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EYES, TEARS, AND TREATMENT: UNVEILING THE SERUM PROTEIN FACTOR

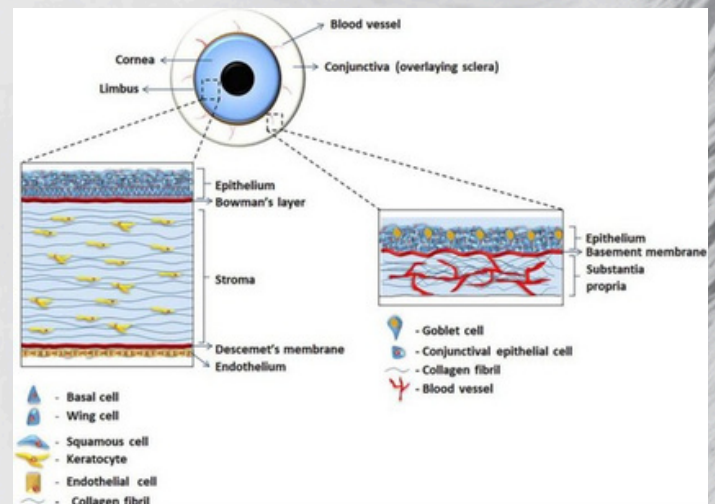
The Role of Tears and How Increased Serum Protein Potentially Impacts Treatment Efficacy



As you will recall from anatomy in veterinary school, the front aspect of the eye has two important structures: the cornea and the conjunctiva. The cornea is a clear avascular structure, covered by a layer called the corneal epithelium that sits on top of a collagenous tissue called stroma. The conjunctiva is a pink mucous membrane that lines the anterior sclera ('white of the eye') and the inner surface of the eyelids.

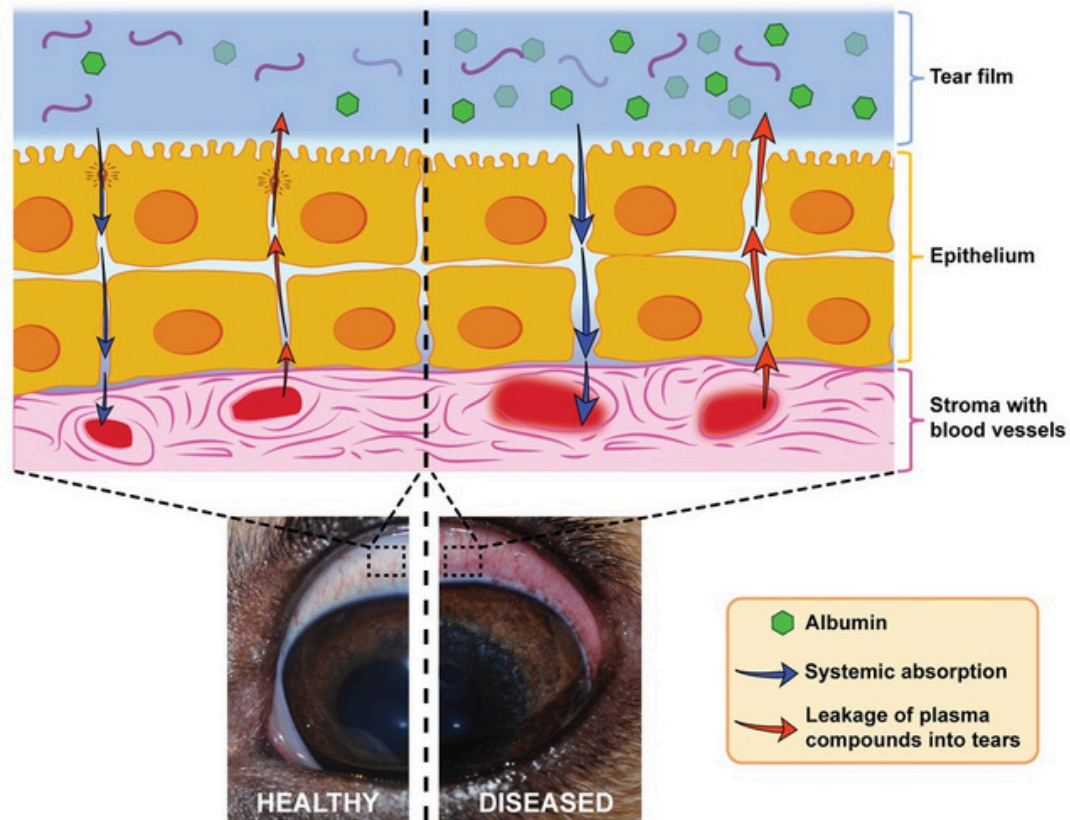
Veterinarians are quite familiar with the functions of the corneal barrier:

- Prevents invasion of pathogens
- Keeps the cornea relatively dehydrated
- Limits penetration of drugs and toxins



(Image Source: Ramos T, Parekh M, Meleady P, O'Sullivan F, Stewart RMK, Kaye SB, Hamill K, Ahmad S. Specific decellularized extracellular matrix promotes the plasticity of human ocular surface epithelial cells. Front Med (Lausanne). 2022 Nov 15;9:974212.)

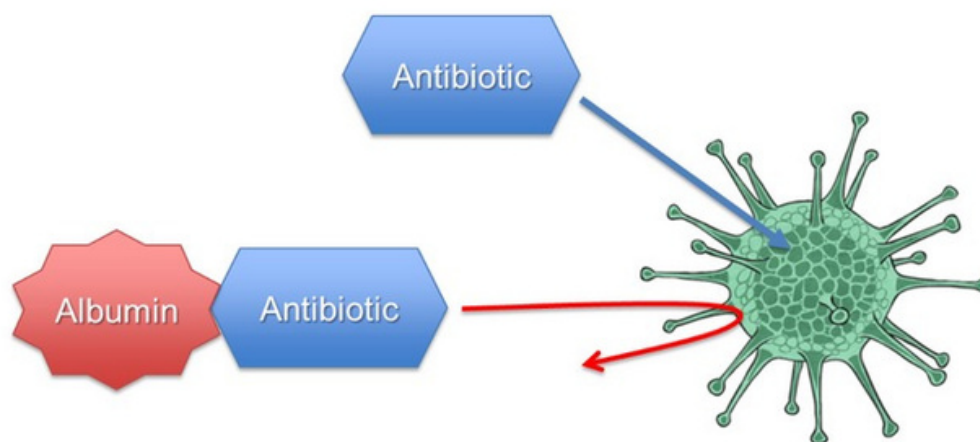
But what happens when the conjunctiva is affected? In dogs, cats, horses, and other veterinary species, conjunctival inflammation (conjunctivitis) is very common, either as a primary condition, or as bystander to other diseases in the eye such as corneal erosion (ulcer), inflammation inside the eye (uveitis), elevated pressure inside the eye (glaucoma), and more. Physiologically, conjunctival inflammation is known to disturb the blood–tear barrier and thus affects the tear film stability and composition as depicted in the image below.¹



In a recent study, researchers examined the levels of total protein content and serum albumin levels in the tears of dogs with both healthy and diseased eyes.² They found that the protein composition in canine tears varied based on ocular health status. Specifically, they observed that the presence of ocular disease was associated with elevated serum albumin levels in canine tears. Similar findings were observed in horses.² These studies suggest serum albumin serves as a valuable biomarker for ocular insult.¹⁻³

Increased serum albumin in the tear film indicates a disruption of the blood-tear barrier:

1. Mechanisms of Barrier Breakdown: Ocular disease leads to the disruption of the blood-tear barrier through mechanisms involving vascular permeability and the disruption of epithelial tight junctions in the conjunctiva.
2. Plasma Compounds in Tear Film: The breakdown of the blood-tear barrier allows plasma compounds, including proteins and drugs, to diffuse into the tear film at elevated levels.
3. Serum Albumin: In healthy individuals, serum albumin concentration in tears is typically low. However, in disease states, it increases significantly.
4. Clinical Implications: Breakdown of the blood-tear barrier can have several clinical implications in veterinary patients¹, including:
 - a. Decreased antimicrobial efficacy as only unbound antibiotic is microbiologically active (see image below)
 - b. Reduced drug bioavailability of topical medications, as only unbound drug can penetrate through the cornea
 - c. Enhanced diffusion of systemically administered medications into the tear film



Of particular significance, another study showed that even a minimal concentration of 0.5 mg/mL of albumin in dogs resulted in a reduction in antibacterial activity, with the extent of reduction being dose-, bacteria-, and antibiotic-dependent.⁴ This finding underscores the importance of considering albumin's binding effect when applying antibiotics to the eye.

In other words, part of the antibiotic you apply to your veterinary patient is being lost to binding by serum albumin in the tear film. In another recent study presented at ACVO 2022⁵, both fresh and frozen canine and equine serum/plasma were tested for 17 topical antibiotics against the three most common bacterial pathogens in corneal ulcers (*Staphylococcus* sp., *Streptococcus* sp., *Pseudomonas* sp.). The addition of serum or plasma increased the minimal inhibitory concentrations (MICs) of the tested antibiotics. Higher MICs mean that the antibiotics applied topically might not reach therapeutic levels, or remain above MIC for a shorter duration.

In a recent Dômes Pharma-sponsored study⁶, tear film concentrations of cefazolin compounded with two different lubricants were compared. The two lubricants studied were Refresh (1.4% polyvinyl alcohol, PVA) and Oculenis (0.75% cross-linked hyaluronic acid, XHA). The study concluded that XHA greatly improved tear film concentrations of cefazolin over 8 hours when compared to PVA. As such, compounding cefazolin with Oculenis might help reduce dosing frequency, and improve clinical outcomes of veterinary patients with bacterial keratitis; however, future experiments are still needed to assess XHA-cefazolin in canine patients, and determine clinical breakpoints for cefazolin against common bacterial pathogens.

In conclusion, leaky conjunctiva in inflamed eyes has important clinical implications on the effect of topical medications. Ocular inflammation leads to excess albumin in the tear film, which can bind to topical drugs and reduce their efficacy.¹ Looking for potential alternatives to serum/plasma might be helpful, as these blood products are helpful against collagenolysis (melting) but are also rich in proteins that could negatively affect other drugs. For now, it is advised to wait at least 10 minutes between the last eyedrop and administration of serum/plasma, especially when using topical antibiotics.

¹Sebbag L, Mochel JP. An eye on the dog as the scientist's best friend for translational research in ophthalmology: Focus on the ocular surface Med Res Rev. 2020 Nov;40(6):2566-2604.

²Sebbag L, Allbaugh RA, Weaver A, Seo YJ, Mochel JP. Histamine-Induced Conjunctivitis and Breakdown of Blood-Tear Barrier in Dogs: A Model for Ocular Pharmacology and Therapeutics. Front Pharmacol. 2019 Jul 9;10:752.

³Terhaar HM, Allbaugh RA, Mochel JP, Sebbag L. Serum albumin and total protein concentration in the tear film of horses with healthy or diseased eyes. Vet Ophthalmol. 2021 Jan;24(1):20-27.

⁴Sebbag L, Broadbent VL, Kenne DE, Perrin AL, Mochel JP. Albumin in Tears Modulates Bacterial Susceptibility to Topical Antibiotics in Ophthalmology. Front Med (Lausanne). 2021 Nov 30;8:663212.

⁵Kubai MA, Allbaugh RA, Stinman CC, Kenne DE, Moniot JM, Baum DH, Roy MM, Sebbag L. Canine and equine serum/plasma modulate the effect of topical antibiotics against common bacterial pathogens in dogs with infectious keratitis. Abstract, Annual congress of the American College of Veterinary Ophthalmology, 2022.

⁶Sebbag L, Ortaeskinaz E, Goncharov Y, Ofri R, Arad D. Cross-linked hyaluronic acid enhances tear film concentrations of cefazolin sodium in canine eyes. Abstract, Annual congress of the American College of Veterinary Ophthalmology, 2023.