Physiology of the Tear Film and Adnexa
2018 William Magrane Basic Science Course
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“Lacrimal Functional Unit” (LFU)

- Multi-organ functional unit which keeps the corneal surface balances
  - Lacrimal gland
  - Tear film
  - Ocular surface epithelium
  - Cornea, conjunctiva, Meibomian/tarsal glands
  - Eyelids
  - Interconnecting sensory and motor nerves
Control of the Tear Film

- **Traditional View**
  - Tears are due to intrinsic lacrimal gland activity and only reflex tears involve neural input

- **Current View**
  - On-going homeostatic regulation of the ocular surface
  - Constant level of neural input that may mediate lipid and mucin
Control of the Tear Film: Let’s Start with Nerves

- Afferent nerves from the cornea and conjunctiva
- Central nervous system relay nuclei
- Efferent nerves comprise the autonomic innervation to secretory tissues that contribute to the tear film
Innervation of the Lacrimal Gland: Afferent

- Cornea stimulates afferent nerves traveling along lacrimal nerve
  - Ophthalmic division of the trigeminal nerve
  - Sensory nuclei in trigeminal ganglion (TG)
    - Lacrimal nerve – smallest branch of the ophthalmic nerve
    - Courses laterally within the orbital cavity above and along the upper border of the lateral rectus muscle

*Miller’s Anatomy of the Dog*
Innervation of the Lacrimal Gland: Afferent

- Trigeminal ganglion is connected to lacrimal nucleus of facial nerve in pons by internuncial neurons

- Impulses are directed via the trigeminal ganglion to the lacrimal nucleus

Plate 788, Gray’s Anatomy. https://commons.wikimedia.org/wiki/File:Gray788.png
Lac rimal Gland Innervation: Afferent Pathway of Trigeminal Ganglion-Mediated Reflex

Lacrimal gland

Corneal conjunctiva

stimulus

Lacrimal nerve

Ophthalmic nerve

Trigeminal ganglion

Maxillary nerve

Mandibular nerve

Pons

Internuncial neurons

Lacrimal nucleus of the facial nerve in the pons

Slide courtesy of Dr. Juri Ota-Kuroki, former MU ophthalmology resident
Innervation of Lacrimal Gland: Additional Sensory

- Afferent innervation
  - Also provided by ipsilateral superior vagal ganglion (SVG)
  - Superior glossopharyngeal ganglion (SGG)
  - SVG and SGG may c
- In primates hypothalamus and lacrimal nucleus mediate emotional tears
- Olfactory system and lacrimal nucleus ("wasabi tears")
Innervation of the Lacrimal Gland: Sympathetic

- Postganglionic fibers from cranial cervical sympathetic ganglion
- Is the uppermost ganglion of the sympathetic trunk
- Fibers travel in the plexus of nerves around internal carotid artery
- Join maxillary nerve, zygomatic nerve, zygomaticotemporal nerve and finally lacrimal nerve
Innervation of the Lacrimal Gland: Sympathetic

- Post-ganglion fibers on maxillary nerve, zygomatic nerve, zygomaticotemporal nerve and finally lacrimal nerve
  - Distributed to interstitium surrounding acini and to vascular smooth muscle fibers of glands
  - Sympathetic nerves contain norepinephrine and neuropeptide Y
  - Sympathetic stimulation increases tear secretion by affecting vascular supply to lacrimal gland
  - Activates G protein pathway
In some species, the sympathetic nervous system may influence tear secretion, not only by modulating blood flow to the gland and its distribution within it, but also by direct effects on the secretory acini.
Innervation of the Lacrimal Gland: Parasympathetic

- Cranial nerve VII parasympathetic nuclei
- Travel with the greater petrosal nerve (pass through petrous temporal bone and Eustachian tube)
- Move to the nerve of the pterygoid canal (with postganglionic sympathetic and sensory fibers)
- Synapse in pterygopalatine ganglion in the orbit
Innervation of the Lacrimal Gland: Parasympathetic

- Passes to caudal nasal nerve (maxillary division of cranial nerve V)
- Innervated **lateral nasal gland**

- Lacrimal gland secretion inhibited by LeuEnkephaline (L-Enk)
  - Neuropeptide interacts with inhibitory G Proteins
  - Interferes with activation adenylate cyclase by G stim proteins

*Photo courtesy of Dr. Carrie Breaux*
Parasympathetic ganglion involved in the neural regulation of lacrimal gland secretion

- Ciliary ganglion
- Otic ganglion
Parasympathetic innervation

Lacrimal nucleus (CN VII)

Trigeminal ganglion

Maxillary nerve

Mandibular nerve

Ophthalmic nerve

Zygomatic nerve

Sphenopalatine nerve

Pterygopalatine (Sphenopalatine) ganglion

Post-ganglionic parasympathetic fibers

Pre-ganglionic parasympathetic fibers

Greater petrosal n.

Vidian n.

Pterygoid canal

Electrolyte and water secretion

Lacrimal gland acinar cells, tubules, ductules, and vascular wall

Zygomatico-temporal nerve

Maxillary nerve

Mandibular nerve

Pons

Lacrimal nucleus CN VII

Slide courtesy of Dr. Juri Ota-Kuroki, former MU ophthalmology resident
Neurogenic KCS

- Lesion along efferent arm
  - Parasympathetic nucleus CN VII
  - Main trunk CN VII
  - Geniculate ganglion
  - Major petrosal nerve
  - Nerve of pterygoid canal
  - Pterygopalatine ganglion
  - Postganglionic parasympathetic fibers

- Xeromycetria (dry nose) common finding
Neurogenic KCS - the Petrous Temporal Bone

- Otitis
  - KCS
- Concurrent disease
  - Neurotropic keratitis
  - Eyelid anesthesia
  - Homer’s syndrome
  - CN VII paralysis
Petrus Temporal Bone Lesions

- Xeromycteria (CN VII)
- Xerostomia (CN VII, CN IX)
- Facial nerve paralysis (CN VII)
- Facial anesthesia (CN V)
- Nasal cavity anesthesia (CN V)
- Loss of taste (CN VII, CN IX)
- Homer’s syndrome
  - Often due to subclinical otitis media
Neurogenic KCS: Lesions of Middle Fossa and Major Petrosal Nerve

- Result of erosive lesions
- Similar findings to petrous temporal bone lesions
  - No facial nerve paralysis/paresis

Pharmacological Reviews June 2003, 55 (2) 271-324;
Neurogenic KCS: Lesions in Extraperiorbital Sheath and Pterygopalatine Ganglion

- Temporal muscle myositis/cellulitis/abscess/dental abscess
- Preganglionic
  - KCS
  - Xeromycteria
  - Eyelid anesthesia
- Postganglionic
  - KCS
  - NO Xeromycteria
  - Eyelid anesthesia
Neurogenic KCS: Lesions in Periorbital and Zygomaticotemporal Nerve

- Orbital Trauma
  - KCS
  - Periocular anesthesia
  - NO xeromycteria
Neurogenic KCS

- Denervation Hypersensitivity of the Lacrimal Gland
  - Exogenous neurotransmitter in form of oral 2% pilocarpine
    - 2 drops/2%/BID increasing daily until toxicity then decrease
  - Topical 0.5% pilocarpine
    - BID
I’m here to make you cry

- Normal Tears
  - Prevent surface infection
  - Provide pure optical surface for light refraction
  - Maintain homeostatic environment
  - Now described as the Lacrimal Functional Unit (LFU)
    - Embraces the relationship between the ocular surface and lacrimal glands
    - Tear film, ocular surface epithelium, eyelids, interconnecting sensory and motor nerves
Tear Film Anatomy & Physiology

- **Lipid**
  - Superficial
  - Stabilize & prevent evaporation
  - Made by Meibomian glands

- **Aqueous**
  - Intermediate
  - Corneal nutrition, remove waste
  - Made by lacrimal and gland of 3rd eyelid

- **Mucus**
  - Interface of tear film with hydrophobic cornea
  - Source of secretory IgA
  - Produced by conjunctival goblet cells
Tear Film – Functional Anatomy

- Thickness
  - SO MANY PAPERS AND DIFFERENT MODALITIES OF MEASUREMENT!
  - Prydal et al IOVS 1992: 35-40um
  - Danjo et al, Jpn J Ophthal 1994 – 11um
  - Ewen King-Smith et al IOVS 2000 – 3(!)um
  - J Cataract Refract Surg – 6-20um
  - Werkmeister et al IOVS 2013, OCT – 5um
Tear Film Thickness

- Actually changes during stages of blinking and area of the cornea

Werkmeister et al. (2013). Measurement of Tear Film Thickness Using Ultra-high-Resolution Optical Coherence Tomography. IOVS. 54. 10.1167/iovs.13-11920.
Tear Film the New Model

Figure 1 Models of tear film structure

1940s-1970s: Three-layer model
- Lipid
- Aqueous
- Mucin
- Microvilli
- Squamous cells

Updated structure: multiple blended phases
- Lipid: polar and non-polar layer
- Aqueous: proteins, soluble mucins, lipids, electrolytes plus other organic components
- Mucins: membrane bound and secreted

Tear Film the New Model

- Layers are a continuum
- Lipid most anterior to aqueous and mucin

Tear Film Content

- Many discrepancies!
  - Tears difficult to sample
  - Inconsistent sampling
  - Qualitative vs. quantitative techniques

### Chemical Composition Of Human Tears & Plasma

<table>
<thead>
<tr>
<th></th>
<th>TEARS</th>
<th>PLASMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) water</td>
<td>98.2g%</td>
<td>94g%</td>
</tr>
<tr>
<td>2) solids, total</td>
<td>1.8%</td>
<td>6g%</td>
</tr>
<tr>
<td>3) Na+</td>
<td>142meq/l</td>
<td>137-142meq/l</td>
</tr>
<tr>
<td>4) K+</td>
<td>15-29meq/l</td>
<td>5meq/l</td>
</tr>
<tr>
<td>5) Cl-</td>
<td>120-135meq/l</td>
<td>102meq/l</td>
</tr>
<tr>
<td>6) HCO3-</td>
<td>26meq/l</td>
<td>24.3meq/l</td>
</tr>
<tr>
<td>7) Ca2+</td>
<td>2.29mg/100ml</td>
<td></td>
</tr>
<tr>
<td>8) Glucose</td>
<td>3-10mg/100ml</td>
<td>80-90mg/100ml</td>
</tr>
<tr>
<td>9) Total proteins</td>
<td>0.6-2gm/100ml</td>
<td>6.78g/100ml</td>
</tr>
<tr>
<td>10) Aminoacids</td>
<td>8gm/100ml</td>
<td></td>
</tr>
<tr>
<td>11) Urea</td>
<td>0.04mg/100ml</td>
<td>20-40mg/100ml</td>
</tr>
</tbody>
</table>
Tear Film Function

- Protect cornea from desiccation and lubricate
- Maintain refractive power
- Protect against infections (specific and nonspecific)
- Supply oxygen/nutrients and remove by-products
- Avoid corneal dehydration due to hyperosmolarity
- Remove foreign materials
- Provide WBCs/Other immune cells with access to cornea/conjunctiva
Tear Film Lipid

- Tarsal (Meibomian) Glands
  - Holocrine modified sebaceous glands
  - Arranged linearly within the tarsal place of the eyelid margin
- Secretions
  - Wax monoesters
  - Sterol esters
  - Hydrocarbons
  - Triglycerides
  - Diglycerides
  - Free sterols (cholesterol)
  - Fatty acids
  - Polar lipids
Tear Film Lipid Layer

- Meibomian glands
  - MW of Meibomian lipids is higher and polarity is lower making meibum fluid at lid temperature
  - May act in combination with the tear film like a lung surfactant
Tear Film Lipid Layer

- Dog: 20-40 glands per eyelid
  - Located in tarsal plate
  - Aggregates of secretory acini
  - Visible through palpebral conjunctiva
- Acini open into ductules at right angles to eyelid margin
- Deliver lipid to surface of eyelid
- “Gray line” anatomic landmark for surgery
Tear Film Lipid Layer

- Thickness varies through day
  - Max when waking up
  - Differs between individuals and with age
- Compression of eyelids during blinking extrudes meibum
  - Neural and hormonal regulation as well
Tear Film Lipid Layer

- Secretion Influences
  - Mechanical (blinking)
  - Nervous (altered if CNV sectioned)
  - Hormonal (androgens stimulatory, estrogen inhibitory)
  - Physical (feedback regulation based on surface tension)
Tear Film Aqueous Layer

- Secreted by lacrimal glands of orbit and third eyelid
- Provides most of the cornea's metabolic needs
  - Glucose, electrolytes, oxygen
- Lubricates cornea and conjunctiva
- Removes metabolites (CO2 and lactic acid)
- Flushes away debris and bacteria
Tear Film Aqueous Layer

- 98.2% water, 1.8% solids
  - Water, electrolytes, glucose, urea, surface-active polymers, glycoproteins and tear proteins
    - Globulins (IgA, IgG, IgM)
    - Albumin, lysozyme, lactoferrin, lacritin, interleukins
    - Lipocalin, epidermal growth factor, transforming growth factors
    - Antibacterial Properties
      - Antibodies, immunoglobulins, lysozyme, lactoferrin, transferrin,
Tear Film Aqueous Layer: Proteinases

- A balance of Proteinase inhibitors and Proteinases
  - Proteolytic activity increased after corneal wounding
  - Melting ulcers have maintained increased protease activity
Tear Film Aqueous Layer

- Lacrimal glands of orbit and 3rd eyelid are tubuloacinar and histologically similar
  - Ductules deliver aqueous secretions into conjunctival fornices
  - Dogs: 3-5 ductules from orbital lacrimal gland to dorsolateral fornix
  - Dogs: multiple ductules between lymphoid follicles on bulbar third eyelid
Tear Film Aqueous Layer

- Humans: pH 7.14-7.82, osmotic pressure of 305 mOsm/kg, refractive index 1.357
- Cattle pH 8.32
- Dog pH 8.05
- Horse pH 7.84
Tear Film Aqueous Layer

- Relative contributions to reflex tear
  - Varies considerably between individuals
  - Orbital lacrimal gland was majority in some but not all
  - Removal of either increases production in the other
  - Removal of both led to complete dryness
Role of gland in basal versus reflex tearing unclear

- Destruction of lacrimal gland decreases tears 23-46%
- Destruction of third eyelid gland decreases tear 12-26%
Tear Film Aqueous Layer: Control

- Chemical mediators of lacrimal gland secretion
  - Cholinergic agonists
  - Sympathetic agonists (norepinephrine)
- Neurotransmitters activate signal transduction
  - Effects myoepithelial, acinar and duct cells, blood vessels
- Other protein stimuli
  - EGF, neuropeptide Y, substance P, calcitonin and hormones
Harderian Gland

- Lacrimal gland in amphibians, reptiles, birds and mammals
- 5 types: serous, mucous, seromucoid, mixed and lipid glands
- Found on nasal side of orbit and duct opens on bulbar third eyelid
- Contiguous with gland of 3rd eyelid except:
  - Rabbits - below and medial to lacrimal gland
  - Pigs - separate from 3rd eyelid gland
  - Rodents (rat, hamster, gerbil) - posterior to globe and produces porphyrin creating a reddish color that fluoresces under ultraviolet light
Tear Film Mucus Layer

- Deepest tear film layer
- Adheres firmly to underlying epithelial cells
- Thickness 0.8um over cornea to 1.4um over conjunctiva
- Facilitates adherence of aqueous layer to surface of epithelial cells
Mucin

- Heterogeneous group of hydrated o-linked oligosaccharides linked to protein
- Proteins synthesized in endoplasmic reticulum of goblet cells
- Saccharide branches added in golgi apparatus
- Glycoproteins condense and stored in secretory granules at apical side of goblet cells
- Secretagogues, serotonin, epi, phenylephrine, dopamine, antigen, immune complexes, mechanical action all stimulate release
Tear Film Ocular Immunity

- Lysozyme, betalysin, lactoferrin and antibody (cattle have low lysozyme)
- Cortisol increases with systemic levels
- IgA & IgG secreted by lacrimal glands
- Serum proteins
  - Derived from vascular compartment by filtration
  - 1% of total proteins in absent of infection
  - Albumin, haptoglobin, IgG, IgA, IgM, IgE, alpha2-macroglobulins, complement-derived proteins, transferrin, alpha1-antitrypsin, beta2-microglobulin
Function of cortisol in stress

Short-term stress
- Nerve impulses
- Spinal cord
- Preganglionic sympathetic fibers
- Adrenal medulla (secretes amino acid-based hormones)
- Catecholamines (epinephrine and norepinephrine)

**Short-term stress response**
1. Increased heart rate
2. Increased blood pressure
3. Liver converts glycogen to glucose and releases glucose to blood
4. Dilation of bronchioles
5. Changes in blood flow patterns leading to decreased digestive system activity and reduced urine output
6. Increased metabolic rate

More prolonged stress
- Hypothalamus
- CRH (corticotropin-releasing hormone)
- Corticotroph cells of anterior pituitary
- To target in blood
- ACTH
- Adrenal cortex (secretes steroid hormones)
- Glucocorticoids
- Mineralocorticoids

**Long-term stress response**
1. Retention of sodium and water by kidneys
2. Increased blood volume and blood pressure
3. Proteins and fats converted to glucose or broken down for energy
4. Increased blood glucose
5. Suppression of immune system
So, does native cortisol have an influence on the eye?
Question 1: is cortisol present in the tears of healthy horses
Designing the study

- Challenges: collecting tears
- Diurnal cycling
- Sensitive assay reliable on small sample size
Detection of endogenous cortisol in equine tears and blood at rest and after simulated stress

![Graph showing cortisol concentrations over time in serum and tears.](http://onlinelibrary.wiley.com/doi/10.1111/vop.12128/full#vop12128-fig-0003)
What about horses with PPID?
Canine Tear Cortisol

![Graph showing cortisol concentration in serum and tears with statistical significance markers.](image-url)
Keratoconjunctivitis Sicca

- “Abnormality in either the quantity or quality of any primary tear component” (C Moore, 1999)

- “Dry eye is a disorder of the tear film due to tear deficiency or excessive tear evaporation” (NEI NIH)
KCS: Immunopathogenesis in dog

1. Lymphocyte-associated cytotoxicity
2. Apoptosis of glandular epithelial cells
3. Cytokine release from inflammatory cells
4. Inflammatory cells/associated cytokines or autoantibodies may influence neurotransmitter function in lacrimal gland and inhibit neurologic stimulation of tear secretion

**BUT**

- Could the new model of Dry Eye Disease be part of the story?
Epithelial cells of the conjunctiva regularly secrete antigens or cellular proteins. In normal states, these antigens are recognized by antigen-presenting cells and then presented to effector lymphocytes on the ocular surface. In parallel, regulatory lymphocytes which are also present block the action of effector lymphocytes, thus preventing the development of an inflammatory state and maintaining local immunohomeostasis (SMITH, 2005). This pathway is believed to be regulated by androgens which are responsible for the regulation of the production of transforming growth factor β (TGF-β) whose function is to reduce local prolactin concentration. Conditions such as senility and idiopathic factors may reduce the production of androgen hormones, leading to changes in conjunctival immunohomeostasis due to the reduced production of TGF-β and increased prolactin concentration in conjunctival epithelial cells. This in turn would alter local antigen detection, favoring the effector lymphocyte pathway and thus triggering local inflammation (SMITH, 2005).

Causes of KCS

- Immune-mediated is most common in the dog
  - With breed disposition

*Table 16.2 Causes of Keratoconjunctivitis Sicca (KCS) in the Dog*

<table>
<thead>
<tr>
<th>Chronic blepharoconjunctivitis</th>
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<tbody>
<tr>
<td>Congenital</td>
</tr>
<tr>
<td>Pug</td>
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<tr>
<td>Yorkshire Terrier</td>
</tr>
<tr>
<td>Drug-induced</td>
</tr>
<tr>
<td>Topical/general anesthesia</td>
</tr>
<tr>
<td>Topical/parenteral atropine</td>
</tr>
<tr>
<td>Drug toxicity</td>
</tr>
<tr>
<td>5-Aminosalicylic acid</td>
</tr>
<tr>
<td>Phenazopyridine</td>
</tr>
<tr>
<td>Sulfadiazine</td>
</tr>
<tr>
<td>Sulfasalazine</td>
</tr>
<tr>
<td>Trimethoprim/sulfonamide</td>
</tr>
<tr>
<td>Immune-mediated</td>
</tr>
<tr>
<td>Local</td>
</tr>
<tr>
<td>Systemic</td>
</tr>
<tr>
<td>Breed disposed (see Table 16.3)</td>
</tr>
<tr>
<td>Irradiation</td>
</tr>
<tr>
<td>Neurogenic</td>
</tr>
<tr>
<td>Surgically induced</td>
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<tr>
<td>Excision of lacrimal/nictitans glands</td>
</tr>
<tr>
<td>Evisceration/prosthesis</td>
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<tr>
<td>Systemic disease</td>
</tr>
<tr>
<td>Canine distemper</td>
</tr>
<tr>
<td>Metabolic disease</td>
</tr>
<tr>
<td>Trauma to the eye and orbit</td>
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</tbody>
</table>

Based on the Physiology of the Tear Film: Why does KCS look like this?
Qualitative Dry Eye

- Tear film breakup time
- Tear osmolality measurement
- Tear ferning: reflects tear components, no diurnal variation
- Meibometry to assess lipid
The Tear Film Breakup Test

- Tear film flooded with fluorescein
- Eyelids opened and timing starts
- Test concludes at first dry spot
- The average TBUTs reported are 16.7 s +/- 4.5 in normal dogs, 19.7 s +/- 5 in sedated dog, >13 s in cats; 8.3 +/- 1.3 in normal horses.
  (Cullen et al. 2005), sedated dogs (Moore 1990) and horses (Monclin 2010)

Topical anesthetic shortens the TBUT
Tear Ferning

- Extract tears via capillary tube or STT strips and centrifuge out
- Place on glass slide and allow to dry
- Can correlate with STT (not exact)
- "normal" dogs can have abnormal ferning (temp dependent, sample handling?)

http://dx.doi.org/10.4314/ovj.v7i3.11
Meibometry

- Hard to reproduce
- Ofri et al reports 179 ± 60 Meibometer units in normal dogs (lower in Schnauzers)
- Meibometer MB550 unreliable (Benz et al 2008)
  - Very different readings between observers
Qualitative KCS

- Loss of goblet cells
  - Chronic inflammatory cell infiltrates in the conjunctiva
  - Symblepharon post ulcerative conjunctival disease and fibrosis
  - Hypoplasia (reported once)
- Dysfunction of the Meibomian glands
  - Seborrhea
  - Autoimmune diseases effecting Mucocutaneous junctions
  - Post cryotherapy/Distichia treatment
  - Eyelid agenesis
Treating KCS: honestly you get a whole other lecture about this so...

- Problem: inflammation of lacrimal gland
- Problem: conjunctivitis – proliferation of goblet cells
- Problem: lack of aqueous tears
- Problem: disruption of microenvironment and bacterial overgrowth