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Flow of Aqueous Humor in Humans

[The Friedenwald Lecture]

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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,[1] but several important observations laid this misconception to rest. The first was the experiment done by Seidel[2,3] 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,[4] but it was Ascher[5] 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.[6-8] In 1951, an aqueous vein was identifed 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.[9]

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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.[10] 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.[11-18] 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.[19-25] 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[26-29] and based on the theoretic work of Friedenwald.[30,31] Variations of tonography such as the perilimbal suction cup technique[32] or PV tonography[33] 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

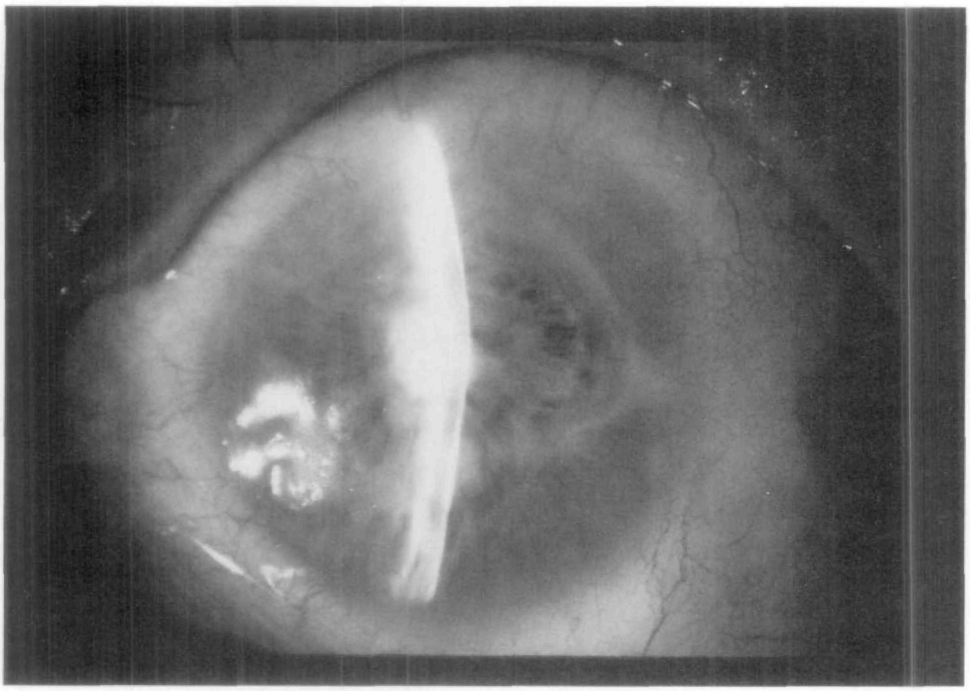

Fig. 1. Human eye that lacks aqueous humor formation. Cornea is thickened, anterior chamber is absent, iris is atrophic, and crystalline lens is cataractous.

injected into the anterior chamber, and the rate of disappearance of gamma radiation was observed by means of an external scintillation counter.[34] 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.[35,36] A method of photographing the "pupillary bubble" in eyes was devised after instillation of fluorescein and pilocarpine.[37-39] 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.[40]

Corneal Depot Method of Maurice

Others introduced fluorescein into the eye by topical administration.[41] 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.[42] Later, a more accurate instrument was developed for clinical work.[43] 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.[19,44] Others later devised a detailed multicompartmental pharmacokinetic model of the eye that included the vitreous, the posterior chamber, and the anterior chamber.[20,45] Friedenwald,[46] 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.[21]

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[40] 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[40] or by applying a high concentration in the conjunctival cul-de-sac.[47] Having penetrated the epithelium and entered

Table 1. Aqueous flow in human eye measured with topically applied fluorescein

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

* Technique requires use of miotic.

† Calculated from k_o assuming chamber volume = 200 μl and $k_o = k_f$.

the stroma, fluorescein, which is not metabolized in the eye, can disappear in one of three ways: (1) by rediffusing through the corneal epithelium and flowing away with the tears, (2) by diffusing laterally into limbal tissue, and (3) by penetrating the endothelium and entering the aqueous humor. The third pathway offers the least resistance and consequently is the major route of loss from the stroma. Once in the anterior chamber, the tracer is washed away by flowing aqueous or diffuses into the iris. This diffusional loss has been shown to account for less than 10% of the total clearance.[10]

The advantages of Maurice's technique are that it is safe, repeatable, and objective. Studies of the human eye can span 18–24 hr after the application of a single dose of fluorescein. The procedure disturbs the eye minimally, and the subject need not be constrained during the interval of measurement. Furthermore, the technique is reliable even when the rate of flow is not constant.

In one variation of Maurice's technique, fluorescein is introduced into the stroma by iontophoresis or more simply by instilling drops.[48,49] A waiting period of 6 hr or more allows the dye to become distributed uniformly in the stroma. Fluorescence is measured in the stroma and in the anterior chamber at the beginning and end of an interval. Flow is the clearance of the dye during the interval minus the diffusional loss. The technique is not applicable to eyes that lack an iridolenticular barrier between the anterior and posterior chambers. Also, the technique measures only that portion of secreted aqueous that passes into the anterior chamber.

Aqueous Flow Measured With Topically Applied Fluorescein

Table 1 summarizes the rate of aqueous flow measured with topical fluorescein by a number of workers. The principle of one of these techniques is based on the rate of appearance of a "pupillary bubble" as described previously.[38] 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,[50] 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.[34]

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).[51] Flow was calculated from clearance of fluorescein applied 6 hr earlier.[52] 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

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.[53,54] 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.[55] Thus, the relative rates of plasma flow and aqueous formation determine the upper limit of the concentration of ascorbate in the anterior chamber.[56] 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.

Table 3. Variation in flow, normals, daytime

Comparison	*Coefficient of variation*
OD vs. OS, same subject, same time	15%
OD vs. OD, same subject, diff. times	21%
One eye vs. another eye, different subjects	23%

Table 4. Sample size for comparison ($\alpha = 0.05$, $1\text{-}\beta = 0.95$)

% True difference	*R vs. L, same time**	*R vs. R, diff. time**	*Age-matched, diff. people**
10	33	61	138
20	10	17	36
30	6	9	17
40	5	6	10
50	5	5	7

* Number of subjects required.

Others determined, by microsphere injection in monkeys, that the blood flow to the ciliary processes per minute is about 227% of their weight.[57] From anatomic measurements,[58] the volume of the pars plicata in humans is approximately 68 μl. If the ratio observed in monkeys is applicable to humans, we would expect the blood flow in the pars plicata of humans to be 154 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.[59,60] 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.[61–63]

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.

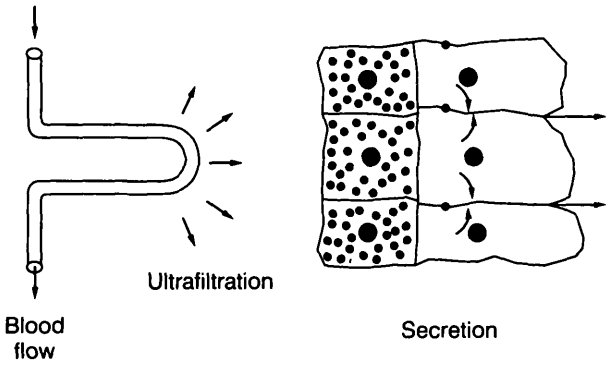

Fig. 2. Sequence of steps in the formation of aqueous humor. (Reprinted by permission of Grune and Stratton, Inc.[331])

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,[64] whereas the blood flow to the pars plicata is about twice its weight per minute.[60] 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.[65]

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

Parameter	*Kidney*	*Eye*
Blood flow, % of weight/min	420%	227%*
Percent of plasma filtered	24%	4%*
Concentration of protein	filt = 0.03%	aq = 0.03%
Oncotic gradient	With flow	Against flow
Vol. transport/min/cell vol.	0.72	0.31
Vol. transport/cm^2/day	1.2 ml	0.6 ml

* From Bill, ref. 60.

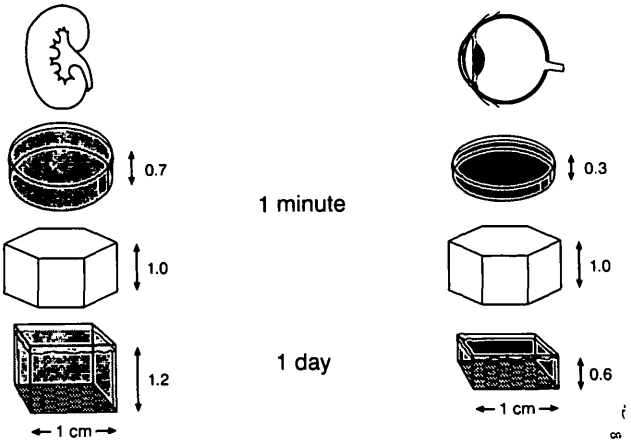

Fig. 3. Comparison between the rate fluid transport by the renal tubular system on the left and the pars plicata of the ciliary body on the right. In 1 min, the average renal tubular cell transports 70% of its cell volume; the average ciliary epithelial cell transports 30% of its volume. In 1 day, renal tubular cells transport 1.2 cm^3 per cm^2 area; ciliary epithelial cells transport 0.6 cm^3 per cm^2 area.

In 1950, the synthesis of a compound (2 acetylamino, 1,3,4 thiadiazole-5-sulfonamide)[66] 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.[67]

Investigators wasted no time in determining the systemic effects of acetazolamide. It ameliorated congestive heart failure[68] and suppressed gastric[69] and pancreatic secretion.[70] It was known that carbonic anhydrase was present in the anterior uvea of the rabbit eye.[71] Also, Friedenwald,[72] 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.[73–75] 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

Table 6. Aqueous flow: drug effects (insignificant effects)

Drug	*Study*
Thymoxamine	Lee[78,336]
Pilocarpine	Nagataki[50]
Phenylephrine	Lee[76]
Dexamethasone	Anselmi[90]
	Rice[92]
$PgF_{2\alpha}$-IP-ester	Kerstetter[89]
Caffeine	Adams[337]

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.[50] Adrenergics, such as phenylephrine[76] (an α_1-selective agonist) and thymoxamine[77,78] (an α_1-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 β-adrenergic activity and systemically administered corticosteroids, have been shown to stimulate the rate of aqueous humor flow in humans. Stimulation of flow by β-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 β-adrenergic action on the outflow apparatus and most probably the trabecular cell.[103–110]

Several studies showed that the acute administration of β-adrenergic agonists is associated with an *increase* in the rate of clearance of fluorescein from the anterior segment.[111–114] These investigators concluded that β-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[40,128] 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 oubain,[129] cholera toxin,[130–132] vanadate,[133–137] phenobarbital,[138] prazosin,[139] halothane,[139] δ-9-tetrahydrocannabinol,[140–145] demeclocycline,[146] colforsin (forskolin),[147–155] atrial naturietic peptide,[156–161] 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 β-adrenergic antagonists, and the α_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 β-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

applicable carbonic-anhydrase inhibitors to avoid the side effects associated with systemic administration.[177-180] 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.[181] 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.[182] In 1967, propranolol, a β-adrenergic antagonist, when administered systemically to humans, lowered intraocular pressure.[183] The following year, others reported that propranolol applied topically to humans lowered intraocular pressure.[184] Some years later, it was shown that timolol lowered intraocular pressure in a glaucomatous rabbit model.[185,186] About the same time, others demonstrated that timolol was an effective topical ocular hypotensive agent in humans.[187,188] 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.[47,189] Other β-adrenergic antagonists have a similar effect.[190,191] 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).[192]

Table 7. Aqueous flow: drug effects (β adrenergic antagonists, adaptation to effect)

Treatment	*Flow, μl/min*	*Difference*
Placebo*	2.57 ± 0.38	
Timolol 1/2%, single dose*	1.68 ± 0.32	35%
Washout†	2.54 ± 0.71	
Timolol 1/2%, 1 week†	1.35 ± 0.53	47%
Timolol 1/2%, 1 year†	1.87 ± 0.57	26%

* Coakes.[189]
† Brubaker.[192]

Table 8. Aqueous flow: drug effects (β adrenergic antagonists, timolol withdrawal)

Treatment	*Flow, μl/min (experimental eye)*	*Difference*
Timolol, average 4 years	1.92 ± 0.43	
Discontinue timolol exptl. eye		
2 days	2.01 ± 0.45	5%
4 days	2.08 ± 0.51	8%
7 days	2.15 ± 0.56	12%
13 days	2.29 ± 0.51	19%
Discontinue timolol both eyes		
6 weeks	2.51 ± 0.59	31%

Data from Schlecht.[193]

This effect was observed in another study in which subjects receiving timolol for 4 years discontinued use of the drug for 6 weeks.[193] 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).[193] These data suggest that the terminal half-life of timolol's effect is at least 1 week.

A similar study of levobunolol was done.[191] 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.[191] 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.[174-176] By contrast, the acutely treated timolol-suppressed eye does not respond to apraclonidine, which normally suppresses aqueous formation.[194] 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.[195] 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 α_2-adrenergic activity. A few years later, it was administered systemically to human subjects, and intraocular pressures were

lowered.[196] Administered topically, it was observed that lowering of intraocular pressure occurred, attributed to an improvement in the facility of outflow.[197] 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.[198-204]

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).[205] Subsequently, a derivative of clonidine, apraclonidine (p-aminoclonidine), was found to lower intraocular pressure[206] and to be useful in preventing a spike of intraocular pressure after laser treatments.[207-210] Apraclonidine, like its parent compound, lowered the rate of aqueous humor formation (Table 9).[211] The discovery of the effect of β-adrenergic antagonists and α_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.[212] Flow was calculated by indirect means, namely by a combination of tonometry and tonography using Goldmann's[10] formula:

Flow = (intraocular pressure − 10 mmHg) × (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 (α_2 adrenergic agonists)

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

* Lee.[205]
† Gharagozloo.[211]
‡ Koskela.[194]

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

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

In 1981, we studied a group of 113 normal volunteers ranging in age from 20–83 yr.[213] 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%,[214] and others found a 3.5% loss of corneal endothelial cells per decade.[215] 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.[216]

Table 10 is a summary of flow by decade of age. The remarkable fact is that aqueous flow is almost steady throughout adult life. An implication of this finding is that the more common infirmities of the eye are probably not a result of an age-dependent reduction of the rate of flow of aqueous humor. However, virtually nothing is known about the possibility that certain pathologic conditions of the eye could be caused by alteration of aqueous composition. Much additional work needs to be done to study the aqueous flow and composition of persons with specific ocular abnormalities.

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.[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."[218] Subsequent work showed that this phenomenon exists in anesthetized monkeys[13,219] and in human volunteers,[220,221] but the effect is small and transient.[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.[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[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 trabeculoplasty (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.[225] These investigators found no change in the rate of aqueous formation, even though intraocular pressure had been lowered significantly.[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).[229] Patients were studied with myotonic dystrophy, a condition in which spontaneous intraocular pressures below 10 mmHg are encountered frequently (Table 13).[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.[217]

Table 11. Effect of intraocular pressure on flow (trabeculoplasty)

Laser trabeculoplasty	*Effect on IOP*	*Effect on flow*
Brubaker and Liesegang[226]	Lowered	None
Araie et al[227]	Lowered	None
Yablonski et al[228]	Lowered	None

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

Pigment dispersion syndrome	*IOP (mmHg)*	*Flow (μl/min)*
Affected eyes		
IOP > 22 (n = 25)	26.2 ± 3.6	2.86 ± 0.56
IOP < 22 (n = 49)	17.3 ± 2.8	2.85 ± 0.69
Control eyes (n = 80)	16.3 ± 2.9	2.60 ± 0.42

Data from Brown.[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 perilimbal suction cup.[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.[232] These findings were a result of a true circadian rhythm driven by light, a rhythm that persists in constant darkness.[233]

This rhythm also was studied in humans.[175,234] 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)

Myotonic dystrophy	*IOP (mmHg)*	*Flow (μl/min)*
Patients (n = 26)*	7.1 ± 2.21	2.51 ± 0.62
Controls (n = 37)*	14.6 ± 3.5	2.54 ± 0.74

* Walker.[230]

Table 14. Aqueous flow during sleep

Conditions	*Flow, μl/min*	*Difference*
Daytime, awake (n = 19)	3.10 ± 0.60	
Night, asleep	1.60 ± 0.50	48%
Daytime, awake (n = 6)	3.10 ± 0.60	
Night, asleep	1.40 ± 0.19	55%
Night, sleep deprived	2.30 ± 0.30	26%

Data from Reiss.[234]

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] Controverting this conclusion is the fact that acetazolamide (Table 18)[176] and apraclonidine (Table 19)[194] are able to suppress the rate of aqueous flow in the sleeping eye.

Table 15. Aqueous flow: circadian cycle (human eye)

Hours	*Rate, μl/min*	*Volume, μl*	*% Day's total*
600–1200	3.0	1087	33%
1200–2200	2.7	1602	50%
2200–600	1.2	585	17%
Total 24 hours	2.3	3274	100%

Data from Koskela.[235]

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

Catecholamine	*Day*	*Night*
Epinephrine*	15%	47%
Isoproterenol†	22%	34%
Terbutaline‡	2%	15%

* Topper.[175]
† Larson.[113]
‡ Gharagozloo.[114]

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.

Disease

A few studies were done with fluorophotometry in persons with ocular or systemic diseases. Goldmann[10] concluded that patients with chronic simple glaucoma had a reduced rate of aqueous flow (normal rate, 2.18 mm^3/min; glaucoma simplex rate, 1.67 mm^3/min) and those with chronic congestive glaucoma had a higher rate (4.1 mm^3/min). The rate of flow was measured in patients during a glaucomatocyclitic crisis.[243] The loss coefficient from flow, k_{fa},

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

	Flow, μl/min	
Treatment	*Daytime*	*Night*
Placebo (n = 19)*	2.26 ± 0.86	1.61 ± 0.40
Timolol 1/2%	1.58 ± 0.49	1.66 ± 0.40
Difference	30%	None
Pretreatment (n = 18)†	2.61 ± 0.82	1.08 ± 0.59
Timolol 1/2%	1.60 ± 0.28	1.13 ± 0.28
Difference	39%	None

* Topper.[175]
† McCannel.[176]

Table 18. Aqueous flow: drug effects, day vs. night (acetazolamide)

	Flow, μl/min	
Treatment	*Daytime*	*Night*
Oral placebo (n = 18)	2.61 ± 0.82	1.08 ± 0.59
Acetazolamide 500 mg	2.07 ± 0.57	0.82 ± 0.32
Difference	21%	24%

Data from McCannel.[176]

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] The 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 fluorphotometric 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 transsection 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.[12,153,259-266] 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[239] (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)

	Flow, μl/min	
Treatment	*Daytime*	*Night*
Placebo (n = 20)	2.84 ± 0.61	1.15 ± 0.40
Apraclonidine 1%	2.00 ± 0.54	0.84 ± 0.28
Difference	30%	27%

Data from Koskela.[194]

Table 20. Horner's syndrome

Condition	Aqueous flow, µl/min: Normal eye	Horner's eye	Difference
Daytime (n = 21)*	2.14 ± 0.57	2.21 ± 0.54	ns
Daytime (n = 12)†	2.13 ± 0.71	2.43 ± 0.60	ns
Night (n = 12)†	1.31 ± 0.31	1.51 ± 0.65	ns
Difference	47%	38%	ns

* Wentworth.[239]
† Larson.[113]

Table 21. Melatonin and aqueous flow

Normal subjects, daytime (mean ± SD, n = 19)	Urinary melatonin (µg)	Aqueous flow, µl/min
Without melatonin	4.0 ± 3.5	2.80 ± 0.66
With melatonin	353 ± 81	2.71 ± 0.64
Percent change	8700%	3%
P, type I error	<0.0001	0.4
P, type II error		<0.05

Data from Heinrich.[289]

mor between the affected eye and the unaffected eye of these patients. In addition, these eyes responded normally to the β-adrenergic antagonist timolol but responded differently to epinephrine. Epinephrine suppressed aqueous flow in the denervated eye and stimulated it in the innervated eye. The suppressive effect was attributed to hypersensitivity of the eye to α-adrenergically induced vasoconstriction.

This research was continued by another investigator who showed that persons with Horner's syndrome have a normal pattern of circadian rhythm of aqueous flow (Table 20).[113] These patients were found to be more sensitive to a selective β-adrenergic catecholamine, isoproterenol, that stimulated flow during sleep to a greater extent than in the denervated eye. We concluded from these studies that the rate of aqueous flow, the circadian rhythm of flow, and the response to β-adrenergic antagonists in humans do not depend on complete sympathetic innervation of the eye. Some of the findings in these studies may have resulted from the ability of the eye to adapt to chronic denervation.

Hormonal Regulation

Renewed interest in the circadian rhythm of aqueous flow and the recognition that nocturnal suppression of flow is as great as suppression by any known therapeutic agent has led investigators to look for endogenous hormones that may play a role in this rhythm. A comprehensive review of the subject was published.[276] Many hormones have been studied including atrial naturietic peptide,[156-161] corticosteroids,[90,91,119,277-281] gonadotropins,[282-285] growth hormone,[286] melatonin,[235,287-289] progesterone,[290-293] serotonin,[294] thyrotropin-releasing hormone,[263,295] and vasopressin or one of its analogues.[296-302] We studied three of these hormones—melatonin, progesterone, and desmopressin—none of which had any significant effect on aqueous humor flow.

Melatonin: At one time, melatonin was thought to have an effect on the circadian rhythm of aqueous humor flow. It was observed that intraocular pressure could be altered in human beings by administration of melatonin.[288] The effect on flow in human subjects was studied at a time of day when melatonin normally would be absent from plasma.[289] Large doses of melatonin were given, but no measurable effect on aqueous flow in human subjects was observed (Table 21).

Vasopressin: The effect of vasopressin on aqueous flow in the rabbit eye also was investigated. In one study, the vasopressin analogue, desmopressin, was shown to increase the rate of aqueous flow by 57%.[302] However, previous studies in humans did not look at flow directly.[299,301] A group of persons with diabetes insipidus of neural origin and normal renal function was examined.[303] While receiving desmopressin, these subjects were able to live without the excessive thirst and diuresis that are part of the syndrome, and aqueous flow was studied when these subjects were taking their usual doses of desmopressin. The drug was discontinued, and the patients were observed to have changes in plasma and urine osmolarity and urine volume. A small change in the rate of aqueous flow was observed that might have been a result of a direct effect of desmopressin on the eye. The effect was small and could have been related partly to the removal of water by the hypertonic plasma during the period of excessive thirst and diuresis. This report shows that the effect of desmopressin on the eye is small and that vasopressin is not likely to account for large changes of aqueous flow, such as the change observed in the normal circadian rhythm (Table 22).

Table 22. Desmopressin and aqueous flow

Diabetes insipidus (mean ± SD, n = 17)	Plasma olsmolarity (MOSM) 2:00 PM	Urine osmolarity (MOSM) 2:00 PM	Aqueous flow, µl/min 12:00-4:00 PM
Without desmopressin	299 ± 8	92 ± 52	2.34 ± 0.69
With desmopressin	291 ± 6	619 ± 284	2.53 ± 0.78
Percent change	3%	573%	8%
P	<0.0001	<0.0001	0.05

Data from Heinrich.[303]

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.[159,316] 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] The 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]

Table 23. Progesterone and aqueous flow

Day of estrus cycle, normal, nonpregnant women (n = 20)	*Progesterone, ng/dl (mean ± SD)*	*Aqueous flow, µl/min (8:00–12:00)*
1	86 ± 146	3.12 ± 0.76
7	95 ± 130	3.27 ± 0.73
14	474 ± 502	3.33 ± 0.58
21	620 ± 511	3.12 ± 0.69

From Gharagozloo, unpublished data.

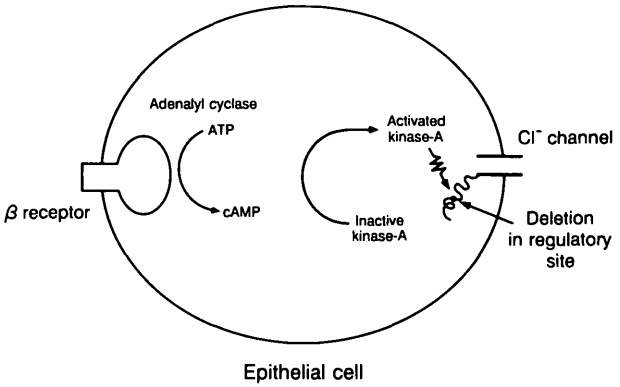

Fig. 4. Hypothesized defect in cystic fibrosis. Activation of β receptor of epithelial cell results in synthesis of cAMP and activation of kinase-A, but chloride-selective channel fails to open in response to the stimulus.

Failure of this signal transduction pathway in affected individuals accounts for the clinical manifestations of this disease (Fig. 4).[249,327]

The findings in cystic fibrosis are interesting in view of the suggestion[325,327,330] 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.

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 α_2-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 the flow of sleeping subjects.
6. Acute doses of β-adrenergic antagonists and α_2-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 (eg, 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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