Aqueous and Lens Physiology

Lisa Ostrin, OD, PhD, FAAO
Room 2153
lostrin@uh.edu
Aqueous and Lens Physiology

• Aqueous humor
  – secretion
  – outflow

• Crystalline lens
  – Embryology
  – Structure
  – Function
Gross Anatomy

- Anterior chamber
- Posterior chamber

vs

- Anterior segment
- Posterior segment
Anterior Segment Anatomy

Anterior and posterior chambers
Ciliary Body

- Triangular in cross section
- Anteriorly attached to scleral spur and iris
- Posteriorly attached to choroid and retina
- Externally abuts sclera (but not attached)
- Internally in contact with vitreous
Ciliary Body Functions

- Production of aqueous humor – made in pars plicata
- Aids in aqueous removal through canal of Schlemm with contraction
- Accommodation
- Aids in restoration of vitreous
• Pars plicata – aka corona ciliaris
  – Anterior portion
  – About 70 meridional ridges known as ciliary processes
  – Connected to base of iris, extend into posterior chamber
  – Contains zonules that connect to lens capsule
Ciliary Body Ridges

View from posterior chamber
Zonular Fibers

Hogan’s figure 7-6, pg. 272
Ciliary Body Regions

- Pars plana – aka orbicularis ciliaris aka vitreous base
  - Posterior flattened portion
  - Continuous choroid and retina posteriorly
  - Ora serrata is where pars plana and retina meet
    - Scalloped appearance
    - Also called dentate processes
Ora Serrata

- Pars plana
- Ora seratta
- Retina
Ciliary Body Layers

1. Suprachoroidal space
2. Ciliary muscle
3. Layer of vessels and ciliary processes
4. Basal Lamina
5. Epithelium
6. Internal limiting membrane
1. Suprachordal Lamina

- Suprachoroidal space
- Resembles the choroid posteriorly, but more serous anteriorly
2. Ciliary Muscle

- Multi-unit smooth muscle
- Innervated by CN III, parasympathetic fibers
- 3 parts
  - Longitudinal – aka Brucke’s muscle, outer layer, runs from anterior to posterior along length of ciliary body
  - Circular – aka Muller’s muscle, innermost bundles, run circumferentially, major arterial circle is just anterior
  - Radial – few in number, between longitudinal and circular
3. Layer of Vessels

- Ciliary processes are essentially blood vessels
  - Fold of connective tissue with vascular core covered by double layer of ciliary epithelium
- Most vascular part of eye
- Direct communication with choroid
4. Basal Lamina

- Continuation forward of Bruch’s Membrane of choroid
- Internal surface has thickened ridges
  - Forms sockets
  - Provides firm anchorage
  - Helps withstand traction of zonular fibers
5. Epithelium
Aka pars ciliaris retinae

- Pigmented ciliary epithelium
  - between basal lamina and unpigmented ciliary epithelium
  - continuous with RPE posteriorly
  - Continuous with anterior epithelium/dilator of iris anteriorly
  - Desmosomes and tight junctions with unpigmented ciliary epithelium
5. Epithelium
Aka pars ciliaris retinae

- **Unpigmented ciliary epithelium**
  - Continuation of neural retina
  - Single cell layer
  - Continuous with posterior/pigmented epithelium of iris anteriorly
  - Firmly attached to pigmented epithelium
Ciliary Body Epithelium
6. Internal Limiting Membrane

- Continuation forward of internal limiting membrane of retina
- More tightly adherent to vitreous than most of retina
IRIS

CILIARY BODY

Layer of vessels → Choroid vessels

Basal Lamina → Bruch’s membrane

Anterior epithelium → Pigmented epithelium → RPE

Posterior pigmented epithelium → Unpigmented epithelium → Neural retina

POSTERIOR GLOBE

ILL

ILL
Ciliary Body Blood Supply

- Primary supply is from long posterior and anterior ciliary arteries from the ophthalmic artery
- Major arterial circle (of iris) gives off branches to ciliary body

Blood Supply and Drainage of the Ciliary Processes:
The major arterial circle sends branches into the ciliary processes, where they divide into large capillaries. The capillaries run at several levels of depth in the processes, but the large marginal capillaries run back along the ridges of the processes. The capillaries merge as small veins running back through the pars plana into the drainage fields of the vortex veins. (After Funk and Rohan 1990 and Morrison and Feddo 1996.)

Ciliary Body Blood Supply
Venous Drainage and Lymphatics

• **Venous system**
  - all drain to episcleral veins → into the ophthalmic vein

• **Lymphatics**
  - medial trunk to submaxillary nodes
  - lateral trunk to pre-auricular nodes
Ciliary Body and Iris Innervation

• **Motor**
  
  – Long and short ciliary nerves (same as iris)
    
    • **SHORT**: Parasympathetic from EW nucleus via inferior division of CN III, enter eye via short ciliary nerves – *increases aqueous outflow, no effect on aqueous production*
    
    • **Parasympathetic from VII through pterygoplatine ganglion**, increases IOP likely due to increased episcleral venous pressure
    
    • **LONG**: Sympathetic from cervical sympathetic trunk, synapse in superior cervical ganglia, enter eye via long ciliary nerves – *can affect inflow and outflow*
• Sensory (LONG CILIARY NERVES)
  – Nasociliary branch of ophthalmic division of CN V
  – run in long ciliary nerves
  – Enter ciliary body, terminate in iris, cornea and ciliary muscle
Ciliary Body and Iris Innervation

**Motor parasympathetic**

EW nucleus \(\rightarrow\) Ciliary ganglion \(\rightarrow\) Short ciliary nerves \(\rightarrow\) Ciliary Body/Sphincter/BV/TM (muscarinic receptors)

**Motor sympathetic**

cervical sympathetic trunk \(\rightarrow\) Superior Cervical ganglion \(\rightarrow\) Long ciliary nerves \(\rightarrow\) Dilator/BV (\(\alpha_1, \alpha_2\) receptors) Trabecular Meshwork (\(\beta_2\) receptors)

**Sensory**

CN V, ophthalmic division, nasociliary branch, Long ciliary nerves \(\leftrightarrow\) Iris, cornea, cb
Adrenergic Receptors (Sympathetic)

- **Alpha 1 receptors**
  - mydriasis, vasoconstriction
  - decreases facility of outflow

- **Alpha 2 receptors**
  - vasoconstriction
  - agonists
    - involved in glaucoma therapy
    - increases outflow facility; useful in inflammation
    - examples: clonidine, brimonidine, apraclonidine
  - Reduces production by decreasing cAMP
Adrenergic Receptors
(Sympathetic)

• Beta 2 receptors
  – (1 mostly in the heart)
  – 2 (most of receptors in trabecular meshwork and CB)
    • Adrenergic activation of receptor
    • Stimulations intramembrane G-protein
    • G-proteins activates adenylate cyclase
    • This increases cAMP (2nd messenger) and leads to phosphorylation of a kinase
    • Increases aqueous production
Aqueous Humor
Functions

- Positive internal pressure of eyeball (maintain IOP)
- Nourishment of avascular cornea and lens
- Removal of metabolic waste
- Transport neurotransmitters
- Can be used for drug delivery
Aqueous Humor

general characteristics

- Hyperosmotic
- Acidic
- Excess ascorbate (slight excess Cl⁻, lactic acid)
- Deficit of protein (slight deficit glucose, Na⁺, bicarbonate, CO²)
Aqueous Humor Flow

- From the posterior chamber, through pupil to anterior chamber
- Temperature gradient creates flow pattern in anterior chamber

Gabelt, & Kaufman, 2011
Aqueous Humor Flow

- Blood flow to ciliary processes is 125 µl/min
- 4% of plasma enters pars plicata
- Aqueous turnover – has diurnal variation
  - 3.0 µL/minute in morning
  - 2.4 µL/minute in afternoon
  - 1.5 µL/minute at night
- Posterior chamber volume 60 µL
- Anterior chamber volume 150 µL
- Complete turnover every 30 (PC) and 120 (AC) minutes
Aqueous Humor Composition

- $\text{H}_2\text{O}, \text{O}_2, \text{CO}_2$
- Organic and inorganic ions
- Carbohydrates
- Glutathione (antioxidant)
- Urea
- Amino acids and proteins (low)
- Ascorbate (high)
  - active secretory mechanism
  - may be used as an antioxidant
  - Diurnal animals have higher levels possibly to protect against UV
Aqueous Humor Composition

- **Proteins**
  - IgG > IgM and IgA
    - in low concentrations 3 mg/100ml
    - little to no IgA, IgM, IgD
  - lens and vitreous proteins in low concentration
  - Lactate dehydrogenase
    - high in patients with retinoblastoma

- **Lipids**
  - low in aqueous
Blood Aqueous Barrier

- Aqueous is substantially different than plasma due to BAB that provides selective permeability
- BAB formed by epithelium of CB
  - NPE cells have zonula occludens (major barrier), gap junctions and desmosomes
  - Minor barrier by other junctional complexes between NPE and PE
Blood Aqueous Barrier

- Ciliary body blood vessels NOT part of BAB
  - large fenestrations that allow substances from 300-1000 angstroms to penetrate
  - material diffuses into stroma, moves between the PE and is stopped by the ZO of the NPE
- Iris blood vessels ARE part of BAB, have tight junctions and no fenestrations
- Substances that penetrate the BAB
  - lipids
  - medium MW
Blood Aqueous Barrier

• Use of hyperosmotics can make use of BAB
  – After a rise in IOP, patient ingests a hyperosmotic
    • This sets up an osmolality difference between aqueous and blood
    • Fluids are drawn out of the aqueous
    • Good for short-term treatment only

• Other theories: decrease vitreous volume and hypothalamus influence
Mechanisms of Aqueous Formation

• Diffusion (minor) – lipid soluble substances, passive, moves down concentration gradient

• Ultrafiltration (minor) – includes water, water soluble substances, through fenestrations in ciliary capillaries, dialysis, passive, osmotic gradient

• Active secretion (major) – 80-90% of aqueous, active transport in NPE, generation of an osmotic gradient, two major enzymes:
  – Na-K ATPase in NPE
  – Carbonic Anhydrase (CA) in PE & NPE
G-protein Coupled Energy from ATP
Ion Transport in Ciliary Epithelium

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

- **Symport**: Na\(^+\), K\(^+\), 2Cl\(^-\)
- **Antiport**: Na\(^+\), K\(^+\), Cl\(^-\)

[Diagram showing ion transport and equilibrium reactions.]
Aqueous Humor Formation

- **Active secretion**
  - 80-90% of aqueous
  - Main site is NPE
  - Evidence
    - concentrations of ascorbate, lactate, amino acids are higher than Gibbs-Donnan equilibrium
    - Na and bicarb higher than G-D
    - effects of metabolic inhibitors or temperature decrease will decrease secretion (IOP)
    - Membrane permeable to K+
Aqueous Humor Active Secretion

- Net direction is secretion across the epithelium, but hydrostatic and oncotic forces favor reabsorption.
- Active transport produces an osmotic gradient across the ciliary epithelium, which promotes the movement of other plasma constituents by ultrafiltration and diffusion.
Aqueous Humor Formation – Active Transport

1. Molecules penetrate thru PE to active sites on NPE
   Na\(^+\) enters NPE from stromal side by diffusion or Na/H ion exchange.
   Na\(^+\) is the main cation involved in aqueous formation
   In lateral intercellular channels (LIC) via Na/K ATPase transport system

2. Bicarbonate formed from hydration of CO\(_2\), reaction is catalyzed by carbonic anhydrase
Aqueous Humor Formation – Active Transport

3. Chloride enters ICS by HCO$_3^-$/Cl$^-$ exchanger or active transport

4. Hypertonic fluid is created in LIC
   - water enters via osmosis
   - diluted as it enters posterior chamber
Calcium-activated Potassium Channels


• showed “super” K channels
  – large conductance, voltage
  – activated by Calcium
  – play a role in aqueous humor secretion
  – did not show role to be related to other known roles in other epithelial types
Carbonic Anhydrase

• Responsible for formation of H\(^+\) and HCO\(_3^-\)
  – enhances aqueous secretion
  – CA inhibitors block formation of H\(^+\) and HCO\(_3^-\)
    • H\(^+\) used in ion exchange with Na\(^+\)
  – Topical CA antagonist for glaucoma: dorzolamide (Trusopt)
Aqueous Humor Formation

Kiel et al 2011
Factors Affecting Aqueous Formation

- Innervation – *generally*...
  - sympathetics decrease
  - parasympathetics increase
  - Blood pressure increases then increase IOP
  - others decreasing formation: exercise, aging, anesthetics, inflammation, acidosis, hypothermia
Adrenergic Receptors and cAMP

- Reducing inflow
  - alpha agonists decrease cAMP
  - beta blockers decrease cAMP
Aqueous Outflow

• Conventional pathway
  – Fluid movement through tm → schlemm’s canal → collector channels → aqueous veins → episcleral veins
  – Passive, moves down pressure gradient
  – Dependent on IOP
  – Giant vacuoles and pores move aqueous out

• Unconventional pathway
  – Movement through uveoscleral TM → suprachoroidal space → sclera
  – Passive, independent of IOP
Trabecular Meshwork

• Dense, consists of type I and III collagen, elastin
• Progressing from anterior chamber to Schlemm’s canal, the spacing between bands decreases
• Three regions
  – Uveal meshwork
  – Corneoscleral meshwork – most extensive portion
    • has β2 receptors
    • when ciliary muscle contracts--pulls scleral spur and opens spaces in TM, especially the corneoscleral portion
  – Juxtacanicular meshwork – most resistance
Trabecular Meshwork

• Aqueous must pass through....
  – 3 layers
    1. Endothelial layer of TM
    2. Collagenous layer of canal wall
    3. Endothelial wall of canal

• vacuoles
Trabecular Meshwork is Phagocytic
Trabecular Meshwork

- MMPs – matrix metalloproteinases facilitate outflow by regulating synthesis and degradation of ECM
- TIGR – TM glucocorticoid inducible response protein, also called myocilin (Myoc).
  - Many patients with POAG have TIGR/MYOC mutations, including children
Schlemm’s Canal

- Endothelial layer of juxtacanicular TM comprises inner wall of Schlemm’s Canal
- Lumen diameter is 350 to 500 µm and it undulates. Increases the surface area which aides in aqueous absorption
- Aqueous prior to canal has gone through an irregular path, creates a percolating effect
- Possesses internal collector channels
Schlemm’s canal: transport of aqueous via giant vacuoles
Drainage

- **Intrascleral collector channels**
  - 25-35 of them to episcleral veins
  - Indirect

- **Aqueous veins**
  - Directly to episcleral veins

![Diagram of the aqueous outflow system](image)
Identification and Assessment of Schlemm’s Canal by SD-OCT
Kageman L, et al. IOVS 2010
Aqueous Outflow

- routes for flow out:
  1) pinocytosis
  2) diverticuli
    - outpockets
    - increase surface area
  3) pores and vacuoles
    - on the inner endothelial wall
    - relationship between IOP and # of pores
    - POAG fewer pores
Aqueous Outflow in POAG

• Pores and vacuoles – on the inner endothelial wall
  – Relationship exists between IOP and # of pores
  – POAG fewer pores

• Pore number and size are decreased in glaucoma
  – 1437/mm² in normal eyes vs 489/mm² in glaucoma
  – pore larger in normal eyes (0.91µm vs 0.85 µm)

• Outflow facility
  – 0.25 in normals
  – 0.11 in POAG; facility= 1/ resistance
Schlemm’s Canal

FIGURE 4. (A) Mechanism of Schlemm’s canal (SC) endothelium (SCE) attachment to underlying trabecular lamellae. Cytoplasmic processes of SCE attach to juxtanacanalicular cell (JCC) processes. Juxtanacanalicular cell processes (JCC) in turn attach to trabecular lamellae (TL). Intertrabecular cell processes rather than collagen attachments limit excursions of adjacent trabecular lamellae. IOP-induced tissue loading (hollow arrows). (B) Region of scanning electron micrograph in (C) (white square). Numerous cytoplasmic processes arise from juxtanacanalicular cells creating extensive attachments to the undersurface of SCE. (D) Appearance and relationships of SCE to juxtanacanalicular cells and trabecular meshwork when IOP is low. (E) Appearance and relationships at physiologic IOP. SCE attachments to the underlying TM modulate SCE distention into SC. When IOP progressively increases, structural elements responsible for resistance to pressure respond by configuration changes as illustrated by the configuration change from D to E. Configuration changes illustrated in transition from D to E in response to IOP-induced tissue loading, place resistance to aqueous outflow at SCE. The juxtanacanalicular space enlarges, extracellular matrix material density reduces and cell processes (CP) restraining the inner wall endothelium change from a parallel to perpendicular configuration. Signs of pressure-induced cell stresses are present at cell process origins where cytoplasm and nuclei of both juxtanacanalicular cells and SCE undergo deformation toward respective processes; cytoplasm and nuclei of SCE undergo elongation and attenuation. Evidence of SC endothelial cell tethering by extracellular matrix material is absent. Trabecular lamellae, which participate in modulating and restraining SCE distention into SC progressively separate from one another as seen in Fig. 3. (A) From: Johnstone MA. The morphology of the aqueous outflow channels. In: Drance SM, ed. Glaucoma: Applied Pharmacology in Medical Treatment. New York: Grune & Stratton, 1984. “Copyright © (1984) Grune & Stratton. All rights reserved.” (D and E) From: Johnstone MA. Pressure-dependent changes in nuclei and the process origins of the endothelial cells lining Schlemm’s canal. Invest. Ophthalmol. & Vis. Sci. 1979;18(1):44–51. Copyright © (1979) IOVS. All rights reserved.
Schlemm’s Canal

FIGURE 9. Scanning electron microscopy (SEM) Human eye. The surface of the endothelial lining (ET) of a SC valve spans between the trabecular meshwork (TM) and the corneoscleral wall (CSW) of Schlemm’s canal. The wall of the SC valve is continuous with the lining of the inner and outer wall of SC. (cc) collector channel, (AC) anterior chamber. An opening is visible at the distal end of the SC valve where it attaches to the external wall.
Aqueous Outflow

• Internal Collector channels

• Aqueous Veins
  – connect the canal directly to episcleral veins contains aqueous but sometimes find blood in aqueous veins or even in canal of Schlemm
  – If episcleral pressure is greater than IOP
Pressure Gradients

- Ciliary arterial system – 60 mmHg
- IOP – 13-17 mmHg
- Resistance in conventional outflow pathway – 3-4 mmHg
- Episcleral veins – 8-10 mmHg
  - if episcleral venous pressure increases will increase IOP but with time adapts and decreases aqueous inflow
  - IOP must be greater than episcleral vein pressure for outflow
Unconventional Aqueous Outflow

• Uveoscleral outflow
  – Another route for aqueous
  – out through choroid
  – can go to lymphatics or vitreous
  – Approximately 10-50% of aqueous leaves by this route
  – pressure independent
Outflow

• Total flow in and total flow out must = 0
• Inflow and outflow operate as parallel flow rather than in series
• Inflow consists of two parts
  – one driven by pressure (ultrafiltration, diffusion)
  – second is active secretion

• Outflow has two parts
  – through TM which is pressure dependent ($P_i = \text{IOP and } P_e$)
  – uveoscleral outflow
Aqueous Flow

Facility = 1/resistance \quad F_u = \text{Uveoscleral outflow}

\[ F_{in} = F_{out} = 2.5 \ \mu\text{L/min} = C_{tm}(P_i - P_e) + F_u \]

\[ 2.5 \ \mu\text{L/min} = 0.3(16 - 9) + 0.4 \]

\[ P_i = 16 = \text{intraocular pressure in mmHg} \]
\[ P_e = 9 = \text{episcleral venous pressure in mmHg} \]
\[ C_{tm} = 0.3 \ \mu\text{L/min/mmHg} = \text{facility of trabecular outflow} \]
\[ F = 2.5 \ \mu\text{L/min} = \text{aqueous flow in } \mu\text{L/minute (at steady state, } F_{in} = F_{out} = F) \]

\[ P_i - P_e \text{ needs to be positive to get outflow} \]
Population distribution of IOP

Clinical Applications in Glaucoma

- Beta blockers – suppress aqueous humor production by decreasing cAMP (timolol, levobunolol)
- Adrenergic (alpha_2) agonists – suppress production by decreasing cAMP (apraclonidine, brimonidine)
- Carbonic anhydrase inhibitors – suppress production (dorzolamide, brinzolamide)
- Prostaglandin analogs – increases uveoscleral outflow (bimataprost, travaprost, latanaprost)
- Parasympathetic agonists – increases outflow by opening angle (pilocarpine)
- Hyperosmotic agents – decrease vitreous volume (glycerine, mannitol)
# TABLE 1: PHARMACOTHERAPY OPTIONS FOR PRIMARY OPEN-ANGLE GLAUCOMA

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>b-adrenergic blockers</td>
<td>• betaxolol ophthalmic</td>
</tr>
<tr>
<td></td>
<td>• carteolol ophthalmic</td>
</tr>
<tr>
<td></td>
<td>• levobunolol</td>
</tr>
<tr>
<td></td>
<td>• metipranolol</td>
</tr>
<tr>
<td></td>
<td>• timolol</td>
</tr>
<tr>
<td>a-adrenergic agonists</td>
<td>• apraclonidine</td>
</tr>
<tr>
<td></td>
<td>• brimonidine</td>
</tr>
<tr>
<td>carbonic anhydrase inhibitors</td>
<td>• brinzolamide</td>
</tr>
<tr>
<td></td>
<td>• dorzolamide</td>
</tr>
<tr>
<td>prostaglandin analogues</td>
<td>• bimatoprost</td>
</tr>
<tr>
<td></td>
<td>• latanoprost</td>
</tr>
<tr>
<td></td>
<td>• tafluprost</td>
</tr>
<tr>
<td></td>
<td>• travoprost</td>
</tr>
<tr>
<td></td>
<td>• unoprostone</td>
</tr>
<tr>
<td>miotic agents</td>
<td>• carbachol</td>
</tr>
<tr>
<td></td>
<td>• pilocarpine</td>
</tr>
</tbody>
</table>

Adapted from reference 29.
Effects of Prostaglandins on the Aqueous Humor Outflow Pathways

Fig. 1. IOP before prostaglandin F$_{2\alpha}$ 1-isopropylester (PGF$_{2\alpha}$-IE) treatment (day 0) and before and after the 7th dose of PGF$_{2\alpha}$-IE on the 4th day of unilateral topical treatment. Time 0 occurs \( \sim \) 17 hours after the 6th PGF$_{2\alpha}$-IE dose (given at \( \sim \) 3:00 PM on day 3) and immediately before the 7th dose (given at \( \sim \) 8:30 AM on day 4). Data are mean \( \pm \) SEM IOP for six animals, each contributing one treated (solid circle) and one untreated (open circle) eye. Symbols on abscissa indicate significant difference between treated and control eyes by the two-tailed two-sample t-test. (Reprinted from Crawford and Kaufman, with permission of Investigative Ophthalmology & Visual Science.)
The Crystalline Lens
Functions

- Absorption/transmittance
- Transparency
- Refraction
- Accommodation
The Crystalline Lens

- Transparent biconvex structure behind iris, in front of vitreous
- Contributes about 15D of the total power of the eye, ~58D
- Grows throughout life, from 6.5mm diameter at birth to 10mm diameter as adult
The Crystalline Lens

- Center point of anterior and posterior surfaces are the anterior and posterior poles, the “axis” is the line joining the poles
- Anterior surface (10mm radius of curvature) is less convex than posterior surface (6mm radius of curvature)
- Outer edges are the equator
Lens Optics

• index of refraction
  – cortex = 1.386
  – n of nucleus = 1.406
  – overall = 1.413

• Transparency
  – regular fiber arrangement
  – lamellar style proteins
  – avascularity
  – thin epithelium

• Absorption
  – absorbs UV < 360 nm

• Scatter
  – scatters about 5% of light due to “n” variation of proteins and membranes.
  – Scatter will increase with age due to increase separation of lens fibers and increase in “n”
Layers of the Lens

- Lens capsule – outermost elastic layer
- Epithelium – only on the anterior surface
- Lens fibers
  - Cortex
  - Nucleus – innermost
Lens Position

- Lens equator is encircled by ciliary processes.
- Suspended within the ciliary ring by zonules, suspensory ligaments attached to ciliary processes and lens equator, spanning about 0.5mm space, space decreases as lens grows with age.
- Lens is suspended and under tension during distance viewing.
- During accommodation the ciliary muscle contract, releases zonular tension, and allows lens to fatten.
Lens Composition

- 60% water, 40% proteins (crystallins, increase with age)
- High concentration of glutathione (GSH)
  - Maintains protein sulfhydryl groups in reduced form
  - composed of glycine, cystein and glutamic acid
  - Protects from oxidative damage by detoxification of peroxide
  - Removes xenobiotics by conjugation with hydrophobic compounds having an electrophilic center
  - highest concentration in cortex, lowest in nucleus
Lens Biochemistry

- Proteins
- Amino acids - glycine, alanine, leucine, taurine, (actively transported into cells)
- Inorganic Ions
  - Sodium - 17 meq/Kg; low compared to aqueous, actively extruded, concentration high during cataract
  - Potassium - 25 meq/Kg, Na in and K out, low in cataract formation
  - Calcium and Magnesium
  - Phosphate
  - Chloride - 30 meq/Kg
  - Others: Mn, Cu++, Fe etc
Lens Biochemistry

• Lipids
  – Cholesterol - concentration increases with cataract formation
  – Phospholipids
    • mainly sphingomelin- increases with age
    • Sphingomyelin and cholesterol
    • May have some effect on accommodation
Lens Biochemistry

• Carbohydrates
  • mainly glucose
  • If levels in aqueous too high get lens swelling
  • May have facilitated transport with unique transporter

• Organic phosphate - ATP
  – Ascorbic acid - high levels..... eye from UV...
  – Inositol - high concentration in lens, actively transported
Lens Capsule

- Acellular basement membrane that completely envelopes lens
- Thinnest at poles and equator
- Thickest in midregions
- Formed by lens epithelium anteriorly and lens fibers posteriorly
- Consists of about 40 lamella made up of reticular fibers (netlike or entangled) embedded in sulfated glycoaminoglycan
- Composed of collagen type IV
- Forms a barrier to bacteria and inflammatory cells
- Will allow diffusion of smaller molecules
Lens Epithelium

- Single layer of simple cuboidal epithelium over anterior surface (under capsule), not found posteriorly
- Become columnar towards equator and convert into lens fibers
- Cells are not shed because of capsule
- Has sodium potassium pumps
Lens Epithelium Zones

- **Central zone**
  - Cells are flattened and hexagonal

- **Germative or proliferative zone**
  - Cells are columnar and smaller in area, higher in density
  - New cells generated here – nuclei divide and migrate posteriorly to become new lens fibers in cortex
  - Layers are laid down in close contact through “ball and socket” like joints

- **Transition Zone and Equator**
  - Cells elongate and rotate so long axis is parallel to cortical surface
• New superficial fibers are nucleated
• Nuclear bow forms as nuclei move anteriorly and fibers pass deeper into the lens to form the cortex
• Deeper fibers are anucleate (true fibers)
• Preceding generations of cells are pushed deeper into center of lens
Lens Fibers
Microscopic Anatomy

• Fibers
  – produced by epithelial cells at
    equator
  – elongation epithelial cell proceed
    anteriorly
    • basal end proceeds posteriorly
  – Interdigitations
    • more like ball and socket
    • responsible for flexibility of
      lens
  – zonular fibers
    • insert into ILM of CB and 1.5
      mm on either side of equator
Lens Embryology

- Lens placode noticed at 22 days gestation
- Primary lens fibers - cells of posterior wall of lens elongate, lose nuclei, become transparent lens fibers, make up embryonic nucleus (innermost part of lens), attach to apical surface of anterior lens
Lens Embryology

- Secondary lens fibers – new fibers added (throughout life) and grow around embryonic nucleus to make up the fetal nucleus
- Secondary fibers form Y sutures
  - Formed by fusion of lens fibers
  - Anterior Y suture is erect
  - Posterior Y suture is inverted
Lens Embryology

- Cortex – after birth new fibers continue to form, produced by mitosis of epithelial cells in equitorial region
- Fibers elongate to surround existing nucleus
- Development of cortical fibers form the “nuclear bow” or “lens bow”
Lens Embryology

- Hyaloid artery supplies lens in developing fetus
- Regresses prior to birth
- Small remnant can remain
  - Mittendorf’s dot on the posterior lens surface
  - Bermeister’s papilla in posterior pole
Abnormalities during Development

- Mittendorf Dot
- Persistent Hyaloid Artery
- Bergmeister’s Papilla
Fig. 3. Schematic scale diagrams showing the asymmetric, oblate, spheroidal shapes (approximately the ratio of anterior/posterior sphericity) of a variety of vertebrate lenses. A: Fish (80°/90°); B: rat (45°/55°); C: chicken (20°/30°); D: human (15°/30°); E: rabbit (40°/55°); and F: bovine (35°/60°). In all of these schematic drawings the anterior surface is to the right.
Lens Growth and Suture Formation
Lens Growth and Suture Formation
Lens “Circulation”

- **Gap Junctions** – connexins, move nutrients between cells
- **Aquaporin 0** – important for water transport
- **Na/K ATPase pump**
  - in lateral apical membranes
  - near equitorial differentiating fibers
  - important in maintaining transparency and water balance
  - pump leak mechanism
Lens Cytoskeleton – Alpha Crystallin

- Horwitz J. Eye 13:403, 1999
  - major lens protein whose structure helps maintain transparency
  - alpha crystallin is a chaperone
    • binds with unfolded/denatured protein
    • suppresses aggregation therefore maintains transparency
    • subunits alpha A:B in ratio of 3:1
      - preserves thermal stability
    • chaperone properties are better served in higher temperatures ie 37°C vs 20°C
Lens Cytoskeleton

• Quinlan RA et. al. Eye 13:409, 1999
  – lens contains microtubules, microfilaments and intermediate filaments
  – intermediate filaments
  • CP49 and filensin
    – found complexed with alpha and beta crystallin
    – found in all stages of lens fiber differentiation
    – precise role unknown
    – implicated in cataract formation
Lens Metabolism

- Primarily anaerobic through glycolytic pathway
- Continuous supply of ATP needed, uses only small amount of energy, made in epithelium
  - Growth
  - Synthesis
  - Pumping nutrients in and waste out
Lens Metabolism - Glucose

Energy production based on glucose metabolism
  – glucose enters lens by simple and facilitated diffusion
  – rapidly metabolized

1. **anaerobic metabolism** – 85% of glucose metabolism
2. aerobic metabolism – kreb’s cycle, 3% of glucose metabolism
3. Hexose monophosphate shunt – 5%, source of NADPH for sorbitol and glutathione pathways
4. Sorbitol pathway – 5%, obtained by reduction of glucose, sorbital sets up osmotic gradient
Changes during diabetic cataract

**Chemicals and processes involved:**
- Glucose
- Na+
- K+
- Inositol
- GSH
- ATP
- Water
- Sorbitol
- Fructose

Diagram showing the flow of these chemicals and processes during diabetic cataract.
Lens

- **Key enzymes against oxidative insult**
  - Glutathione peroxidase
    - $2 \text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2 \text{H}_2\text{O}$
  
  - Catalase
    - $\text{H}_2\text{O}_2 + \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2$
  
  - Superoxidase Dismutase
    - $\text{O}_2^\cdot + \text{O}_2^\cdot + 2 \text{H} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$
  
  - Glutathione S-Transferase
    - $\text{GSH} + \text{RX} \rightarrow \text{Mercapturic Acid}$
Lens

• Transport and Permeability
• Cation Balance
  – lots of Potassium in lens
  – try to decrease Calcium and Sodium via Na/K pumps
  – decrease in lens temperature causes \( \uparrow \text{Na} \) and \( \downarrow \text{K} \)
  – Electrophysiology
    • lens has a -70 mV compared to Aqueous
Na = 25 mM  
K = 140 mM

Na = 163 mM  
K = 4 mM

Na = 144 mM  
K = 8 mM

Posterior Lens

Anterior Lens

Diffusion

Facilitated diffusion

ATPase

Glucose

Ca++

ATPase

DA
Age Changes in the Lens

• Water content
  – nuclear content increases
  – changes occur around cataracts

• Electrolytes
  – increase sodium, calcium
  – decrease potassium
  – Mg constant

• Protein changes
  – increase in insoluble and decrease in soluble
    • Increase in albuminoid protein
Age Changes in the Lens

• Lipid changes
  – Increase
  – Increase in cholesterol

• Metabolic changes
  – decrease in hexokinase
  – increase aldose reductase
  – decrease in Na/K ATPase
  – Glutathione in oxidized state

• Gross
  – increase in lens size
  – increase in lens weight and thickness
  – increase in lens nucleus

• Microscopic
  – Increase in lens density
  – Optical changes
  – Zones of discontinuity not as clearly visible
  – yellowing - brunescence
Any Questions?

lostrin@uh.edu