Flow of Aqueous Humor in Humans

[The Friedenwald Lecture]

Richard F. Brubaker

Figure 1 shows a human eye without its aqueous circulation. The cornea is thickened, the anterior chamber is absent, the iris is partly atrophic, and the lens is cataractous. The picture serves as a reminder of the dependency of the health of the eye on the continuous supply of aqueous humor that circulates through its chambers. It is surprising that it was not known until recently that aqueous humor was formed continuously and drained.

Does Aqueous Humor Circulate?

Early in this century, the aqueous humor was regarded as a stagnant fluid, but several important observations laid this misconception to rest. The first was the experiment done by Seidel and published in 1921 in which he infused indigo carmine from a reservoir through a cannula into the anterior chamber of the rabbit eye. When the reservoir was lowered, aqueous humor entered the cannula and displaced the dye. When the reservoir was raised to create a pressure over 15 mmHg, the dye entered the anterior chamber and appeared in the episcleral veins. Seidel concluded correctly that aqueous humor was formed continuously and drained.

Boerhaave may have been the discoverer of the aqueous veins, but it was Ascher in 1942 who observed a clear fluid in veins of the episclera and demonstrated by means of external compression with a glass rod that these veins were interconnected with veins containing blood. In 1946, it was shown that these vessels contained aqueous humor by injecting fluorescein intravenously and observing the dye enter the anterior chamber and subsequently the aqueous veins. In 1951, an aqueous vein was identified in a living human eye, and after enucleation, it was demonstrated with a neoprene cast that there was a direct connection between that vessel and Schlemm's canal.

The major hurdle in studies of the aqueous circulation now had been cleared, and scientists during the rest of this century busied themselves answering other questions about the system, such as: (1) what is the rate of aqueous flow, (2) how is aqueous formed, (3) does flow vary with conditions, and (4) how is flow regulated?

What Is the Rate of Aqueous Flow?

By the middle of the century, a technique was described for quantifying the rate of flow of aqueous humor in the human eye. This method was accomplished by measuring the kinetics of unbound fluorescein in the plasma and the fluorescence of the anterior chamber after intravenous injection of fluorescein. It was the first quantitative method of measuring aqueous flow that was suitable for human subjects.

Techniques of Measuring Flow in Humans

About the time of this classic experiment, other investigators were devising ingenious techniques for measuring the rate of aqueous humor flow in the living eye. Many of these techniques used a needle or cannula that could be connected to the anterior chamber, permitting either drainage of aqueous humor at various pressures or infusion of the fluid at measured rates and pressures. A common technique was to infuse a tracer, either systemically or intraocularly, and observe its appearance or disappearance from the eye or its appearance in the systemic circulation. Many of these techniques had limited application in living human eyes, thus stimulating the development of alternatives that required neither punctures nor tracers.

The most noteworthy of these was tonography, developed by Grant and based on the theoretic work of Friedenwald. Variations of tonography such as the perilimbal suction cup technique or PV tonography were devised by others. However, Grant’s technique became the standard for measuring outflow resistance and was the most convenient method of estimating aqueous humor flow in humans.

Several other techniques were devised for use in the human eye. In one, radioactively labeled albumin was...
injected into the anterior chamber, and the rate of disappearance of gamma radiation was observed by means of an external scintillation counter. Goldmann, as mentioned previously, did studies in which systemically administered fluorescein was observed to enter and leave the anterior chamber. The concentration of unbound fluorescein was monitored in the plasma, and the rate of aqueous flow was calculated by a complicated algorithm. Goldmann’s technique was used and enhanced by other investigators. A method of photographing the “pupillary bubble” in eyes was devised after instillation of fluorescein and pilocarpine. Using geometry, the rate of flow of aqueous humor was calculated from the posterior chamber into the anterior chamber. An important development in the measurement of aqueous flow in humans was the invention of a technique for measuring the rate of clearance of topically applied fluorescein.

Corneal Depot Method of Maurice

Others introduced fluorescein into the eye by topical administration. Their work was hampered, however, by two problems. The first was the problem of measuring fluorescence in the cornea and anterior chamber; the second was the problem of deducing aqueous flow from the observed changes in fluorescence. The first problem was solved by the construction of the first objective slit-lamp fluorometer. Later, a more accurate instrument was developed for clinical work. The second problem was studied by a number of investigators who devised several new experimental approaches.

The use of systemic tracers that are cleared rapidly by the kidney was advocated. Flow could be deduced by the rate of disappearance from the eye after renal clearance of the plasma. Others later devised a detailed multicompartmental pharmacokinetic model of the eye that included the vitreous, the posterior chamber, and the anterior chamber. Friedenwald, in his last paper, published posthumously, was the first to include the corneal stroma as a separate and distinct compartment. This work, done with Becker, was the basis for Becker’s subsequent development of a method to measure flow that required a single puncture of both eyes at the completion of the experiment, leaving the eye undisturbed during the critical period.

It was Maurice who first realized that the stroma could serve as a depot from which fluorescein could be introduced slowly into the anterior chamber. His method clarified the important role of the cornea in affecting the kinetics of topically applied drugs and tracers. Maurice’s technique now is used most frequently for measuring the rate of formation of aqueous humor in the human eye. All of the data that follow were acquired with his technique or slight modifications of it.

The corneal depot method of Maurice can be described briefly as follows. Fluorescein is introduced into the cornea either by iontophoresis or by applying a high concentration in the conjunctival cul-de-sac. Having penetrated the epithelium and entered
Table 1. Aqueous flow in human eye measured with topically applied fluorescein

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Method</th>
<th>Year</th>
<th>Ref.</th>
<th>No. of subjects</th>
<th>Flow µl/min (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jones &amp; Maurice</td>
<td>Iontophoresis</td>
<td>1966</td>
<td>40</td>
<td>10</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>Holm</td>
<td>Photogrammetry</td>
<td>1968</td>
<td>38</td>
<td>17</td>
<td>3.1 ± 1.6*</td>
</tr>
<tr>
<td>Holm &amp; Wiebert</td>
<td>Photogrammetry</td>
<td>1968</td>
<td>332</td>
<td>11</td>
<td>3.4 ± 1.7*</td>
</tr>
<tr>
<td>Bloom et al.</td>
<td>Iontophoresis</td>
<td>1976</td>
<td>173</td>
<td>19</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>Yablonski et al</td>
<td>Drops</td>
<td>1978</td>
<td>47</td>
<td>15</td>
<td>2.5 ± 0.8±</td>
</tr>
<tr>
<td>Coakes and Brubaker</td>
<td>Iontophoresis</td>
<td>1978</td>
<td>128</td>
<td>20</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>Brubaker et al</td>
<td>Iontophoresis</td>
<td>1981</td>
<td>213</td>
<td>113</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>Schenker et al</td>
<td>Drops</td>
<td>1981</td>
<td>333</td>
<td>14</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Araie</td>
<td>Drops</td>
<td>1983</td>
<td>334</td>
<td>11</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Coakes and Siah</td>
<td>Iontophoresis</td>
<td>1984</td>
<td>335</td>
<td>22</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>Hayashi et al</td>
<td>Drops</td>
<td>1989</td>
<td>248</td>
<td>12</td>
<td>2.2 ± 0.4</td>
</tr>
</tbody>
</table>

* Technique requires use of miotic. † Calculated from \( k_o \) assuming chamber volume = 200 µl and \( k_e = k_o \).

Aqueous Flow Measured With Topically Applied Fluorescein

Based on the rate of appearance of a "pupillary bubble" as described previously. The assumptions of this technique, which is based on geometry, differ from the assumptions of Jones and Maurice's technique. Also, this technique requires the use of pilocarpine to produce miosis and a well-defined pupillary bubble. Given the fact that pilocarpine has a slight stimulatory effect on the rate of aqueous flow, the agreement between the results derived from the photogrammetric technique and the fluorescein clearance technique are remarkably good. Also, another technique, in which radioactive albumin was injected intracamerally into humans, serves as an independent confirmation of the accuracy of Maurice's method.

A recent analysis of a group of more than 300 normal subjects (3–83 years of age) was done using the scanning ocular fluorophotometer (Table 2). Flow was calculated from clearance of fluorescein applied 6 hr earlier. The rate of aqueous flow, determined from 8 AM to 4 PM in one eye of each subject was 2.75 ± 0.63 µl/min (mean ± standard deviation). The 2.5 percentile of this distribution was 1.78 µl/min, and the 97.5 percentile was 4.26 µl/min. Aqueous flow in the morning from 8 AM to noon was higher, 2.86 ± 0.73, and from noon to 4 PM was lower, 2.63 ± 0.57, an 8% difference (P < 0.001).

Comparisons were made between simultaneous measurements of the two eyes of the same subject. The coefficient of variation of the difference in flow between the two eyes was 15%. When the flow of an eye was significantly lower than the mean, the second eye was measured.

Table 2. Rate of aqueous flow, normals, daytime

| Number subjects | 314 |
| Number eyes     | 314 |
| Age range       | 5–83 years |
| Flow, mean      | 2.75 µl/min |
| SD              | 0.63 µl/min |
| 2.5th percentile| 1.78 µl/min |
| 97.5th percentile| 4.26 µl/min |
eye of a subject was compared with the same eye measured on another day at the same time of day, the coefficient of variation of the difference was 21%. The coefficient of variation in one eye of normal subjects 5 to 38 years old was 23% (Table 3). These coefficients are useful for calculating the probability of a type II statistical error for various sample sizes when comparisons are made in different kinds of study protocols. Table 4 depicts several examples of these calculated sample sizes.

### How Is Aqueous Formed?

The last three decades span a period during which scientists have made great progress in understanding the biophysical basis of water transport. Discoveries and techniques in many tissues have been applied to the problem of determining how aqueous humor is formed.

**Stages of Aqueous Formation**

The formation of aqueous humor involves three processes that occur in series. First, there is flow of blood to the ciliary processes in the anterior uvea. Second, some portion of the plasma that reaches this vascular bed is filtered through the fenestrated capillaries into the interstitial spaces between the vessels and the ciliary epithelia. Third, the major portion of the filtered fluid is secreted by the ciliary epithelia into the posterior chamber (Fig. 2).

**Blood flow to the ciliary processes:** In humans, the concentration of ascorbate in the anterior chamber is approximately 20-fold higher than that in plasma. Humans cannot synthesize ascorbic acid, because of a lack of the enzymes D-glucuronoreductase and L-glucuronooxidase that catalyze the terminal steps of its synthesis. Thus, the relative rates of plasma flow and aqueous formation determine the upper limit of the concentration of ascrobate in the anterior chamber. If the aqueous-plasma ascorbate ratio and the rate of aqueous humor formation are known, then the minimal rate of blood flow to the ciliary processes can be calculated. This calculation yields an estimated rate of 115 μl/min.

**Ultrafiltration in tissues:** It was estimated that approximately 4% of the plasma entering the pars plicata is filtered into the tissue spaces of the ciliary processes. The rate of filtration is therefore 2.7 μl/min, in good agreement with the estimated rate of aqueous formation. This research also suggests that a small portion of the filtrate never enters the posterior chamber but moves through the uvea to leave the eye by the uveoscleral outflow pathway.

**Secretion by ciliary epithelia:** The major portion of the filtrate is available to the ciliary epithelia, involved in the final step of aqueous formation. This process occurs against an oncotic pressure gradient. This energy-consuming secretion is done by approximately 4 million nonpigmented epithelial cells that have an estimated combined volume of 8 μl. If each cell contributes an equal share to the formation of aqueous humor, each cell must secrete a volume of aqueous per minute equal to one third of its own intracellular volume.
There are similarities and differences in the functions of the pars plicata of the eye and the nephron of the kidney, as a comparison of the two tissues will illustrate. The blood flow to the kidney is about four times its weight per minute, whereas the blood flow to the pars plicata is about twice its weight per minute. The percent of the plasma flow to the kidney that is filtered by the glomeruli is approximately 24%. By contrast, only 4% of the plasma flow to the pars plicata is filtered. The 2.4 million nephrons of the kidney must absorb approximately 180 l of fluid per day, fluid that is transferred from the lumen of the nephron into the blood. The average cell along the nephron transports a volume of water per minute equal to two thirds its own volume.

Because the protein concentration of the glomerular filtrate is nearly the same as the protein concentration in aqueous humor, the oncotic pressure across each of these two secreting epithelia is approximately the same. However, the renal epithelium transports water with the gradient whereas the ciliary epithelium transports water against the gradient.

Both the kidney and the pars plicata are vascular organs that transport water rapidly in comparison to their weight. The two organs differ, however, in the percentage of plasma that is filtered; the pars plicata filters a much smaller fraction of its plasma than does the glomerulus. Consequently the rate of blood flow to the kidney is a major determinant of glomerular filtration; the rate of blood flow to the eye is less a determinant of the rate of aqueous formation (Table 5). Most physiologists regard the renal tubular cells as very active transporters. As we can see by this comparison, ciliary epithelial cells are also very active transporters (Fig. 3).

Effect of Drugs

Discoveries stimulate basic research: Studies on the mechanism of formation of aqueous humor were stimulated by experiments in which the effect of various pharmacologic agents on aqueous humor formation were observed, as an example will illustrate.

Table 5. Aqueous formation, comparison to renal absorption

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Kidney</th>
<th>Eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood flow, % of weight/min</td>
<td>420%</td>
<td>227%*</td>
</tr>
<tr>
<td>Percent of plasma filtered</td>
<td>24%</td>
<td>4%*</td>
</tr>
<tr>
<td>Concentration of protein</td>
<td>fittest</td>
<td>aq = 0.03%</td>
</tr>
<tr>
<td>Oncotic gradient</td>
<td>With flow</td>
<td>Against flow</td>
</tr>
<tr>
<td>Vol. transport/min/cell vol.</td>
<td>0.72</td>
<td>0.3</td>
</tr>
<tr>
<td>Vol. transport/cm²/day</td>
<td>1.2 ml</td>
<td>0.6 ml</td>
</tr>
</tbody>
</table>

* From Bill, ref. 60.

In 1950, the synthesis of a compound (2 acetylamino, 1,3,4 thiadiazole-5-sulfonamide) was reported for which American Cyanamid was awarded US patent #2,554,816 the following year. Lederle acquired this compound and referred to it as “compound 6063” (acetazolamide, Diamox). It was found that acetazolamide was an efficient inhibitor of carbonic anhydrase.

Investigators wasted no time in determining the systemic effects of acetazolamide. It ameliorated congestive heart failure and suppressed gastric and pancreatic secretion. It was known that carbonic anhydrase was present in the anterior uvea of the rabbit eye. Also, Friedenwald, in the first Proctor award lecture, suggested that the transport of electrons was linked to solute transport in the formation of aqueous humor, giving bicarbonate a likely role in this process. These facts may have persuaded investigators to test this carbonic-anhydrase inhibitor for its clinical effect in glaucoma.

Several groups tested acetazolamide, publishing their results in 1954. These investigators observed a reduction of intraocular pressure in normal human subjects and in patients with glaucoma without a significant change in the tonographic C value. They concluded that acetazolamide reduced the rate of aqueous humor formation. The discovery of acetazolamide’s effect was not only clinically important but also stimulated research on aqueous secretion.

Drugs with insignificant effects: Over the last 40 years, many drugs have been tested in the human eye for their effect on aqueous humor formation. The results of these studies indicate that most drugs have insignificant effects. Table 6 lists drugs that affect the
pupil or intraocular pressure but have no clinically significant effect on flow.

Pilocarpine, the oldest treatment for glaucoma, may have a slight stimulating effect on aqueous humor formation.\(^5\) Adrenergics, such as phenylephrine\(^5,7\) (an \(\alpha_1\)-selective agonist) and thymoxamine\(^7,8\) (an \(\alpha_2\)-selective antagonist), have no significant effect on aqueous humor formation when applied topically to the human eye. The prostaglandins, recently demonstrated to lower intraocular pressure\(^79-86\) by improving outflow,\(^87,88\) have no measurable effect on aqueous flow.\(^89\) Topically applied corticosteroids can raise intraocular pressure but have no effect on aqueous flow in the human eye.\(^90-92\)

**Stimulating drugs:** Two classes of drugs, catecholamines with \(\beta\)-adrenergic activity and systemically administered corticosteroids, have been shown to stimulate the rate of aqueous humor flow in humans. Stimulation of flow by \(\beta\)-adrenergic agonists is not seen consistently in all species or under all experimental conditions. Thus, there has never been a consensus as to their effects. Likewise, the evidence that systemic corticosteroids can increase the rate of aqueous flow is not compelling.

Epinephrine was suggested to be essential for the formation of aqueous humor.\(^93\) When bilateral adrenalectomies were rabbits, aqueous flow into a cannula was *stimulated* by the intravenous administration of this hormone. However, other workers concluded, on the basis of the clinical effects of epinephrine, that its ocular hypotensive effect could be attributed partly to an improvement of outflow of the aqueous humor\(^94,95\) and partly to a *reduction* of the rate of aqueous humor formation.\(^96\) Goldmann\(^96\) briefly mentioned the possibility that "Glaucosan" might have an *inhibitory* effect on secretion.

The idea that epinephrine suppressed flow was supported by clinical studies in which flow was calculated indirectly from tonography.\(^97-100\) This idea also gained additional support from early fluorophotometric studies.\(^101,102\) It is now known, on the basis of clinical studies and tissue culture studies, that epinephrine’s ocular hypotensive effects can be attributed to its \(\beta\)-adrenergic action on the outflow apparatus and most probably the trabecular cell.\(^103-110\)

Several studies showed that the acute administration of \(\beta\)-adrenergic agonists is associated with an *increase* in the rate of clearance of fluorescein from the anterior segment.\(^111-114\) These investigators concluded that \(\beta\)-adrenergic agonists stimulate the rate of aqueous humor formation in humans, a conclusion that is supported by evidence from experiments in other primates.\(^115,116\) As will be discussed later, this pharmacologic class has greater activity in sleeping subjects than in awake subjects.

Topical application of corticosteroids has no effect on aqueous flow,\(^90-92\) but some studies suggest that systemic steroids can have an affect.\(^91,117-126\) Recently, eight normal subjects were studied by fluorophotometry, and it was concluded that flow was doubled by administering oral hydrocortisone.\(^127\) Surprisingly, intraocular pressure did not change. The only fluorophotometric change was an increase in the rapidly decaying exponent (coefficient "B" of Jones and Maurice), an exponent that can be difficult to determine\(^90,128\) by the method used. The evidence for corticosteroid influence deserves a fresh look with the best available techniques.

**Inhibiting drugs:** By contrast to the dearth of compounds that stimulate aqueous formation, many have been discovered that inhibit formation. These include such diverse agents as ouabain,\(^129\) chola toxin,\(^130-132\) vanadate,\(^133-137\) phenobarbital,\(^138\) propranolol,\(^139\) halothane,\(^139\) \(\delta\)-tetrahydrocannabinol,\(^140-145\) demeclocycline,\(^146\) colforsin (forskolin),\(^147-155\) atrial natriuretic peptide,\(^156-162\) phorbol esters,\(^162\) metyrapone,\(^117,126\) and cyclic guanosine monophosphate.\(^163\) The concentration, route of administration, or toxicity of most of these compounds either has not permitted clinical trials in humans or was insufficiently effective at maximal doses.

Three pharmacologic classes are clinically useful. These are the carbonic-anhydrase inhibitors, the \(\beta\)-adrenergic antagonists, and the \(\alpha_2\)-adrenergic agonists.

Before the technique of Jones and Maurice was used widely, other techniques showed the aqueous suppressing effects of acetazolamide and other carbonic-anhydrase inhibitors.\(^164-172\) Later, the effect was confirmed with the technique of fluorescein clearance. For example, the effect of acetazolamide was tested, and a 38% suppression of flow was observed.\(^173\) In addition, a 27–40% suppression was found, and it was observed that the carbonic-anhydrase inhibitor was partly additive to the \(\beta\)-adrenergic blocker, timolol.\(^174\) In other groups of normal subjects, approximately a 20% suppression of flow was seen.\(^175,176\)

Of current interest is the development of topically

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**Table 6. Aqueous flow: drug effects**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymoxamine</td>
<td>Lee, Nagataki</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>Anselmi, Lee</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>Rice, Lee</td>
</tr>
<tr>
<td>Dexmethasone</td>
<td>Kerstetter</td>
</tr>
<tr>
<td>(\mathrm{Pgf}_2\alpha)-ester</td>
<td>Adams</td>
</tr>
<tr>
<td>Caffeine</td>
<td></td>
</tr>
</tbody>
</table>

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\(^{\text{Vol. 32 INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE / December 1991}}\)
applicable carbonic-anhydrase inhibitors to avoid the side effects associated with systemic administration. It will be interesting to compare the aqueous-suppressing effects of the most potent of the topical agents to the systemic ones. A direct comparison using a sensitive flow-measuring technique should be able to separate the local effects of this class of drugs from any renal or other systemic effects on intraocular pressure.

In 1958, an inhibitor of adrenergic receptors, dichloroisoproterenol, was described. It was found that this β-adrenergic antagonist reduced intraocular pressure and resistance to outflow in the rabbit eye but had no effect on aqueous flow. In 1967, propranolol, a β-adrenergic antagonist, when administered systemically to humans, lowered intraocular pressure. The following year, others reported that propranolol applied topically to humans lowered intraocular pressure. Some years later, it was shown that timolol lowered intraocular pressure in a glaucomatous rabbit model. About the same time, others demonstrated that timolol was an effective topical ocular hypotensive agent in humans. Timolol subsequently became the leading drug for the treatment of glaucoma, a position that it still maintains. Before competition from other β-adrenergic antagonists, as many as 7 million prescriptions for timolol were written yearly in the United States.

When we measure the effect of timolol on the clearance of fluorescein, we observe consistent effects in human subjects. Other β-adrenergic antagonists have a similar effect. Aqueous flow in the fellow eye of a timolol-treated eye can be suppressed as much as 10%, perhaps as a result of systemic distribution of the drug. Thus, the percent suppression in a given experiment depends on whether a treated eye is compared with a placebo-treated fellow eye at the same time or with placebo treatment on a different day. As will be discussed later, the effect of timolol is dependent on the time of day at which it is tested.

There is a slow adaptation to the effect of timolol after chronic administration. In one study, 50% of the effect was lost after 1-year of treatment (Table 7).

<table>
<thead>
<tr>
<th>Table 7. Aqueous flow: drug effects (β adrenergic antagonists, adaptation to effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>Placebo*</td>
</tr>
<tr>
<td>Timolol 1/2%, single dose*</td>
</tr>
<tr>
<td>Washout†</td>
</tr>
<tr>
<td>Timolol 1/2%, 1 week†</td>
</tr>
<tr>
<td>Timolol 1/2%, 1 year†</td>
</tr>
</tbody>
</table>

* Coakley.† Brubaker.

This effect was observed in another study in which subjects receiving timolol for 4 years discontinued use of the drug for 6 weeks. In these subjects, return of flow to pretreatment rates was gradual, but complete recovery to the normal rate was observed.

The onset of timolol's effect is rapid, but after chronic use, its offset is prolonged. It was observed that it took several weeks before full recovery of flow after timolol was discontinued in chronic users (Table 8). These data suggest that the terminal half-life of timolol's effect is at least 1 week.

A similar study of levobunolol was done. The terminal half-life after 2 weeks' use of this drug was 5 days. By contrast, the effect of the cardioselective β-blocker betaxolol disappeared with a terminal half-life of 2 days.

A dose–response study was done of levobunolol and betaxolol. An effect of levobunolol was observed even after a 30-fold dilution of its clinical concentration (0.5%). For 0.5% betaxolol, a tenfold dilution produced a detectable effect.

Additivity of the three classes of suppressors of aqueous formation has been studied. The timolol-suppressed eye is able to respond to the effects of acetazolamide and vice versa. By contrast, the acutely treated timolol-suppressed eye does not respond to apraclonidine, which normally suppresses aqueous formation. In acute, daytime studies, these two drugs have the same effect in combination as either alone. However, apraclonidine in the chronically timolol-treated eye has a measurable and clinically useful effect. These data suggest that the eye undergoes adaptive changes over a long period of timolol treatment. Understanding these adaptations is a challenge of ongoing research.

In 1962, chemists at Boehringer, Ingelheim synthesized an imidazole derivative that was termed “Catapresan” (clonidine). This pharmacologic agent was found to have relatively selective α2-adrenergic activity. A few years later, it was administered systemically to human subjects, and intraocular pressures were

<table>
<thead>
<tr>
<th>Table 8. Aqueous flow: drug effects (β adrenergic antagonists, timolol withdrawal)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>Timolol, average 4 years</td>
</tr>
<tr>
<td>Discontinue timolol expl. eye</td>
</tr>
<tr>
<td>2 days</td>
</tr>
<tr>
<td>4 days</td>
</tr>
<tr>
<td>7 days</td>
</tr>
<tr>
<td>13 days</td>
</tr>
</tbody>
</table>

Data from Schlecht.
lowered. Administered topically, it was observed that lowering of intraocular pressure occurred, attributed to an improvement in the facility of outflow. Subsequent studies confirmed that this agent lowers intraocular pressure, but it was not clear from these studies whether clonidine improved outflow, suppressed inflow, lowered episcleral venous pressure, or acted centrally on arterial blood pressure.

Fluorophotometry was used to study the effect of clonidine on aqueous humor flow in normal human subjects, and a consistent difference was observed in aqueous flow (2.4 µl/min in the placebo-treated eye compared with 1.9 in the clonidine-treated eye). Subsequently, a derivative of clonidine, apraclonidine (p-aminoclonidine), was found to lower intraocular pressure and to be useful in preventing a spike of intraocular pressure after laser treatments. Apraclonidine, like its parent compound, lowered the rate of aqueous humor formation (Table 9).

The discovery of the effect of &beta;-adrenergic antagonists and &alpha;2-selective adrenergic agonists stimulated additional work into the mechanism of the formation of aqueous humor.

**Does Flow Vary With Conditions?**

One of the current avenues of research is to determine the consequences of varying conditions on the rate of aqueous humor formation.

**Age**

A large number of subjects were studied for the effect of age on aqueous humor formation. Flow was calculated by indirect means, namely by a combination of tonometry and tonography using Goldmann's formula:

\[
\text{Flow} = (\text{intraocular pressure} - 10 \text{ mmHg}) \times (\text{facility of outflow})
\]

The flow of aqueous was steady until age 60 yr, but it dropped appreciably thereafter. Before age 60 yr, the flow was approximately 2.0 µl/min; between 61-70 yr, it was 1.3 µl/min; and after 70 yr, it was 1.0 µl/min.

**Table 9. Aqueous flow: drug effects (α₂ adrenergic agonists)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Flow, µl/min (daytime)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo*</td>
<td>2.40 ± 0.70</td>
<td></td>
</tr>
<tr>
<td>Clonidine 1/8%*</td>
<td>1.90 ± 0.60</td>
<td>21%</td>
</tr>
<tr>
<td>Placebo†</td>
<td>2.66 ± 0.53</td>
<td></td>
</tr>
<tr>
<td>Apraclonidine 1%†</td>
<td>1.76 ± 0.43</td>
<td>34%</td>
</tr>
<tr>
<td>Placebo‡</td>
<td>2.84 ± 0.61</td>
<td></td>
</tr>
<tr>
<td>Apraclonidine 1/2%‡</td>
<td>2.00 ± 0.54</td>
<td>30%</td>
</tr>
</tbody>
</table>

In 1981, we studied a group of 113 normal volunteers ranging in age from 20–83 yr. We observed a slight decrease of flow with age (2.4% per decade) but did not notice the precipitous fall observed earlier. More recently, we examined more than 300 normal volunteers whose ages ranged from 5–83 yr. This group also showed a slight decrease of flow with age (3.2% per decade if those younger than 10 yr were excluded). Thus, from ages 10–80 yr, an average person's aqueous flow would decline approximately 25%.

An age-dependent loss of ciliary epithelial cells in humans has not been described. Such an occurrence could explain the gradual reduction of aqueous flow. Some authors showed a loss of trabecular cells per decade in humans of 5.8%, and others found a 3.5% loss of corneal endothelial cells per decade. The age dependency of the population of ciliary epithelial cells should be studied. If it were found that aqueous formation parallels the number of secreting cells, it would suggest that the normal rate of aqueous formation is dependent on cell count rather than on neural or hormonal stimulation. Alternatively, the decline of aqueous flow could be a result of the changes observed in the fine structure of aging ciliary epithelial cells.

**Table 10. Aqueous flow µl/min, 8 AM–4 PM, normal human subjects (n = 314)**

<table>
<thead>
<tr>
<th>Age</th>
<th>Mean</th>
<th>SD</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–9</td>
<td>2.40</td>
<td>0.67</td>
<td>10</td>
</tr>
<tr>
<td>10–19</td>
<td>2.92</td>
<td>0.60</td>
<td>18</td>
</tr>
<tr>
<td>20–29</td>
<td>2.88</td>
<td>0.67</td>
<td>132</td>
</tr>
<tr>
<td>30–39</td>
<td>2.80</td>
<td>0.57</td>
<td>58</td>
</tr>
<tr>
<td>40–49</td>
<td>2.58</td>
<td>0.48</td>
<td>20</td>
</tr>
<tr>
<td>50–59</td>
<td>2.69</td>
<td>0.50</td>
<td>19</td>
</tr>
<tr>
<td>60–69</td>
<td>2.49</td>
<td>0.61</td>
<td>35</td>
</tr>
<tr>
<td>70–79</td>
<td>2.48</td>
<td>0.51</td>
<td>19</td>
</tr>
<tr>
<td>80–89</td>
<td>2.20</td>
<td>0.68</td>
<td>3</td>
</tr>
</tbody>
</table>

In 1981, we studied a group of 113 normal volunteers ranging in age from 20–83 yr. We observed a slight decrease of flow with age (2.4% per decade) but did not notice the precipitous fall observed earlier. More recently, we examined more than 300 normal volunteers whose ages ranged from 5–83 yr. This group also showed a slight decrease of flow with age (3.2% per decade if those younger than 10 yr were excluded). Thus, from ages 10–80 yr, an average person's aqueous flow would decline approximately 25%.

An age-dependent loss of ciliary epithelial cells in humans has not been described. Such an occurrence could explain the gradual reduction of aqueous flow. Some authors showed a loss of trabecular cells per decade in humans of 5.8%, and others found a 3.5% loss of corneal endothelial cells per decade. The age dependency of the population of ciliary epithelial cells should be studied. If it were found that aqueous formation parallels the number of secreting cells, it would suggest that the normal rate of aqueous formation is dependent on cell count rather than on neural or hormonal stimulation. Alternatively, the decline of aqueous flow could be a result of the changes observed in the fine structure of aging ciliary epithelial cells.

**Intraocular Pressure**

Two mutually exclusive hypotheses have been entertained about the effects of intraocular pressure on
the rate of aqueous humor formation. One hypothesis is that aqueous formation is insensitive to moderate changes of intraocular pressure. Tonography depends on this assumption. The other hypothesis is that the eye regulates its intraocular pressure at a steady level by making compensatory adjustments in the rate of aqueous formation.

The latter hypothesis was addressed in a paper published in 1947, and it was concluded that regulation of intraocular pressure by flow was unlikely to occur.\textsuperscript{217} The author presumed that, in the hierarchy of regulated variables, the rate of aqueous flow would itself require regulation within narrow limits to maintain the health of the crystalline lens. Alternatively, regulation of intraocular pressure could occur by the outflow pathways or their recipient vessels.

Later, this author formulated a theory that predicted, in the absence of neural and humoral regulation of the vascular system of the eye, that small shifts in capillary and tissue fluid exchange in the globe would result in a net suppression of aqueous humor formation during tonography, a phenomenon he termed "pseudofacility."\textsuperscript{218} Subsequent work showed that this phenomenon exists in anesthetized monkeys\textsuperscript{13,219} and in human volunteers,\textsuperscript{220,221} but the effect is small and transient.\textsuperscript{222,223}

The effect of intraocular pressure on aqueous flow was studied by altering the intraocular pressure in human volunteers with a tilt table over periods varying from 30 min to 8 hr.\textsuperscript{224} Small changes were observed in the rate of aqueous flow as measured by fluorophotometry. The changes of fluorescein clearance could be explained entirely by the Friedenwald\textsuperscript{30} pressure-volume relationships of the globe in the absence of any change in the rate of aqueous formation.

The question of homeostasis of pressure also was studied by observing fluorescein clearance in eyes with abnormal outflow resistance. Three groups of investigators looked at aqueous flow after laser trabeculectomy (Table 11). This procedure lowers intraocular pressure by improving the facility of outflow, perhaps by stimulation of phagocytic activity of cells in the filtration portion of the trabecular meshwork.\textsuperscript{225} These investigators found no change in the rate of aqueous formation, even though intraocular pressure had been lowered significantly.\textsuperscript{226–228} In a study of persons with pigment dispersion syndrome, no difference was found between eyes that had elevated pressure and those that had normal pressure (Table 12).\textsuperscript{229} Patients were studied with myotonic dystrophy, a condition in which spontaneous intraocular pressures below 10 mmHg are encountered frequently (Table 13).\textsuperscript{230} There was no demonstrable increase or decrease in the rate of aqueous humor flow. From the results of both acute and chronic experiments, it appears that the eye does not adjust its rate of aqueous humor formation in a direction or to an extent that would have a stabilizing effect on intraocular pressure, a conclusion reached 44 years ago.\textsuperscript{217}

### Table 11. Effect of intraocular pressure on flow (trabeculectomy)

<table>
<thead>
<tr>
<th>Laser trabeculectomy</th>
<th>Effect on IOP</th>
<th>Effect on flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brubaker and Liesegang\textsuperscript{226}</td>
<td>Lowered</td>
<td>None</td>
</tr>
<tr>
<td>Araie et al\textsuperscript{227}</td>
<td>Lowered</td>
<td>None</td>
</tr>
<tr>
<td>Yablonski et al\textsuperscript{228}</td>
<td>Lowered</td>
<td>None</td>
</tr>
</tbody>
</table>

### Table 12. Effect of intraocular pressure on flow (pigment dispersion syndrome)

<table>
<thead>
<tr>
<th>Pigment dispersion syndrome</th>
<th>IOP (mmHg)</th>
<th>Flow (μl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affered eyes</td>
<td>IOP &gt; 22 (n = 25)</td>
<td>26.2 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>IOP &lt; 22 (n = 49)</td>
<td>17.3 ± 2.8</td>
</tr>
<tr>
<td>Control eyes (n = 80)</td>
<td>16.3 ± 2.9</td>
<td>2.60 ± 0.42</td>
</tr>
</tbody>
</table>

Data from Brown.\textsuperscript{229}

### Time of Day

In 1958, a detailed study of aqueous humor flow was done in the human eye at different times of day, using a peribulbar suction cup.\textsuperscript{231} The rate of aqueous flow during sleep was much lower than during waking hours. These findings were confirmed by measurements of light scattering in the anterior chamber of humans and rabbits at different times of day. The fluctuations of scattering from aqueous humor were believed to be a result of variations in the concentration of proteins in the aqueous that, in turn, were related to a circadian rhythm of the rate of aqueous humor formation. Later, others showed that rabbits had a higher rate of aqueous flow at night.\textsuperscript{232} These findings were a result of a true circadian rhythm driven by light, a rhythm that persists in constant darkness.\textsuperscript{233}

This rhythm also was studied in humans.\textsuperscript{173,234} These results indicate that the rate of fluorescein clearance during sleep is approximately one half the rate during the morning hours after awakening. Occlusion

### Table 13. Effect of intraocular pressure on flow (myotonic dystrophy)

<table>
<thead>
<tr>
<th>Myotonic dystrophy</th>
<th>IOP (mmHg)</th>
<th>Flow (μl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n = 26)*</td>
<td>7.1 ± 2.21</td>
<td>2.51 ± 0.62</td>
</tr>
<tr>
<td>Controls (n = 37)*</td>
<td>14.6 ± 3.5</td>
<td>2.54 ± 0.74</td>
</tr>
</tbody>
</table>

* Walker.\textsuperscript{230}
Table 14. Aqueous flow during sleep

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Flow, µ/l/min</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime, awake (n = 19)</td>
<td>3.10 ± 0.60</td>
<td></td>
</tr>
<tr>
<td>Night, asleep</td>
<td>1.60 ± 0.50</td>
<td>48%</td>
</tr>
<tr>
<td>Daytime, awake (n = 6)</td>
<td>3.10 ± 0.50</td>
<td></td>
</tr>
<tr>
<td>Night, asleep</td>
<td>1.40 ± 0.19</td>
<td>55%</td>
</tr>
<tr>
<td>Night, sleep deprived</td>
<td>2.30 ± 0.30</td>
<td>26%</td>
</tr>
</tbody>
</table>

Data from Reiss.234

Table 15. Aqueous flow: drug effects, day vs. night (catecholamine stimulation of flow)

<table>
<thead>
<tr>
<th>Catecholamine</th>
<th>Day</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine*</td>
<td>15%</td>
<td>47%</td>
</tr>
<tr>
<td>Isopropenol†</td>
<td>22%</td>
<td>34%</td>
</tr>
<tr>
<td>Torbutaline‡</td>
<td>2%</td>
<td>15%</td>
</tr>
</tbody>
</table>

* Topper.175
† Larson.176
‡ Gharagozloo.174

Table 16. Aqueous flow: drug effects, day vs. night (catecholamine stimulation of flow)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Daytime</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n = 19)*</td>
<td>2.26 ± 0.86</td>
<td>1.61 ± 0.40</td>
</tr>
<tr>
<td>Timolol 1/2%</td>
<td>1.58 ± 0.49</td>
<td>1.66 ± 0.40</td>
</tr>
<tr>
<td>Difference</td>
<td>30%</td>
<td>None</td>
</tr>
<tr>
<td>Pretreatment (n = 18)†</td>
<td>2.61 ± 0.83</td>
<td>1.08 ± 0.59</td>
</tr>
<tr>
<td>Timolol 1/2%</td>
<td>1.60 ± 0.28</td>
<td>1.13 ± 0.28</td>
</tr>
<tr>
<td>Difference</td>
<td>39%</td>
<td>None</td>
</tr>
</tbody>
</table>

* Topper.175
† McCannell.176

of an eye during the day or reclining during the day does not have the same effect. Although sleep-deprived subjects at night are observed to have reduced aqueous flow, sleeping subjects have the lowest rates (Table 14).234 Sleeping under a bright light at night does not eliminate the nocturnal suppression of flow (Table 15).235 The renewed interest in this circadian rhythm stimulated additional work into the question of how aqueous flow is regulated.

Drugs that affect aqueous flow can have different effects at different times of day. For example, several observers found a greater effect of catecholamines on aqueous flow in sleeping subjects than that observed during the day (Table 16).113,114,175 It has been hypothesized that the lack of an effect during daytime hours is a result of the higher level of endogenous catecholamines obscuring the effect of the topical agent. At night when catecholamine levels are lower, the effect of these drugs is unmasked.

The opposite was observed for the β-adrenergic antagonist timolol. As noted previously, it has a consistent effect when tested during the day. At night, however, it has no measurable effect (Table 17).175,176,236

The lack of an effect of timolol during sleep may be related to the existence of a baseline rate of flow that resists further suppression by any clinically useful pharmacologic agent. However, this lack of effect could be a result of the lack of timolol-blockable activity (such as stimulation by endogenous epinephrine from the adrenal or norepinephrine from ocular sympathetic nerves) in the sleeping eye.237,238

Controversing this conclusion is the fact that acetazolamide (Table 18)78 and apraclonidine (Table 19)194 are able to suppress the rate of aqueous flow in the sleeping eye.

However, humans with Horner's syndrome are still able to respond to timolol during the day.113,237,239 Presumably these eyes lack the major source of local catecholamine stimulation, although they may be hypersensitive to endogenous circulating catecholamines.

An anion-selective channel was identified recently in cultured human nonpigmented ciliary epithelial cells.240,241 The "open" probability of this isolated channel in a black lipid membrane is increased by epinephrine and decreased by β-adrenergic antagonists. These investigators hypothesized that some of the clinical effects of β-adrenergic drugs on aqueous formation can result from direct action of these drugs on this "C" channel.242 If their hypothesis is correct, we might presume from the foregoing discussion that C-channel activity is low during sleep and that it would be fruitful to look for the endogenous regulator of this channel's activity.

Table 17. Aqueous flow: drug effects, day vs. night (timolol)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Daytime</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n = 19)*</td>
<td>2.26 ± 0.86</td>
<td>1.61 ± 0.40</td>
</tr>
<tr>
<td>Timolol 1/2%</td>
<td>1.58 ± 0.49</td>
<td>1.66 ± 0.40</td>
</tr>
<tr>
<td>Difference</td>
<td>30%</td>
<td>None</td>
</tr>
<tr>
<td>Pretreatment (n = 18)†</td>
<td>2.61 ± 0.83</td>
<td>1.08 ± 0.59</td>
</tr>
<tr>
<td>Timolol 1/2%</td>
<td>1.60 ± 0.28</td>
<td>1.13 ± 0.28</td>
</tr>
<tr>
<td>Difference</td>
<td>39%</td>
<td>None</td>
</tr>
</tbody>
</table>

* Topper.175
† McCannell.176
Table 18. Aqueous flow: drug effects, day vs. night (acetazolamide)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Daytime</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral placebo (n = 18)</td>
<td>2.61 ± 0.82</td>
<td>1.08 ± 0.59</td>
</tr>
<tr>
<td>Acetazolamide 500 mg</td>
<td>2.07 ± 0.57</td>
<td>0.82 ± 0.32</td>
</tr>
<tr>
<td>Difference</td>
<td>21%</td>
<td>24%</td>
</tr>
</tbody>
</table>

Data from McCannel.196

was 35% higher in the affected eye than in the unaffected eye. A reduction of flow also was reported in the exfoliation syndrome.244 All except one of these patients had been receiving long-term timolol therapy. This experiment was done after 1-week washout without timolol. It is now known that a longer washout is necessary to permit full recovery of the eye from the flow-suppressing effects of this drug.193 Recently, 40 patients with unilateral exfoliation syndrome were studied who had never been treated with any ocular hypotensive drug.245 Tt; flow in the affected eye was 2.1 ± 0.58 μl/min, and in the unaffected eye, it was 2.3 ± 0.63 μl/min. In concurrent age-matched controls, it was 2.3 ± 0.75 μl/min. The small difference in flow in the affected eye from the other two groups was not statistically significant. The size of the sample was sufficient to detect a clinically significant effect if it were present. We now conclude that flow in the early stages of exfoliation syndrome is normal.

A few other studies were conducted with topical fluorescein to evaluate aqueous flow in abnormal eyes including those with Fuchs' uveitis syndrome,246 pigmentary glaucoma,229 myotonic dystrophy,230-247 and Horner's syndrome.113,239 In none of these studies was an abnormal rate of aqueous humor flow observed. However, two recent studies showed a reduction of flow in insulin-dependent diabetic patients.248,249 In both studies, the reduction of flow was related to the severity of the diabetes. This finding is an important one that could result in a greater understanding of the process of aqueous formation.

The results of most studies of ocular disease indicate that the secretory system of the ciliary body can continue to produce adequate amounts of aqueous humor. However, many classes of disease have not been studied by fluorescein clearance techniques, especially conditions in which inflammation or ischemia are strong components. The study of these diseases is one of the current challenges of fluorophotometric techniques.

How Is Flow Regulated?

The physiologic basis of the regulation of aqueous formation has been an important topic of study in recent years. Investigators have looked at the central and peripheral nervous system and have searched for mediators in the humoral system.

Neural Regulation

A systematic study of the control of aqueous flow in the brain was done earlier.250 Blood pressure and intraocular pressure were recorded in anesthetized cats, and the effects of stimulation of various portions of the diencephalon were examined. In 1969, differences in responses to water drinking in patients who had optic nerve transection were studied, and it was concluded that the optic nerve must serve as a regulatory pathway between a pressure-regulating center in the brain and the eye.251 After careful examination of this phenomenon, it was concluded that the hypothalamus must contain an osmoreceptor that can regulate intraocular pressure in some way.252-256 Others believed that this receptor in the rabbit must be associated with the supraoptic nucleus.257

In more recent years, central regulation of intraocular pressure in the rabbit eye was tested using the technique of ventriculocisternal perfusion.258 These investigators showed complex interactions between the brain and intraocular pressure. Despite the complexities, their results and those of others identify the sympathetic nerves as an important common pathway for signals that affect the flow of aqueous humor, especially those associated with the circadian rhythm.12,13,259-266 However, there are many other potential pathways, and these have been explored for their effects on blood flow or aqueous dynamics.267-272

Information about the role of sympathetic nerves on aqueous flow in humans is sparse. Published studies of Horner’s syndrome were examined that contained measurements of aqueous humor dynamics. There were three papers found containing data on nine eyes.273-275 The techniques used in the three studies were different, and the conclusions were preliminary. Later, 21 cases were collected of unilateral Horner’s syndrome with typical pupillary findings (Table 20). There was little difference between the intraocular pressure and the rate of flow of aqueous hu-

Table 19. Aqueous flow: drug effects, day vs. night (apraclonidine)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Daytime</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n = 20)</td>
<td>2.84 ± 0.61</td>
<td>1.15 ± 0.40</td>
</tr>
<tr>
<td>Apraclonidine 1%</td>
<td>2.00 ± 0.54</td>
<td>0.84 ± 0.28</td>
</tr>
<tr>
<td>Difference</td>
<td>30%</td>
<td>27%</td>
</tr>
</tbody>
</table>

Data from Koskela.194
Hormonal Regulation

The eye is innervated by both sympathetic and parasympathetic nerves. In addition, there are non-cholinergic, non-adrenergic neural pathways that may contribute to aqueous humor flow (Table 21). The ability of the eye to adapt to changes in sympathetic innervation suggests that there is a significant contribution from these pathways. The effect of sympathetic denervation on aqueous humor flow is small and could be explained by the effects of heightened parasympathetic activity or by changes in the extracellular matrix.

Table 20. Horner’s syndrome

<table>
<thead>
<tr>
<th>Condition</th>
<th>Normal eye</th>
<th>Horner’s eye</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime (n = 21)*</td>
<td>2.14 ± 0.57</td>
<td>2.21 ± 0.54</td>
<td>ns</td>
</tr>
<tr>
<td>Daytime (n = 12)†</td>
<td>2.13 ± 0.71</td>
<td>2.43 ± 0.60</td>
<td>ns</td>
</tr>
<tr>
<td>Night (n = 12)‡</td>
<td>1.31 ± 0.31</td>
<td>1.51 ± 0.65</td>
<td>ns</td>
</tr>
<tr>
<td>Difference</td>
<td>47%</td>
<td>38%</td>
<td>ns</td>
</tr>
</tbody>
</table>

* Wenworth.229
† Larson.113

Table 21. Melatonin and aqueous flow

<table>
<thead>
<tr>
<th>Normal subjects.</th>
<th>Urinary melatonin (mean ± SD, n = 19)</th>
<th>Aqueous flow, μl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>daytime</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without melatonin</td>
<td>4.0 ± 3.5</td>
<td>2.80 ± 0.66</td>
</tr>
<tr>
<td>With melatonin</td>
<td>353 ± 81</td>
<td>2.71 ± 0.64</td>
</tr>
<tr>
<td>Percent change</td>
<td>8700%</td>
<td>3%</td>
</tr>
<tr>
<td>P, type I error</td>
<td>&lt;0.0001</td>
<td>0.4</td>
</tr>
<tr>
<td>P, type II error</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Data from Heinrich.289

Table 22. Desmopressin and aqueous flow

<table>
<thead>
<tr>
<th>Diabetes insipidus (mean ± SD, n = 17)</th>
<th>Plasma osmolality (MOSM)</th>
<th>Urine osmolality (MOSM)</th>
<th>Aqueous flow, μl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:00-4:00 PM</td>
<td>2:00 PM</td>
<td>2:00 PM</td>
<td>12:00-4:00 PM</td>
</tr>
<tr>
<td>Without desmopressin</td>
<td>299 ± 8</td>
<td>92 ± 52</td>
<td>2.34 ± 0.69</td>
</tr>
<tr>
<td>With desmopressin</td>
<td>291 ± 6</td>
<td>619 ± 284</td>
<td>2.53 ± 0.78</td>
</tr>
<tr>
<td>Percent change</td>
<td>3%</td>
<td>573%</td>
<td>8%</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Data from Heinrich.301
The same conclusions were reached earlier by others, based on studies of the rabbit eye.304

**Progesterone:** The relationship between endogenous progesterone and the rate of aqueous humor flow in 20 nonpregnant women was examined. Over the span of a single estrus cycle, there was a large change in the plasma concentration of progesterone, but no statistically significant change in the rate of aqueous flow (Table 23). This author also looked for differences in aqueous flow between groups of men and women at various ages. No differences were found. The lack of a finding makes it doubtful that hormones unique to one sex or the other can have a significant role in regulating aqueous formation.

**Intracellular Regulation**

Cyclic adenosine monophosphate was found in the ciliary body of rabbits, and it was thought that it might play a role in the regulation of aqueous humor.305 This hypothesis was explored extensively.304,306-315 It was concluded that cyclic adenosine monophosphate is part of a major pathway in the regulation of aqueous formation. Also, there is a possibility that cyclic guanosine monophosphate is the second messenger in another important pathway for regulation of aqueous formation.315,316 Numerous other intracellular messengers were explored for their role in regulating the process of aqueous formation.317-324 The greatest challenge has been to link intracellular kinetics, measured in isolated cells and tissues, with the net rate of water transport, measured clinically.

A rare opportunity to test a specific signaling pathway came as a result of the discovery of a genetic defect in the human disease cystic fibrosis.325 This genetic defect is the deletion of a single codon for phenylalanine at position 508 in the middle of the long arm of chromosome 7.325 The somatic defect results in failure of the transduction pathway that links β-adrenergic receptors of secretory epithelial cells to a chloride-selective channel in the cell membrane (Fig. 4).326 Both receptor and channel are present, but the abnormal gene product (thought to be the regulatory portion of the channel) is unable to open the channel.326

![Fig. 4. Hypothesized defect in cystic fibrosis. Activation of β receptor of epithelial cell results in synthesis of cAMP and activation of kinase-A, but chloride-selective channel fails to open in response to the stimulus.](image)

Failure of this signal transduction pathway in affected individuals accounts for the clinical manifestations of this disease (Fig. 4).325,327 The findings in cystic fibrosis are interesting in view of the suggestion325,327,330 that chloride-selective channels may play a role in the formation of aqueous humor. Also, as mentioned previously, some claim to have found a C channel in human ciliary epithelium that can be gated directly by epinephrine and inhibited by β-adrenergic antagonists.240,241

We decided to look at a group of patients with cystic fibrosis, as proved by DNA analysis.330 We were interested in determining if the rate of aqueous formation was normal, if these patients had a normal response to a β blocker, and if they had the normal circadian pattern of aqueous flow. What we found was normal flow, a normal circadian rhythm, and a normal response to timolol. We concluded that this particular transduction pathway or its associated chloride channel are not absolute requirements for the formation of aqueous humor. Because there are many ion-conducting channels and regulatory pathways, it would have been fortuitous to have hit the major one. The experiment, as probability would predict, was negative. Perhaps genetic defects, as yet unrecognized, that affect aqueous formation do exist and remain to be discovered.

Although many potential pathways have been described that can influence the rate of aqueous humor formation, no simple system of regulation has been discovered that fits all the observed facts. Despite an incomplete understanding of the physiologic behavior of the living system, therapeutic agents have been developed that can lower intraocular pressure and are clinically useful. Continued research into this system will help the clinician use existing drugs rationally and pave the way for the discovery of new ones.

Table 23. Progesterone and aqueous flow

<table>
<thead>
<tr>
<th>Day of estrus cycle, normal, nonpregnant women (n = 20)</th>
<th>Progesterone, ng/dl (mean ± SD)</th>
<th>Aqueous flow, μl/min (8:00-12:00)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86 ± 146</td>
<td>3.12 ± 0.63</td>
</tr>
<tr>
<td>7</td>
<td>95 ± 130</td>
<td>3.27 ± 0.73</td>
</tr>
<tr>
<td>14</td>
<td>474 ± 502</td>
<td>3.33 ± 0.58</td>
</tr>
<tr>
<td>21</td>
<td>620 ± 511</td>
<td>3.12 ± 0.69</td>
</tr>
</tbody>
</table>

From Gharagozloo, unpublished data.
Summary

Based on clinical experiments with fluorophotometry, several observations can be made about aqueous flow through the chambers of the human eye.

1. The rate of flow is 2.75 ± 0.63 µl/min in normal subjects, as derived from measurements averaged during normal office hours. The normal range (95%) is 1.8 to 4.3 µl/min.
2. There is a circadian rhythm of flow, with the highest rates during morning hours, slightly lower rates during afternoon hours, and rates during sleep that are approximately one half of those during the morning. The hormonal basis for this rhythm is unknown, but it is known to be present in both eyes of persons with unilateral Horner's syndrome.
3. A slight decline of the rate occurs after age 10 yr—3.2% per decade. There is no significant difference in aqueous flow between men and women.
4. Of the hundreds of drugs that are used clinically, most are unlikely to have a significant effect on aqueous flow. Exceptions are the β-adrenergic agonists that, under certain circumstances, are able to increase flow, the corticosteroids that may have a stimulating effect on flow, and three classes of drugs that have therapeutically useful suppressing effects on flow: carbonic-anhydrase inhibitors, β-adrenergic antagonists, and α2-selective adrenergic agonists.
5. Timolol, which has a remarkably consistent suppressing effect on flow during the day, has no effect on the flow of sleeping subjects. By contrast, acetazolamide and apraclonidine are able to reduce flow: carbonic-anhydrase inhibitors, β-adrenergic antagonists, and α2-selective adrenergic agonists.
6. Acute doses of β-adrenergic antagonists and α2-agonists are not additive, but β-adrenergic antagonists and carbonic-anhydrase inhibitors are partly additive.
7. The eye adapts partly to the chronic use of timolol and recovers from its effects when it is discontinued.
8. The rate of disappearance of the effect of β-adrenergic antagonists is longer for the noncardioselective agents, such as timolol and levobunolol, but is relatively short for the cardioselective agent, betaxolol.
9. The rate of aqueous flow is insensitive to moderate changes of intraocular pressure.

Clinical studies can provide suggestive leads for more basic investigations or test specific hypotheses. Biochemical, biologic, and pharmacologic approaches in simpler, more controlled experimental conditions are necessary to determine the fundamental processes that bring about aqueous formation in the living eye. The combination of many disciplines (e.g., studying molecules, cells, tissues, organs, and the intact living system) has the best chance of furthering our understanding of the aqueous circulation.

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