Lens Physiology and Cataractogenesis

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Crystalline Lens

- highly refined biochemical processes designed to maintain transparency
- focuses light rays to a point source on the retina
- high protein content (35 – 40% protein) to provide high refractive index
- relatively low water content (60%)
Accommodation

- of the total refractive power of the eye (~60D), the lens contributes:
  - 13 – 17 D in humans
  - 40 D in dogs
Accommodation

- annular pad is sandwiched between the lens epithelium and the fibres
  - fibres are oriented radially rather than concentrically
Lens – Histology

- capsule – surrounds entire lens
  - basement membrane
  - secreted by lens epithelial cells
  - composed of collagen-like glycoprotein (no elastic fibres)

Streeton BW, 1982
Zonules

- Zonules = suspensory ligament of the lens
- Zonule of Zinn

- Ring of zonule fibres around the lens is triangular in cross section
Zonules

- **classification:**
  - primary keep the lens in place
  - auxillary keep the ciliary body processes in place
Zonules
Lens Luxations
Lens Luxations

- primary (typically Terriers)
- secondary
  - due to glaucoma
  - chronic uveitis
  - neoplasia
  - trauma
  - ageing
- may be seen as phacodonesis or iridodonesis
- treatment depends on the cause, overall ocular health, potential for maintenance of vision
Lens Luxations

Adler's Physiology of the Eye, 2011
Lens Luxations

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Lens Epithelial Cells – Histology

- anterior epithelium
  - single layer of cuboidal cells
Lens Epithelial Cells – Histology

- no posterior epithelium
Sidebar – Lenticular Embryology

A. Optic vesicle stage

B. Lens placode stage

C. Lens pit stage

D. Early lens vesicle stage
Sidebar – Lenticular Embryology

Lenticular Embryology

- lens capsule surrounds the lens prior to the immune system being competent
  - exposure to the lens proteins will result in an inflammatory response
Lens Epithelial Cells – Histology

- anterior epithelium
  - single layer of “cuboidal” cells
  - desmosomes and gap junctions
  - most active part of the lens
Lens Epithelial Cells – Histology

- Function of lens epithelial cells differs depending on location.

Diagram showing different zones:
- Central zone
- Pre-germinative zone
- Germinative zone
- Equatorial zone
- Superior
- Inferior
Lens Epithelial Cells – Histology

- function of lens epithelial cells differs depending on location
Lens Epithelial Cell Proliferation

Hogan, 1971
Lens Epithelial Cell Proliferation

Sikic, 2015
Lens Polarity
Lens Fibre Differentiation

- Apical
- Basal
- Centre

The diagram illustrates the differentiation of lens fibres with apical and basal regions, as well as the central area.
Lens Fibre Differentiation
Lens Fibres Differentiation

- each is an elongated LEC progressively changing
- inhibition of proliferation
  - cyclins regulate cell cycle
  - p57 and p27 binds to lens fibre cyclins to inhibit mitosis

Kuszak, 2004
Lens Fibres Differentiation

- elongation of fibre cells
  - osmotically driven increase in cell volume
  - cytoskeletal rearrangement
  - sustained protein synthesis
  - accumulate large amounts of crystallin proteins (three times higher than in other cells)
  - partially driven by growth factors in the vitreous
Lens Fibre Differentiation
Lens Fibres Differentiation

- role of apoptosis, autophagy, or something else???
  - key features of apoptosis are not met
  - autophagy mediators are present but autophagy-deficient mice suggest autophagy is not required
Lens Bow
Lens Bow
Lens Growth

- grows throughout life without removal of cells
- new growth (terminal differentiation) occurs at the lens bow
Lens Growth

- more LEC are added as the lens develops and grows
  - differentiating cells only migrate as far as the bow region
  - most of fibre elongation occurs apically and basally with the mid-segment of a fibre fixed in position
Lens Growth

a fibre in isolation

Kuzak, et al. 2004
Lens Fibre Organization

Kuzak, 2005
Lens Fibre Size

constant dimensions:
- length: 5 – 15 µm
- width: 1 – 5 µm

changing dimensions:
- length/radius: up to 20 mm
Lens Fibre Shape

“straight” fibres

“opposite end curvature” fibres
Lens Fibres
Lens Sutures
Lens Sutures – Y

- dictating suture pattern is:
  - cell polarity
  - fibre shape
  - fibre length
- mice, rats, guinea pig, cats, dogs, cows, sheep
Lens Sutures – Y

Kuszak et al., 2004
Lens Sutures – Y

- lens fibres meet anteriorly and posteriorly along irregular sutures

Kuszak et al., 2004
Lens Sutures – Comparative

- Y sutures – canine, feline, bovine, etc.
- stellate (star) sutures – primates
- linear suture line – rabbits
- umbilical suture – avian and reptile
Lens Sutures – Stellate (star)

- most complicated of the branched sutures
- primates
- develop a prenatal Y-suture
Lens Sutures – Stellate (star)

- “simple star” suture pattern forms following birth through infancy

Kuszak et al., 2004
Lens Sutures — Stellate (star)

Ageing Baboon
(19 yrs)

Human
37 yrs

Kuszak, 1995
Kuszak and Costello, 2004
Lens Sutures – Linear

- simplest branched suture
- rabbits, frogs...
- fibre length is greater than in other branched suture patterns
- anterior suture is vertical but the posterior suture is horizontal

Kuszak and Costello, 2004
Lens Sutures – Linear

“straight” fibres

“opposite end curvature” fibres

Kuszak et al., 2006
Lens Sutures – Umbilical

arranged end to end (in the anterior-posterior orientation)

branchless suture lenses

Kuszak et al., 2006
Lens Fibre

- lots of gap junctions (30 – 65% of the lens fibre membrane is associated with gap junctions)
  - formed by unique connexins
- joined together by ball-socket-joints
  - plentiful in superficial fibres
  - turn into “membrane furrows” deeper in the lens
Lens Fibre Changes

young lens

old lens
Lens Fibre Composition

- crystallin proteins (40%)
  - not renewed
  - three types present: alpha, beta, gamma
- taxon specific crystallin:
  - delta (avian, reptile)
  - zeta (camel, guinea pig)
  - iota (some diurnal geckos)
  - tau (turtle, lamprey, fish, reptile, avian)
  - epsilon (many birds, crocodiles)
  - rho (frogs)
Lens Fibre Composition

- high cholesterol content
  - increased with age and cataract formation
  - creates rigid membranes
- phospholipids play a role in regulating mitosis and differentiation
Lenticular Metabolism

- aqueous is the main source of nutrients
- epithelium is the main site for energy production for the whole lens
  - nutrients must reach the lens fibres by diffusion through superficial cells
- most metabolism is through glycolysis because the oxygen tension around the lens is low
Lenticular Metabolism

- Some ATP is generated from oxidative phosphorylation, which generates free radicals.
- Energy is expended to regenerate reducing agents (problem for deeper fibres with no mitochondria).
Lenticular Metabolism

- ATP is used for:
  - protein synthesis
  - active transport
  - cell division
  - synthesis of glutathione
  - cellular homeostasis
glucose → glucose-6-PO₄ → glucose-6-PO₄ dehydrogenase → 6-phosphogluconate

hexokinase

fructose-6-PO₄

glycolysis

fructose-1-6-di-PO₄

lactic dehydrogenase

lactate

fructokinase

pentose-PO₄

hexose monophosphate shunt

polyol dehydrogenase aldose reductase

sorbitol pathway

NADPH

NAD

NADH

NADP

NADPH

fructose

sorbitol
Lenticular Metabolism

- glycolysis (80%)
  - hexokinase is the rate limiting factor
  - anaerobic pathways can be used because there is adequate glucose
  - phosphorylation of glucose by hexokinase to form glucose-6-phosphate (uses ATP)
  - pyruvate can:
    - be converted to lactic acid (70 – 75%)
    - be used for aerobic metabolism (i.e. Kreb’s cycle; 5%)
Lenticular Metabolism

- hexose monophosphate shunt (14%)
  - hexokinase forms glucose-6-phosphate
  - glucose-6-phosphate dehydrogenase is needed to create 6-phospho-gluconate
  - pentoses are used in nucleic acid synthesis
  - uses NADP to produce NADPH
    - required for the maintenance of glutathione (oxidative balance)
    - cofactors in sorbitol pathway
Lenticular Metabolism

- sorbitol pathway (5%)
  - aldose reductase and NADPH are used to form sorbitol
  - sorbitol is converted to fructose by polydehydrogenase
    - sorbitol does not diffuse out of the lens
    - fructose diffuses out slowly
  - osmotic gradient causes swelling and cataract
  - increase in glucose increases usage of sorbitol pathway to protect lens from osmotic stress due to glucose levels
Electrolyte Balance

- cation balance
  - the lens tends to accumulate potassium ($K^+$) and it wants to reduce sodium ($Na^+$)
  - the epithelium contains $Na^+K^+$/ATPase activity and passive ion exchanges
  - called the pump-leak system in the lens
Electrolyte Balance

**ANTERIOR**
(AQUEOUS HUMOR)

\[
\begin{align*}
\text{Na}^+ &= 163 \text{ mM} \\
\text{K}^+ &= 4 \text{ mM}
\end{align*}
\]

**POSTERIOR**
(VITREOUS HUMOR)

\[
\begin{align*}
\text{Na}^+ &= 25 \text{ mM} \\
\text{K}^+ &= 140 \text{ mM} \\
\text{Na}^+ &= 144 \text{ mM} \\
\text{K}^+ &= 8 \text{ mM}
\end{align*}
\]
Electrolyte Balance

ANTERIOR
(AQUEOUS HUMOR)

PASSIVE K+ DIFFUSION

INWARD ACTIVE K+ TRANSPORT

OUTWARD ACTIVE Na+ TRANSPORT

PASSIVE Na+ DIFFUSION

INWARD ACTIVE AMINO ACID PUMPS

PASSIVE LEAK
H2O AND SOLUTES

POSTERIOR
(VITREOUS HUMOR)

K+

ADP

ATP

PASSIVE DIFFUSIONAL EXCHANGE OF H2O AND SOLUTES

EPITHELIUM
the highest Na⁺, K⁺, ATPase activity is in the LEC, creating an asymmetry in the ion transport

Na⁺, K⁺, ATPase activity will generate an electrochemical gradient across the cell membranes

electrical potential has been detected around the lens
Ionic Flow and Water Balance

1. NA+
2. gap junctions
3. NA+

Vaghefi, et al. 2013
Water & Electrolyte Balance

- water channels:
  - water content is relatively low (~60 – 65%)
  - created by members of the aquaporin family
  - AQP1 is present in the lens epithelium allowing high membrane water permeability
  - AQP0 is present in the lens fibres and makes up ~50% of the lens fibre membrane spanning proteins
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Cataracts

- defined as any opacity of the lens or its capsule
- results in a loss of transparency
  - etiology quite variable
  - independent of the opacity size, location, or extent
normal vision
cataract
glaucoma
age-related macular degeneration
Lens Capsular Changes

- thinning or thickening
- plaques
- rupture
- wrinkling
Lens Epithelial Cell Changes

- Wedl or bladder cells
- degeneration or necrosis
- proliferation and posterior migration
Fibre Changes

- particulate aggregates/eosinophilia
- vacuolization
- morgagnian globules: cortical liquefactive degeneration of lens fibres
Clinical Classification of Cataracts

- etiology (metabolic, secondary…)
- age of onset (congenital, juvenile, senile…)
- anatomic location (capsular, subcapsular, anterior, posterior, equatorial, cortical, nuclear, axial…)
- biomicroscopic appearance (stellate, punctate…)
- stage of progression (incipient, immature, mature, hypermature)
Tendency to Progress

- nuclear: often static or may get smaller
- cortical: variable but often progressive
- equatorial: often progressive
- subcapsular: often progressive
- highly influenced by age and breed of the animal
Pathophysiologic Changes

- multifactoral!!!

- commonalities:
  - increase in insoluble proteins
  - altered water and oxygen concentration
  - decreased efficiency in metabolic maintenance
  - increase in hydrolytic and proteolytic activity
  - changes in growth factors
Factors that Influence Clarity

Strict and Precise Order
Subcapsular Cataract

- abnormal stimulation of lens epithelial cells
  - physical
  - molecular
  - temporal
Cortical Cataract

- exact etiology unknown
- derangement of electrolyte and water balance
- change in fibre cells arrangement
  - extracellular space increases
  - calcium deposition in the central aspect of the fibre cell
  - higher lipid levels
Factors That Influence Clarity

Age
Lenticular Changes with Age

- decreased elastic-properties in the capsule
- closed system... what do you do with all those cells???
  - increased cortical stiffness due to compression and rearrangement of water and proteins
Lenticular Changes with Age

- nuclear/lenticular sclerosis is the increased density of the central lens
- results in loss of elasticity and pliability (in both the capsule and the lens fibres)
- termed presbyopia
- light scattering but not an ophthalmoscopic obstruction
Lenticular Changes with Age

- natural decrease in antioxidants
- natural increase in metabolic byproducts
Lenticular Changes with Age

- increased oxidative stress
  - limited supply of available antioxidants to protect cells
  - results in damage to proteins, lipids, and DNA
Oxidative Stress

- Main sources include radiation, mitochondrial respiration, and metabolic reactions.
- Thought to be one of the key features in initiated cataracts.
- Induces disulfide crosslinking, loss of protein solubility, and decreased cellular adhesions.
Oxidative Stress

- main endogenous antioxidants are catalase and glutathione
  - most glutathione is maintained in the reduced state (GSH)
  - to protect against oxidation, glutathione will become oxidized (GSSG)
  - glutathione reductase + NADPH + hexose monophosphate shunt are used to reduce oxidized glutathione
Oxidative Stress

- in deeper lens fibres, glutathione (GSH) can form glutathione-oxidized protein complexes (GSH + protein)
- these complexes are reduced by a second glutathione generating two oxidized glutathione molecules

\[ \text{GSH} \quad \text{oxidized} \quad \text{GSH} \]

deeper lens fibres

superficial lens fibres
Age-Related Cataract

- oxidative damage!!!
- post-translation changes reducing the stability of crystallin proteins allowing them to condense into aggregates
  - loss of chaperone activity
  - advanced glycation end products (AGEs)
  - loss of ubiquination activity
- nuclear tend to have less disruption of the fibres while cortical tend so cause greater damage
Age-Related Cataract

- oxygen distribution

Beebe, et al. 2011
Factors That Influence Clarity

Diet
Diabetic Cataract

- Excess glucose saturates the hexokinase pathways
- Accumulation of advanced glycation end products
- Involves the aldose reductase (sorbitol) pathway
  - AR is higher in dogs than in cats
- Typically rapidly developing, bilateral cataractogenesis
Diabetic Cataract

- resulting intumescent lens can initiate uveitis
Diabetic Cataract

- aldose reductase inhibitor (Kinostat™)
  - vehicle treated:
    - lens changes were observed in 20/24 eyes over 12 months
  - AR inhibitor:
    - lens changes were observed in 26/56 eyes over 12 months
    - no cataract in 30/56 eyes or cataract severity was less after 12 months
Nutritional Cataracts

- can arise from:
  - amino acid (arginine) deficiencies
  - tryptophan, phenylalanine, histidine
  - hypoglycemia

- lamellar zone separating anterior and posterior nuclear-cortical junctions

- residual white perinuclear opacity
Hypocalcemia

- most commonly caused by renal failure or hypoparathyroidism
  - multifocal, punctate or coalescing lamellar cortical opacities, bilateral
  - thought to cause a defect in the cation transport in the epithelium leading to swelling in lens fibres
Dietary & Exogenous Antioxidants

- lutein, zeaxanthin, and vitamins E and C are naturally occurring endogenous antioxidants
- thought to protect against ROS-mediated cell damage and act as anti-inflammatory
Dietary & Exogenous Antioxidants

- cell culture and animal models with inducible diseases have been used to demonstrate proof of principle
- no non-human clinical trials demonstrating efficacy
- human clinical trials reveal highly variable results
Factors that Influence Clarity

Family
Heritable Cataracts

- ~160 affected canine breeds have a heritable basis and mode of transmission been suspected or established
- characteristics including age of onset, rate of progression, bilateral presentation can suggest a heritable basis
- most are autosomal-recessive and in dogs, are typically juvenile or middle-age onset
- phenotypic presentation may vary within a breed and may be modified by other genetic or environmental factors
Heritable Cataracts

- heat shock transcription factor (HSF)4 in Staffordshire bull terriers, Boston terriers, and Australian shepherds
  - recessive condition in Staffordshire bull terriers and Boston terriers but dominant in Australian shepherds
  - not all will present the same way

Mellersh, et al. 2006
Heritable Cataracts

- uncommon in cats and horses
  - in cats the mode of inheritance is poorly described
  - Morgan horses have been described to develop bilateral, non-progressive nuclear or perinuclear opacities
- some cattle (Jersey, Herford, and Holstein-Friesian) have documented autosomal recessive congenital cataracts
Factors that Influence Clarity

Inflammation and Injury
Inflammation

- main cause for secondary cataract in horses (includes equine recurrent uveitis (ERU), infectious, and traumatic uveitis)
Inflammation

- main cause for secondary cataract in horses (includes equine recurrent uveitis (ERU), infectious, and traumatic uveitis)
  - anterior uveitis typically presents as focal anterior capsular or subcapsular cataracts
  - posterior uveitis may present with diffuse and irregular posterior capsular or subcapsular cataracts
  - panuveitis can lead to complete cataract
    - ERU-associated typically have periequatorial cortical vacuoles and can progress quickly
Inflammation

- most cataracts in cats are associated with anterior uveitis, glaucoma, and lens luxation
  - typically slow progressing and cortical
Inflammation

- lens induced – phacolytic vs phacoclastic
Inflammation Associated with Injury
Inflammation Associated with Injury

- phacoclastic response
  - prophalactic lens removal has been advocated for tears greater than 1.5 mm or if substantial cortical disruption
Inflammation Associated with Injury

  - Medical management of acute traumatic lens capsule ruptures is possible
    - Average rent size was 7.3 mm with significant fibre disruption
    - 100% of patients were successfully managed (average follow-up was 31.8 months)
    - May be attributed to the fibrinous uveitis
    - Referral time was related to successful outcome
Inflammation Associated with Injury

- septic implantation syndrome:
  - 20 feline and 46 canine eyes with intractable uveitis or glaucoma
  - infectious organisms were identified in 70% of cat lenses and 65% dog lenses

Bell, et al. 2012
Inflammation Associated with Injury

- other penetrating injuries
  - small rents may seal
  - typically results in diffuse cataracts
  - damage (or expulsion) of cortical material may increase the chance of progression or uveitis

- blunt injury may result in compressive forces causing damage to the lens epithelium or displacement of the fibre membranes
Factors that Influence Clarity

Toxicity
Toxicity

- miscellaneous toxins (ketaconozole, DMSO, pefloxicin, digitalis...)
- retinal degeneration – presumably from toxic products (dialdehydes) released from the degreasing retina
  - Miniature and Toy poodles, Labrador
  - typically progressive
Cataracts

- there is only one treatment for cataracts and that is surgery
  - +/- artificial lens implantation
  - earlier is better to reduce the chance of uveitis
Posterior Capsule Opacification (PCO)

- decreases the quality of vision over time
- between 80 – 100% incidence in dogs
Types of PCO
Surgical PCO Prevention

- planned posterior capsulorhexis
- creating an anterior capsulorhexis that is slightly smaller in diameter compared to the IOL optic
- hydrodissection
- irrigation and aspiration
- polishing
Surgical PCO Prevention

capsular tension rings

intraocular lens design
Surgical PCO Prevention

[Graph showing the percent confluence over days for different treatments: Aphakic, Dioptrix, I-Med, and AcriVet.]
Surgical PCO Prevention

![Graph showing PCO scores for different IOL types.](Image)

**Graph Details:**
- **Y-axis:** PCO Score
- **X-axis:** IOL Type (PMMA, hydrophilic acrylic, hydrophobic acrylic)
- **Lines:**
  - observer 1
  - observer 2
  - observer 3
  - observer 4

**References:**
- Apple, et al. 2011
Surgical PCO Prevention

- intraocular lens design
- capsular tension rings
Surgical PCO Prevention

Hoy, et al. 2008
Cataract Surgery Complications


- 66 eyes

- Most frequent histopath diagnoses were glaucoma (76%) and retinal detachment (64%)
  - anterior synechiae, iris bombé, goniodysgenesis
Cataract Surgery Complication
Cataract Surgery Complications

  - 66 eyes
  - most frequent clinical diagnoses were glaucoma (86%) and uveitis (82%)
    - discrepancies with histopath diagnosis possibly due to inclusion of evisceration samples or mild clinical presentation
Questions?