Foundations of Glaucoma: Anterior Segment

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Hydrostatic Pressure

- Hydrostatic pressure is pressure created by the weight of the water.

- Ruptured hull of US submarine Scorpion crushed by hydrostatic pressure.

Hydraulic Pressure

- Hydraulic pressure is pressure created by applying a force to a non-compressible fluid that is within a confined space.

- Blood pressure and IOP are hydraulic pressures.

Atmospheric Pressure

- The pressure created by the weight of air pressing on everything it contacts.

- The higher you go, the thinner the layer of air there is pressing on you.
Atmospheric Pressure and Blood Pressure

- On a good weather day, atmospheric pressure at sea level is around 760 mmHg, while blood pressure is on average 100 mmHg (120/80). Then why do you bleed from cuts? The atmospheric pressure is so much higher, it should prevent the blood from coming out.

So, if your systolic blood pressure is 120 mmHg and you are living in mile-high Denver, where atmospheric pressure is only about 670 mmHg, your actual BP is 790, whereas in Worcester, with an average atmospheric pressure of 760, your BP is 880. So, should people with high blood pressure all go to live in Denver?

Intraocular pressure is also measured RELATIVE to the constantly varying atmospheric pressure

- So, if your IOP is 15 mmHg and you are living in Denver, where atmospheric pressure is usually about 670 mmHg, your actual IOP is 685, whereas in Worcester, with an average atmospheric pressure of 760, your IOP is 775. So, should people with glaucoma all go to live in Denver?

NO. Cells are filled with incompressible water. If you increase the hydrostatic pressure around a cell, the pressure inside simply equilibrates and the cell is not even deformed. Tissue damage is created not by the absolute pressure but by the pressure gradient and its deformability in its location in-situ.

Aqueous Flow

Aqueous Inflow
- Ultrafiltration from capillaries of the ciliary body stroma
- Secretion of aqueous by the PCE and NPCE

Aqueous Outflow
- Trabecular (Conventional) outflow which is pressure dependent
- Uveoscleral Outflow which is largely pressure independent

For IOP to remain stable, aqueous inflow must equal aqueous outflow.
Aqueous humor is made exclusively by the ciliary processes in the pars plicata region of the ciliary body.

- Aqueous humor is a clear nutritive fluid that is very low in plasma protein but contains glucose, amino acids and oxygen (at low levels).
- It is formed at a rate of about 3 µl/min. The volume of the posterior chamber is about 50 µl and the anterior chamber about 250-300 µl. Hence, the total pool of aqueous humor turns over every 100 min.
- Remember that this discloses diffusional losses to the vitreous. Fluid is always being exchanged between the posterior chamber and the core vitreous, across the anterior vitreous face, but generally the net movement of fluid and cells in the vitreous is back to front. Even the dead RBCs after a vitreous hemorrhage, or tumor cells flaking off a retinoblastoma (upper photo), will migrate forward and can even reach the anterior chamber, accumulating at the bottom of the AC.

**Aqueous Inflow**

- Aqueous humor is secreted as a plasma-protein-free fluid. The 1% of plasma protein level found in aqueous in the AC enters from the ciliary body stroma, via the root of the iris. In the MRI left, (*) before intravenous injection of a protein surrogate in a living human, the AC and PC look the same. One hour after injection note accumulation of contrast material (lighter color) in the ciliary body and the AC, but not in the PC (arrows). The inset compares pre and post injection signal level in the AC. This means that the plasma-derived protein in aqueous is not part of aqueous when it is formed but is added to aqueous semi-independently from the ciliary body stroma via the iris root.

**Plasma-derived protein levels in Aqueous Humor are not 0, they are 1% of the level found in plasma itself.**

- This means that reduction of aqueous production does not reduce the amount of protein entering the AC via the iris root. With less aqueous, the protein concentration rises, producing clinical flare. All aqueous suppressants give rise to some flare, not because the blood aqueous barrier is disrupted but purely due to this concentration effect.

**Pseudohypopyon**

- Plasma-derived protein levels in Aqueous Humor are not 0, they are 1% of the level found in plasma itself.

**Pseudohypopyon**

- Following a vitreous hemorrhage, some of the dead and lysed RBCs (aka ghost cells) have migrated from the vitreous into the aqueous humor and have been carried with aqueous flow into the AC, where they sediment out. Note the rusty color distinguishing it from a white cell "true" hypopyon in uveitis. Lysed RBCs will block outflow and elevate IOP in "ghost cell glaucoma."
Aqueous Inflow

Aqueous humor can enter the anterior chamber ONLY through the pupil. If the pupil is bound down to the lens (posterior synechia – picture below, left) in an inflamed eye, continued production of aqueous will elevate IOP and push the iris forward, further elevating IOP by closing off the outflow pathway.

Aqueous humor is a nutritive fluid that both provides metabolites (glucose, oxygen, amino acids, etc) to the avascular tissues of the eye and receives their waste (mostly lactic acid from anaerobic metabolism), aqueous must constantly circulate to mix old with new. This is done using the convective flow driven by the temperature difference between the warmer iris and the cooler cornea (picture at right).

Aqueous inflow is highest in the late morning and lowest in the middle of the night (about 50% less). Nice overview video by Dr. Arthur Sitt (Mayo Clinic) on circadian rhythm: https://youtube.com/watch?v=Of4Bdu06rg0

Ciliary Body - Coronal Section with Lens removed

Suprachoriodal space

Ciliary muscle

Ciliary Body: The plasma protein concentration in the stromal filtrate is 34% of that in plasma itself. But the plasma protein concentration in the PC is essentially zero.

Capillaries of the Ciliary Processes –

A filtrate of plasma is formed in the ciliary body stroma. Vasoconstrictors will reduce filtrate formation and aqueous production.

Fenestrations at arrowheads allow leakage of fluid, ions and even plasma proteins.

The marginal capillaries of the ciliary body are fenestrated. They leak fluid, ions and even plasma proteins. Indeed, the plasma protein concentration in the ciliary body stroma is about 74% of that in the blood itself. It is some of this protein that diffuses into the AC via the iris root.

Black granular surrogate for plasma protein is seen seeping from fenestrated ciliary body capillaries into the stroma.

Zonules running in valley between processes

PCE

NPCE

PC (0%)
The Ciliary Epithelium

Using intravascular tracers, that mimic the behavior of plasma proteins, the intercellular spaces between PCE, melanosome, and NPCE layers are filled with protein-like material (asterisks) leaked from stromal vessels. But when the tracer tried to diffuse between adjacent NPCE cells, it was blocked abruptly by tight junctions (converging arrows) which are the structural analogue of the blood-aqueous barrier in the ciliary body.

NPCE

Melanosomes

PCE

Gap junctions join the cells of the layers of the ciliary epithelium to each other and also join the cells of the two layers together.

Gap junctions allow for electrotonic and metabolic communication between cells.

The result is that all of the ciliary epithelial cells can operate in a coordinated fashion just like a gland.

Gap Junctions

Gap junction surface view via freeze fracture and cross section view of gap junction in inset.

Gap Junctions in Aqueous Production

Intercellular communication and coordination via movement of second messengers through lateral gap junctions.

Ion movement from PCE to NPCE via apical gap junctions.

Posterior chamber

The Diamond-Bossert Model of Standing Gradient Osmotic Flow

In its simplest form, sodium and chloride are pumped into the narrow cleft between PCE cells, making the cleft hypertonic. Water then leaves the NPCE cells to dilute the salt in the cleft.

The tight junction prevents that fluid from going anywhere and toward the posterior chamber.

Critical Enzyme Systems

- Na-K-ATPase
- Carbonic Anhydrase
**Sodium Potassium ATPase Pump**
- The ATPase enzyme (a protein) is localized to non-pigmented ciliary epithelium
- Inhibition with ouabain reduces aqueous secretion

**Na-K-ATPase**
- Immuno electron microscopic studies demonstrate anti-ATPase enzyme antibody binding (black spots) pump sites in the membranes of the non-pigmented ciliary epithelium.

**Inhibition of Na-K-ATPase Reduces Aqueous Flow**
- Ouabain added

**Carbonic Anhydrase**
- Catalyzes hydration of carbon dioxide to carbonic acid leading to liberation of hydrogen and bicarbonate atoms
- Enzyme immunohistochemically localized to ciliary epithelia in major processes of pars plicata region only

\[
\text{H}_2\text{O} + \text{CO}_2 \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+
\]
Carbonic Anhydrase Inhibitors

- NHE-1 (NHE=Sodium hydrogen exchanger) and AE2 (AE=Anion exchanger) antiports on the stromal side of the PCE cell membrane, underlie uptake of Na⁺ and Cl⁻ from the ciliary body stroma, in exchange for H⁺ and HCO₃⁻, respectively. Cytoplasmic carbonic anhydrase II enhances the delivery rate of H⁺ and HCO₃⁻ and thus facilitates Na⁺ and Cl⁻ uptake for use in making aqueous. The Na⁺ and Cl⁻ ions taken up by the PCE are passed into the NPCE via gap junctions where the Na-K-ATPase pumps them into the cleft between NPCE cells.

- Carbonic anhydrase inhibitors used in the treatment of glaucoma likely reduce inflow and IOP by inhibiting the NHE-1 and AE2 antiports in the PCE.

Ion Movements in Aqueous Production

- Aqueous humor outflow is passive flow, along a downhill pressure gradient, from the anterior chamber to the episcleral veins.
Elevation of Episcleral Venous Pressure

- EVP is the ultimate pressure that IOP must overcome in order for AH to leave the eye via the conventional route. The data on whether EVP is elevated in POAG remains equivocal. If we accept data using all methods, it appears that EVP averages approximately 9 mm Hg if the subject is seated and remarkably, does not seem to change with age.

Elevated Episcleral Venous Pressure: With dilated episcleral vessels and elevated IOP, it is important to ask about recent trauma, specifically craniofacial or any head injury, that can suggest a carotid cavernous sinus fistula. A complete past medical history should be obtained to rule out etiologies that may cause venous obstruction, including but not limited to hyperthyroidism, amyloidosis, congestive heart failure, hypercoagulable states, vasculitis, superior vena cava syndrome and Sturge-Weber Syndrome.

Use of the alpha-agonist apraclonidine, the calcium channel blocker, verapamil and the new rho-kinase inhibitors reduce EVP. A case of Carotid-Cavernous Sinus Fistula Post-repair

Elevated EVP in Sturge-Weber

- Elevated EVP is likely the cause of the glaucoma associated with the vascular phakomatosis Sturge-Weber syndrome.
- Elevated EVP has not yet clearly and consistently been shown to play a contributing role in POAG.

Sturge–Weber syndrome, sometimes referred to as encephalotrigeminal angiomatosis, is a rare congenital neurological and skin disorder. It is one of the phakomatoses and is often associated with port-wine stains of the face (A congenital hemangioma composed of excess capillaries around branches of one or more of the three divisions of CN V), glaucoma, seizures, intellectual disability, and posterior leptomeningeal angiomata (cerebral malformations and tumors).

Port wine stain

- Dilated episcleral vessels

- Elevated EVP in Sturge-Weber

- The trabecular meshwork lies within the confines of the red triangle. Note that only the posterior portion appears pigmented.

But clinical observation and science can differ. Forced to a choice, pick science.

The picture shows an example of the kind of controlled ocular perfusion system used for assessment of aqueous outflow. Whether the eye being perfused is a living eye still in the orbit, or an enucleated eye on a bench (with no vascular pulse), the physiological (and even the pharmacological) parameters of trabecular outflow REMAIN THE SAME. The only difference is the episcleral pressure goes from in-vivo 9 mmHg to 0 mmHg and everything else scales down proportionally but maintains the same outflow resistance and IOP. The presence or absence of a vascular pulse makes no difference.

The Case for Pulsatile Aqueous Outflow

- A recent surge of non-NIH reviewed and funded research suggested that there is an ocular pulse-related pumping of aqueous out of the eye and the outflow system is NOT the passive downhill flow just described. It is easy for clinicians who are not scientists to buy into this because, as you have seen, when you touch the tonometer tip to the cornea, the mires get larger and smaller with the pulse. So its easy to believe that that pulse squeezes aqueous out of the eye. Review this on your own: https://www.youtube.com/watch?v=6HcCM6BUN7c

- But clinical observation and science can differ. Forced to a choice, pick science.
Remember that you are viewing a wall sloping toward you, so that which appears on top in this view is also more anterior.

Defining the Anterior from the Posterior Meshwork - Pigment Distribution in Normal Angle

- Only the posterior meshwork has Schlemm’s canal behind it. As such, it is the most direct way out and sees the most flow. With that flow, over many years, named “wear and tear” pigment, which is phagocytized more by the TM cells in the PM than in the AM.

Note abrupt reduction in pigment in the portion of the TM anterior to the end of Schlemm’s canal. This is a normal older angle – NOT PDS.
The Angle

- **UVEAL MESHWORK** (below red line) extends from uveal tissue to Schwalbe's line.
- **CORNEOSCLERAL MESHWORK** (between red and blue line) extends from scleral spur to the deep stroma of the cornea.
- **JUXTACANALICULAR CONNECTIVE TISSUE** (JCT region - between blue line and Schlemm's canal - SC).

Dimensions of the Conventional Outflow Pathway

- As one progresses deeper into proximal portions of the conventional outflow pathway, the sizes of the open spaces are reduced.
- **Uveal meshwork** = 25–75 μm
- **Corneoscleral meshwork** = 2–15 μm
- **JCT region** = 1–2 μm

Given the sizes of the openings in the uveal and corneoscleral meshwork, neither region is considered to contribute any measurable resistance to aqueous outflow.


Ciliary Muscle

- **Longitudinal Bundle**
  - Inserts onto posterior surface of scleral spur but its tendons also extend into the meshwork and even to the inner wall of Schlemm's canal.

Cribriform Plexus - tendons from the tips of muscle cells in the longitudinal bundle do not all attach to the undersurface of the scleral spur (SP). Many extend into the TM and even make direct, elastin-containing connections directly to the inner wall of Schlemm's canal.

Cribriform Plexus...

- Do miotics really decrease IOP by pulling on the scleral spur and "opening up" the meshwork?

Some of the longitudinal bundle attaches to the scleral spur, but most enter the TM and even connect to the inner wall of SC, as we have seen.

As IOP increases, SC wants to collapse. Miotics seem to work mainly by holding open Schlemm's canal, by analogy with the cords (tendinae) attached to the mitral and bicuspid valves of the heart. They brace the inner wall to prevent collapse.

NOTE: If a board question asks about this and only offers the old story about pulling on the scleral spur to increase outflow—pick that one. Just know that it's likely incorrect.

The resistance in those locations is already zero!

Uveal Face of the Trabecular Meshwork by SEM

Most of the Trabecular meshwork is composed of avascular trabecular beams that are attached to Schlemm's canal by analogy with the cornea (tendinae attached to the mitral and bicuspid valves of the heart). They brace the inner wall to prevent collapse.

Structure of the UVEAL AND CORNEOSCLERAL Trabecular Meshwork

- Most of the Trabecular meshwork is composed of avascular trabecular beams that are attached to Schlemm's canal by analogy with the cornea (tendinae attached to the mitral and bicuspid valves of the heart). They brace the inner wall to prevent collapse.

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**Pores** (Two types: I-Pores and B-pores)

- I-pores (Intracellular-left) and B-pores (Border-right) are found as tiny openings through (I) or between (B) inner wall cells. It is through pores that aqueous enters SC.
- Pores of both types decrease significantly in glaucoma but without a change in pore diameter.
- The pores in the inner wall tend to be widely spaced, approximately 20-30 µm apart.

**The Distal Outflow Pathway from Schlemm’s Canal to the Episcleral Veins**

- There are about 30 unevenly distributed collector channels that leave the outer wall of Schlemm’s canal.
- These external collector channels each branch, initially into three vessels (recall this reduces resistance) to join tortuous vessels of the deep scleral plexus.
- These in turn branch some more, joining the more superficial intrascleral venous plexus. These vessels wind their way through the remainder of the scleral thickness to join the episcleral veins on the surface of the sclera.

**Regional Differences**

- If you perfuse the anterior chamber with fluorescent microbeads you will note that some areas receive a lot of flow (a) and some almost none (b). As we increase IOP, areas of good flow become more and more restricted, invariably the last areas to close are right near collector channel ostia.
- If you perfuse the AC and the distal pathway with fluorescein intra-operatively (Huang et al) you can see striking non-uniformity of outflow (right). And the hi flow regions can change to low flow regions over time.
The Distal Pathway in Glaucoma

We have long assumed that the added resistance that elevates IOP in glaucoma was the result of adding more resistance to sites of resistance in the normal eye. But there is now data suggesting that at least part of the added resistance is related to the collector channels.

As pressure is increased in normal eyes, portions of JCT and inner wall are forced into the openings of collector channels. But for greater numbers of collector channel openings are blocked for herniations in age-matched eyes with POAG. Surgeons doing canalostomies now commonly believe that the frequent points of resistance they must break through when passing the catheter through Schlemm’s canal are these fused herniations.

FLOW, PRESSURE AND RESISTANCE

Flow is determined by 2 factors:
- Pressure difference between two ends of a vessel
- Resistance of the vessel to flow (including resistance from fluid viscosity and resistance created by the vessel wall)

- In the vascular system, if properly regulated, the physiology is set up to ensure CONSTANT FLOW, allowing pressure and resistance to vary up and down to ensure this constancy
- In the eye, if properly regulated, the physiology is set up to ensure CONSTANT PRESSURE, allowing resistance and flow to vary up and down to ensure this constancy

- Glaucoma is a dysregulation of this system

Goldmann Equation

\[
F = Ctm \times (P_i - P_e)
\]

- \(P_i\) = Intraocular pressure in mm Hg
- \(P_e\) = Episcleral venous pressure in mm Hg
- \(Ctm\) = Facility of trabecular outflow in microliters / min / mmHg
- \(F\) = Aqueous Outflow in microliters/min

At Steady State \(F_{in} = F_{out}\)

- \(\Delta P\) = Pressure difference (mmHg)
- \(R\) = Resistance (mmHg/ml/min)

Remember that outflow facility is simply the mathematical inverse of outflow resistance.
Goldmann Equation
\[ F_{in} = F_{out} = Ctm (Pi - Pe) + F_u \]
\[ 2.5 = 2.5 = 0.3 (16 - 9) + 0.4 \]

Assuming:
- \( F_{in} = F_{out} = 2.5 \text{ microliters/min} \)
- \( Ctm = 0.3 \text{ microliters/min/mm Hg} \)
- \( Pi = 16 \text{ mm Hg} \)
- \( Pe = 9 \text{ mm Hg} \)
- \( F_u = 0.4 \text{ microliters/min} \)

This is the slightly more complex version that takes uveoscleral facility of outflow into account (\( F_u \)).

So Where is the Resistance and How is it Created?

When vessels branch, resistance decreases, cleverly serving to prevent loss of flow, even as the volume is now distributed into multiple branches. The addition of additional branches reduces further decrease resistance thus maintaining flow, (slightly by the sum of least resistance) in all branches and does not increase resistance. The converse is equally true.

In the eye, recall that many areas of Schlemm's canal are receiving no flow at any given moment. What this means is that outflow resistance (and therefore pressure) will DECREASE if we can open up more of Schlemm's canal to flow. It is the equivalent of branching vessels. And leaving JCT, collector channels further divide. As those vessels further divide, resistance keeps decreasing all the way to the episcleral veins.

How can we change the meshwork to achieve this decrease in resistance (i.e. increase in facility of outflow)?

The Funneling Effect
Rho-Kinase (ROK) Inhibitors

Rho-kinase inhibitors such as Rocklatan affect actinomyosin cytoskeletal networks and have been shown to significantly increase outflow facility (C) in without obvious toxicity. They alter cytoskeleton of TM and inner wall cells such that as pressure attempts to collapse SC, the JCT region becomes distended. The "cherry-picker" arms on the JCT cells limit this expansion. This is the anatomical correlate for the increase in outflow facility produced by this class of medications.


How does Distention of the JCT region Produce an Increase in Outflow Facility?

By loosening the cytoskeleton, the JCT region distends. When it does, that disrupts the funneling effect. This is the equivalent of increasing the branching in a vascular system, or increasing the amount of the inner wall of SC available for flow. The result is a decrease in total resistance.

Dividing Conventional From Uveoscleral Outflow

- Aqueous that enters the uveal face of the meshwork "above" the scleral spur enters the conventional outflow pathway
- Aqueous that enters the uveal face of the meshwork "below" the scleral spur enters the uveoscleral outflow pathway

NET is a monoamine transporter and is responsible for the sodium-chloride (Na+/Cl−)-dependent reuptake of extracellular norepinephrine (NE) from the synaptic cleft.

NET is a monoamine transporter and is responsible for the sodium-chloride (Na+/Cl−)-dependent reuptake of extracellular norepinephrine (NE) from the synaptic cleft. This category is also used in the treatment of ADHD.

Dexedrine, Adderall and Ritalin fall roughly into this category. They block reabsorption of norepinephrine through monoamine transporters (including NET), thereby sustaining levels of these neurotransmitters in the synaptic cleft.

Overabundance of NET in the brain is associated with ADHD. The notion is that by reducing norepinephrine re-uptake in adrenergic neurons supplying the vessels of the ciliary body, vasoconstriction is sustained. By doing that, there is reduced blood flow and thus less fluid made available from which the ciliary body can make aqueous humor.

In about 10-25% of cases patients will develop (vorticeal keratopathy)
Uveoscleral Outflow Pathway

Aqueous entering the ciliary body band, flows along the connective fascicles that interweave among the smooth muscle cells of the longitudinal bundles until it reaches the supraciliary space.

The supraciliary and suprachoroidal spaces are the same space with a name change at the ora. Aqueous continue along the suprachoroid to finally leave the eye through the sclera or the emissarial canals through the sclera used by the vortex veins (i.e. uveovortex outflow).

Current estimates suggest that uveoscleral outflow accounts for up to 22% total in normals and decreases with age and in POAG.

What Regulates Conventional Outflow Resistance?

MMPs and TIMPS and eNOS

In this normal eye, the system responds to elevated pressure by sensing tissue strain (i.e. stretch) within the JCT region and shear stress along the wall of SC.

The compensatory changes to either increase or decrease resistance, as needed, are translated into action by an array of signaling pathways and matricellular proteins that modify intracellular cytoskeletal rigidity, the amount and tensioning of the extracellular matrix, or both. All of these are now targets for therapeutic intervention in glaucoma.

Final effectors of these pathways include altering the balance between MMPs and TIMPS, with MMPs degrading matrix to increase facility or being inhibited from doing so, resulting in more resistance. The shear within SC causes release of eNOS, and then nitric oxide, a vasodilator. Shear also modifies endothelial cytoskeletal rigidity to further relax SC reducing shear and increasing flow.

Reference Resources
