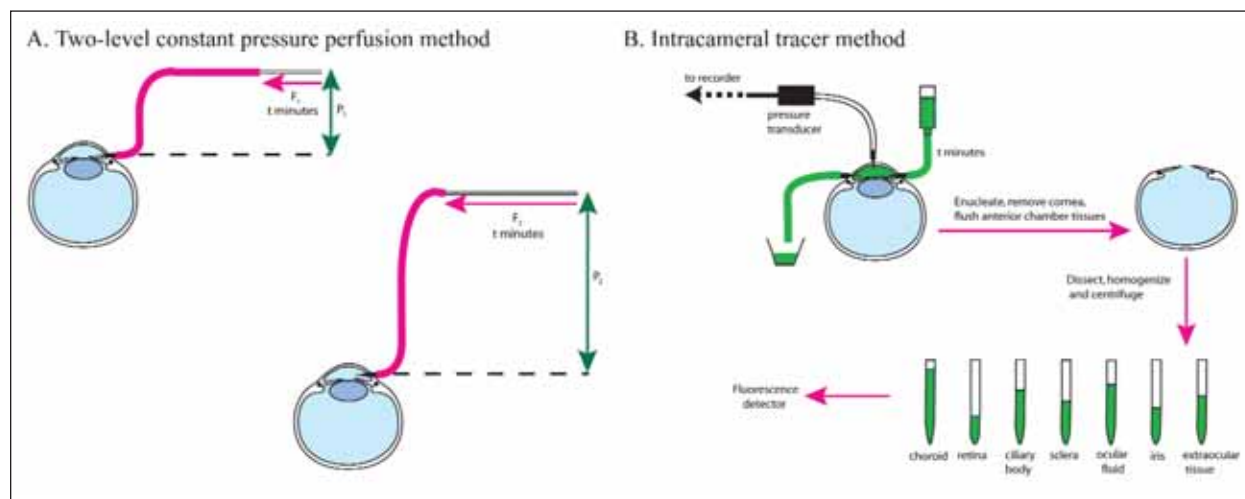


Figure 3. During the measurement of outflow facility by the two-level, constant-pressure perfusion method (Ctot), a cannula connected to a reservoir filled with mock aqueous humor is inserted into the anterior chamber. The end of the cannula is placed at an exact height above the eye to establish a specific pressure (P1) above the spontaneous IOP. The amount of fluid flowing into the eye during time t to maintain P1 is F1. Next, the cannula's end is raised to a new height to establish a new pressure (P2), and, during the next time interval (t), the flow of fluid into the eye to maintain P2 is F2. C is calculated as $(F2-F1)/(P2-P1)$ (image A). During the measurement of uveoscleral outflow with infusion of an intracameral tracer, a tracer of large molecular weight such as fluorescein isothiocyanate dextran is infused into the anterior chamber of an anesthetized animal at a pressure close to spontaneous IOP. The IOP is monitored with a pressure transducer and recorder. After a 30- to 60-minute time interval (t), infusion is stopped, the eye is enucleated, the cornea is removed, and the surfaces of the iris and lens are rinsed to remove all of the intracameral tracer. The eye is dissected into the tissues as shown. The supernatant from each homogenized and centrifuged sample is measured with a fluorescence detector. Uveoscleral outflow (F_u) is calculated by dividing the sum of all the tissue-tracer values, expressed as equivalent volumes of aqueous (ΣV), by the infusion time (t), $F_u = \Sigma V/t$ (image B). (This image was redrawn with permission from Figure 16 and 18 in Toris CB, Yablonski ME, Tamesis R. Aqueous humor dynamics. In: Choplin N, Lundy C, eds. *Atlas of Glaucoma*. 2nd ed. Colchester, Essex, UK: Informa UK Ltd.; 2007:13-27.)

the blood through the uveoscleral pathway and vortex veins, thus resulting in an overestimate of trabecular outflow.

UVEOSCLERAL OUTFLOW

Overview

Uveoscleral outflow is the drainage of aqueous humor from the anterior chamber into the ciliary muscle, where it seeps out of the eye in several different directions (Figure 1). The route of uveoscleral outflow is anatomically ill defined, and its flow rate is relatively independent of pressure.

Techniques for Measuring Uveoscleral Outflow

Mathematical Calculation

Currently, the only noninvasive means by which to assess uveoscleral outflow (F_u) is via mathematical calculation using Equation No. 3:

$$F_u = F - C(IOP - P_v).$$

Aqueous humor flow (F) is measured by fluorophotometry, outflow facility (C) by one of the methods

described earlier, IOP by tonometry, and episcleral venous pressure (P_v) by venomanometry.⁹ A commercially available venomanometer (Eyetechn Ltd., Morton Grove, IL) attaches to a slit lamp. One places the membrane at the device's tip on the conjunctiva near the limbus. The user identifies the episcleral veins underlying the conjunctiva with the aid of the slit-lamp biomicroscope. One raises the pressure within the membrane until the episcleral veins collapse. The pressure required to cause the vessels' collapse is read off the dial on the side of the device; it is a measure of episcleral venous pressure.

One limitation of the calculation method for uveoscleral outflow is the large standard deviations generated because of the inherent variability in each parameter in the equation. Many subjects are needed to achieve sufficient power to detect clinically relevant differences between experimental and control groups. Another limitation is that calculated uveoscleral outflow can vary tremendously depending on which value of episcleral venous pressure is used in the equation. It

is difficult to obtain an accurate measurement of Pv. For this reason, a value of 9 or 10 mm Hg¹⁰ is often used in the equation with the assumption that the value is unchanged during the course of a study. If Pv were to change, one might draw erroneous conclusions concerning the cause of a response in IOP.

Despite its limitations, the mathematical calculation of uveoscleral outflow has provided reasonable explanations for differences in IOP with respect to aging, pharmacological drugs, clinical syndromes, and surgical procedures. In the end, it is the relative changes in uveoscleral outflow, not necessarily its absolute value, that are of greater clinical importance. For example, research has shown exfoliation syndrome to be associated with reduced uveoscleral outflow when compared to age-matched, healthy control subjects.¹¹ From a physiological perspective, it would be preferable to treat the area of pathology than simply to prescribe the drug with the best effect on IOP. As a class, prostaglandin analogs may be a good treatment for exfoliation syndrome, because uveoscleral outflow is increased in patients treated with these drugs.¹²

Invasive Methods

Two invasive methods are used to measure uveoscleral outflow. They are more direct than the mathematical calculation, but they cannot be used in clinical studies. The "intracameral tracer method" (Figure 3B) involves infusing a radioactive or fluorescent tracer into the anterior chamber at a set pressure and for a specific period of time. The total amount of tracer found in the uvea and sclera during the specified time interval is assumed to be uveoscleral outflow. If the time interval is excessive, some tracer can exit the globe and be lost to analysis. Under these circumstances, uveoscleral outflow would be underestimated. Enucleation of the eye makes this method unrepeatable.

The "indirect isotope method" involves infusing a radioactive tracer in the anterior chamber and monitoring the rate of the tracer's appearance in the blood (trabecular outflow) and the rate of the tracer's disappearance from the anterior chamber (aqueous flow). Uveoscleral outflow is the difference between aqueous flow and trabecular outflow. This method is advantageous in that changes in uveoscleral outflow can be assessed over time. Its invasive nature, however, precludes its use in clinical studies.

SUMMARY

Many methods are available to assess aqueous humor outflow. The noninvasive methods are indirect, highly variable, and fraught with many limitations and

assumptions. The invasive methods require anesthesia, may damage the eye, are usually terminal, and are also laden with limitations and assumptions. Nevertheless, these methods are valuable tools in the study of outflow in the healthy and the diseased eye. They have provided clinicians with a better understanding of diseases that affect and treatments that reduce IOP. Such information may be useful in selecting specific treatments or combinations of treatments for glaucoma or ocular hypertension. □

This work was supported in part by an unrestricted grant from Research to Prevent Blindness, Inc. (New York, NY).

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