

## Tears Production: Implication for Health Enhancement

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### Abstract

Tears are liquid produced by the body to lubricate the eyes. The fluid secreted by the lacrimal glands is a complex solution of ions and proteins produced by two resident secretory cell populations: the plasma cells of the immune system and the acinar duct cells of the secretory epithelium of the gland. These cells are found in the interstitial spaces of the gland and migrated from lymphoid organs such as the gut-associated lymphoid tissue (GALT). These plasma cells secrete immunoglobulin A (IgA) which is important in protecting the ocular surface from infection. The acinar cells of the secretory epithelium have three main functions: to synthesize and secrete a number of tear-specific proteins, to secrete water, and to transport the IgA secreted by the plasma cells from the interstitial compartment into the lumen of the gland. The lacrimal gland-specific proteins found at highest concentrations in the tears are lactoferrin, tear-specific pre-albumin (TSP or lipocalin), and lysozyme. Other proteins occurring at lower concentrations are amylase, peroxidase, plasminogen activator, prolactin, epidermal growth factor (EGF), transforming growth factor- $\beta$  (TGF- $\beta$ ), endothelin-1, and retinol. Lactoferrin, TSP, peroxidase and lysozyme as well as IgA which function to protect the cornea from viral and bacterial infections. This is important because the cornea is a wet, warm surface and thus is an ideal pathway for pathogens to invade the body and to affect the cornea. Retinol, derived from vitamin A, is necessary for the health of the cornea. This paper is intended unravel the health benefits of shedding tears.

**Keywords:** Tear film; Lacrimal gland; Secretory cells; Health enhancement; Neurotransmitters

### Introduction

Tears are a liquid produced by the body to lubricate the eyes. The word lacrimation from Latin Lacrima (meaning tears) also spelled lacrimation may also be used in a medical or literary sense to refer to crying. The process of yawning may also result in lacrimation though most people associate tears with crying, the production of tears is essential for the proper functioning of the eyes. In mammals including humans, tears are normally produced in the "tear film" coating the eye. The eye is covered by a thin fluid film called the tear film. The primary function of tears is to protect the cornea and conjunctiva, which together compose the ocular surface. Tears are secreted in response to environmental stresses and protect the ocular surface by providing nourishment to the avascular cornea. The lacrimal gland is a tubuloacinar exocrine gland that functions to synthesize, store, and secrete proteins and electrolytes into the tear film, leading to the formation of the aqueous layer of tears. If this secretion is altered in either amount or composition, a spectrum of diseases called dry eye syndromes results. In these types of diseases, symptoms of itchiness, irritation, and feelings of dryness occur. In severe dry eye, vision-threatening conditions such as corneal ulcers and loss of corneal transparency can occur. Dry eye syndromes are a leading cause of ocular difficulties, with more than 3.2 million women in the United States alone diagnosed with these disorders. The lacrimal gland is composed of three main cell types: the acinar cells which are the main secretory cells; the ductal epithelial cells which line the ducts and modify the fluid by secreting water and electrolytes; and myoepithelial cells, which surround the acini with long processes. The lacrimal gland is highly innervated with parasympathetic and sympathetic nerves, and the neurotransmitters released from these nerves are potent stimuli of protein secretion. Cholinergic agonists released from parasympathetic nerves bind to  $M_3$  muscarinic receptors. These receptors are G-protein-

coupled receptors coupled to phospholipaseC- $\beta$  (PLC $\beta$ ). Activated PLC hydrolyzes phosphatidylinositol 4, 5-bisphosphate to generate inositol 1,4,5-trisphosphate (1, 4, 5-IP $_3$ ) and diacylglycerol (DAG). 1,4,5-IP $_3$  causes the release of Ca $^{2+}$  from intracellular Ca $^{2+}$  [Ca $^{2+}$ ] $_i$  stores that can stimulate secretion, either on its own or through enzymes such as Ca $^{2+}$ /calmodulin-dependent protein kinases and protein kinase C (PKC). DAG is necessary for the activation of PKC.  $\alpha_1$ -adrenergic agonists released from sympathetic nerves also stimulate lacrimal gland protein secretion, though the signaling pathway is not as well characterized as the pathway used by cholinergic agonists. The specific types of  $\alpha_1$ -adrenergic receptors present in the lacrimal gland are unknown, as is the primary G protein activated used by these agonists. The phospholipase activated by the G-proteins is also unknown. It is known that  $\alpha_1$ -adrenergic agonists cause release of [Ca $^{2+}$ ], though to a much lesser extent than cholinergic agonists.  $\alpha_1$ -adrenergic agonists are also known to activate PKC. The tear film that covers the anterior surface of the mammalian eye has varieties of constituents that are essential for the maintenance of the avascular transparent corneal epithelium. The tear film is a complex mixture of secreted fluid, proteins, ions, glycoproteins and lipids that lubricates and protects the ocular surface. Stability and functionality of the tear film plays an important role in ocular surface diseases. The tear film keeps the

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cornea wet, thus allowing gas exchange between the air and epithelium. It cleans debris from the transparent surface, providing a clear optical path to the retina. It protects the ocular surface from invasion by bacteria and viruses. It serves as a barrier to the outside environment. The tear film also provides essential metabolites such as retinol which serves to preserve the transparent nature of the epithelium. The tear film is responsible for the clearance of DNA from both human and microbial sources after degradation by endonuclease (lipocalin) [1].

Structure of the tear film

The tear film is structurally complex with three distinct layers: which are outermost layer: a surface of lipid/superficial lipid layer (0.1-0.2 μm thick). It contains oils and provides a coating to the inner layers which contain water. This layer slows down evaporation of the inner layer; next to this is the middle aqueous layer (7-8 μm thick). It contains water, proteins and other substances. Since the outer layer of the eyes does not contain any blood cell this layer keeps the eye moist. Lastly, the inner mucus layer is present whose thickness is about 30 μm thick. This layer contains mucin, and covers the cornea. The various functions as well as the secretors of the three distinct layers of the tear film are summarized in table 1 shows the source of tear film [2].

The nature of tear fluid

The fluid secreted by the lachrymal glands is a complex solution of ions and proteins produced by two resident secretory cell populations: the plasma cells of the immune system and the acinar and duct cells of the secretory epithelium of the gland. The plasma cells are found in the interstitial spaces of the gland and migrate into it from lymphoid organs such as the gut-associated lymphoid tissue (GALT). These plasma cells secrete immunoglobulin A (IgA) which is important in protecting the ocular surface from infection. The acinar cells of the

secretory epithelium have three main functions: to synthesize and secrete a number of tear-specific proteins, to secrete water, and to transport the IgA secreted by the plasma cells from the interstitial compartment into the lumen of the gland. The lacrimal gland-specific proteins found at highest concentrations in the tears are lactoferrin, tear-specific pre-albumin (TSP or lipocalin), and lysozyme [3]. Other proteins occurring at lower concentrations are amylase, peroxidase, plasminogen activator, prolactin, epidermal growth factor (EGF), transforming growth factor-β (TGF-β), endothelin-1, and retinol. Lactoferrin, TSP, peroxidase, and lysozyme as well as IgA function to protect the cornea from viral and bacterial infections. This is important because the cornea is a wet, warm surface and thus is an ideal pathway for pathogens to invade the body and to affect the cornea. Retinol, which is derived from vitamin A, is necessary for the health of the cornea. The growth factors, TGF, EGF, and endothelin-1, are thought to be involved in the wound healing process in response to corneal abrasion or ulceration. The osmolarity of the lacrimal fluid is about 300 mosM and contains Na<sup>+</sup> (128.7 mM), K<sup>+</sup> (17 mM), Cl<sup>-</sup> (141.3 mM), and bicarbonate (HCO<sub>3</sub><sup>-</sup>; 12.4 mM) [4]. This fluid has about the same osmolarity as plasma but has lower Na<sup>+</sup> (140 mM plasma) and higher K<sup>+</sup> (4 mM plasma) and much higher Cl<sup>-</sup> (100 mM plasma). The higher K<sup>+</sup> and Cl<sup>-</sup> are a reflection of the way in which water is moved across the epithelium and into the gland lumen [5].

The Roles of Tears Production as Health Enhancers

The importance of tears can best be recognized by seeing what happens when someone does not have them. One of the most obvious functions of tears is to lubricate your eyeball and eyelid, but they also prevent dehydration of your various mucous membranes—and anyone with the ‘dry eye’ problem knows how painful this can be. A severe lack of this lubrication produces a condition requiring medication

Name	Container(s)	Secretors	Functions
1.Lipid layer	Oils	meibomian glands (or tarsal glands)	Coats the aqueous layer; provides a hydrophobic barrier that evaporates and prevents tears spilling onto the cheek. These glands are found among the tarsal plates. Thus, the tear fluid deposits between the eye proper and oil barriers of the lids.
2.Aqueous layer	Water and other substances such as proteins (e.g. tear lipocalin, lactoferrin, lysozyme and lacritin)	Lacrimal gland	Promotes spreading of the tear film; promotes the control of infectious agents; promotes osmotic regulation
3.Mucous layer	Mucin	Conjunctival goblet cells	coats the cornea; provides a hydrophilic layer; allows for even distribution of the tear film; covers the cornea

Table 1: The Three Distinct Layers of the Tear Film.

Category	Description
Basal tears	In healthy mammalian eyes, the cornea is continually kept wet and nourished by basal tears. They lubricate the eye, and help to keep it clear of dust. Tear fluid contains water, mucin, lipids, lysozyme, lactoferrin, lipocalin, lacritin, immunoglobulins, glucose, urea, sodium, and potassium. Some of the substances in lacrimal fluid (such as lysozyme) fight against bacterial infection as a part of the immune system. Lysozyme does this by dissolving a layer in the outer coating, called peptidoglycan, of certain bacteria. It is a typical body fluid with a salt content similar to blood plasma. Usually, in a 24-hour period, 0.75 to 1.1 grams (0.03-0.04 ounce avoirdupois) of tears are secreted; this rate slows with age.
Reflex tears	The second type of tears results from irritation of the eye by foreign particles, or from the presence of irritant substances such as onion vapors, tear gas or pepper spray in the eye's environment, including the cornea, conjunctiva, or nasal mucosa. It can also occur with bright light and hot or peppery stimuli to the tongue and mouth. It is also linked with vomiting, coughing or yawning. These <i>reflex tears</i> attempt to wash out irritants that may have come into contact with the eye.
Crying or weeping (psychic tears)	It increases lacrimation due to strong emotional stress, suffering, mourning, or physical pain. This practice is not restricted to negative emotions; many people cry when extremely happy. In humans, <i>emotional tears</i> can be accompanied by reddening of the face and sobbing—cough-like, convulsive breathing, sometimes involving spasms of the whole upper body. Tears brought about by emotions have a different chemical makeup than those for lubrication; emotional tears contain more of the protein-based hormones prolactin, adrenocorticotrophic hormone, and leucine enkephalin (a natural painkiller) than basal or reflex tears. The limbic system is involved in production of basic emotional drives, such as anger, fear, etc. The limbic system, specifically the hypothalamus, also has a degree of control over the autonomic system. The parasympathetic branch of the autonomic system controls the lacrimal glands via the neurotransmitter acetylcholine through both the nicotinic and muscarinic receptors. When these receptors are activated, the lacrimal gland is stimulated to produce tears.

Table 2: Category, Description and the Composition of Different Types of Tears.

or therapy to save the victim's eyesight [6] A thin layer of oil on the exposed eye reduces evaporation of tears, keeping eye tissue moist and soft tears form a complex tri-layered (or tri-phased) film consisting of an inner mucin dominated layer, an aqueous layer, and outer lipid (oil) layer [7,8]. The total thickness varies from the top to the bottom of the cornea, from before and after blinking, and the output of the tear glands. The thickness is estimated to be an average of 3 mm [9-11]. The secretions in each layer are tightly regulated [7,12,13]. The thin layer (usually around 0.2 mm thick) of oil on top of the aqueous layer reduces tear evaporation, keeping eye tissue moist and soft [14]. Much of the lipid part of the layer is near the eye surface and is produced in the tarsal gland (often called the Meibomian glands) located in both the upper and lower eyelids, and some by the glands of Zeis and Moll [15]. Even with normal tear production, lack of this lipid oil would soon result in dry and painful eyes due to evaporation. A severe lack of tear lubrication produces a condition called the *keratoconjunctivitis sicca* requiring medication and sometimes surgery (tarsoraphy) to save the victim's eyesight. The innermost tear layer (about 0.5 mm) contains primarily mucins, which are sticky carbohydrates that allow tears to adhere to the eye surface and produce a thin, even coat [16]. The mucin also serves as a wetting agent by coating and wetting the microvilli of the corneal epithelium. Mucin is secreted by a specialized cell type called conjunctival goblet cells [17]. Tears called 'basal' or 'continuous' tears normally flow constantly in both humans and animals, and routinely drain into the *lacrimal punctua* located at the nasal aspect of the upper and lower lid margins at the nasal border of the eye. A tear flow is visible on the cheeks when the tear production is *greater* than the drainage system can handle, and the overflow runs down the cheek (a condition called epiphora). Tears constantly bathe each cornea, not only preventing the eyes from drying out, but also helping to wash out foreign bodies (like dust) that are an omnipresent part of air [18]. Extra tears called 'reflex' or 'irritant' tears are commonly elicited by mechanical irritation of the eye, infections, or even illness. The lacrimal glands automatically provide the proper level of tears for lubrication and protection when needed. The system works so well that [19] concluded reflex or irritation weeping appears to be designed as an emergency protection mechanism. Onions trigger tears because the chemical they release turns into sulphuric acid on contact with the eye surface-a chemical that could damage the eye if it was not for the tear reflex that renders the sulphuric acid largely harmless.

### The antibacterial function of tears

Another important function of tears is that they bathe the eyes in a very effective antibacterial and antiviral agent, an enzyme called lysozyme. Lysozyme, from the word lysos (to split) and enzyme (because it is an enzyme that chemically splits certain chemical compounds), is a major source of the tear anti-germ ability. Amazingly, lysozyme inactivates 90-95% of all bacteria in a mere 5 to 10 minutes [20]. Without it, severe eye infections would be common. Tears also contain immunoglobulin A and  $\beta$ -lysin (a bactericidal protein) to defend against bacteria [17]. As noted, "the importance of tears can best be recognized by seeing what happens when someone does not have them" [19]. As people get aged, the tear film often becomes thinner and can interfere with tear effectiveness. Victims of *Sjogren's syndrome* lack sufficient tears because of poorly functioning lacrimal glands, or the gland becomes non-functional as a result of an autoimmune disorder, a condition called dry-eye syndrome. The inability to secrete enough tears produces eye-burning sensations and redness. Light itself becomes very bothersome, and the eyes constantly itch and have a gritty feeling. One sufferer described the condition as similar to having sand in the eyes. In time, if severe, it can cause blindness [21-25]. Ulcers eventually develop

on the cornea, and loss of its transparency often occurs as well. The ideal solution is to treat the cause of the lack of tears, but use of artificial tears such as methyl cellulose eye drops--can help patients cope with the problem. Another partial solution is to wear aviator's goggles to keep out irritants and to help the eyes retain as much moisture as possible. In extreme cases punctal occlusions (surgery to block tear drainage) or tarsorraphy (other surgery) is required.

### Emotional tears: unique to humans

One of the most amazing discoveries is that tear production actually may be a way of helping a person to deal with emotional problems. This finding lends some basis to the expression, 'crying it out will help you feel better'. Emotional tears are a response unique to humans, because only humans can weep. All animals that have eyes and live in the atmosphere produce tears to lubricate their eyes, but no creatures except humans possess the marvelous system that causes crying. Interestingly, crocodiles secrete tears while eating their prey for reasons that are yet unknown. Scientific studies have found that many people feel better both physically and physiologically after crying; conversely, suppressing tears usually causes people to feel worse [26,27]. Persons who suffer from diseases that prevent them from crying tears-such as the rare, inherited disease called *familial dysautonomia*-tend to deal with stressful events very poorly. This finding also highlights one of the differences between humans and animals. A study at the St. Paul Ramsey Medical Center in Minnesota compared tears caused by simple irritants to those brought on by emotion. Volunteers were caused to cry, first from watching sad movies, and then from freshly cut onions. The researchers found that the tears caused by the movie (called emotional tears) contained far more toxic biological byproducts than irritant tears. Frey et al. [27] found that *stress-induced* tears remove many kinds of toxic substances from the body, and thus concluded that weeping is an excretory process that removes such substances that can build up during times of emotional stress. The simple act of crying also reduces the body's manganese level (a mineral that affects mood and is found in up to 30 times greater concentration in tears than in blood serum). The researchers also found that emotional tears contain a 24% higher protein concentration than tears caused by eye irritants. Frey and his coworkers concluded that chemicals built up by the body during stress were removed by tears, thereby actually *lowering stress*. These chemical include endorphin called *lucine-enkephalin*, which helps to control pain, and *prolactin*, a hormone that regulates mammalian milk production. One of the more important compounds removed by tears is *adrenocorticotrophic hormone* (ACTH), which is one of the best-known indicators of stress. Research indicates that suppressing tears increases stress levels and can contribute to those diseases that are aggravated by stress, such as high blood pressure, heart problems, and peptic ulcers. Although the exact role of these chemicals in lowering stress is not fully clear, clearly, a good cry can be a healthy response to stress. Emotional and irritation tears are stimulated by different sympathetic and parasympathetic nerves. The fifth cranial nerve, for example, is involved in reflex tears. A topical anesthetic applied to the surface of the eye can inhibit both reflex and irritant tears (the type triggered due to an eye irritant), but not emotional tears. Emotional tears evidently are initiated in the limbic system of the brain, that part which is responsible for emotions-both sad and happy or painful and pleasant.

### Tears as part of human communication

Tears are also an extremely effective method of communication, and usually can illicit sympathy, far much faster than any other means.



Montagu [20] concluded that weeping contributes not only to the individual's health, but also to the group's sense of community; 'it tends to deepen involvement in the welfare of others. Tears effectively convey that one is sincere, and anxious to deal with a problem. Although it is often assumed that boys are less likely to weep (and thus to keep their emotions within themselves) because of social condition. research found that adult women have serum prolactin levels almost sixty percent above the average male [27]. This difference may help to explain why women as a whole cry more frequently [27] found out that women cry four times more often. Before puberty, the serum prolactin levels are the same in both sexes, and studies have found that the crying level of boys and girls is much more similar before puberty [28].

## Components of Tear

Water, mineral salts, proteins, antibodies & lysozyme (bactericidal enzyme) are the major components of the human tears. The principal electrolytes are  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$  with lower levels of  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  as secreted, tears are isotonic with serum although the proportions of ions are somewhat different, especially  $\text{K}^+$  [27]. Many of the small molecules, such as glucose, lactate, urea, etc., also occur in serum but at different levels. More proteins can be detected [29], (some estimates suggest as many as 80–100) but only four are present in large amounts, secreted from the lacrimal gland, its ductal epithelium and associated plasma cells (lysozyme, lactoferrin, lipocalin, and sIgA); lipids come both from the Meibomian glands and lipocalin-associated, apparently delivered with the protein from the lacrimal gland; various mucins of both secreted and epithelial types have been reported. Another interesting discovery about the content of tears was made by [27], a biochemist at the St. Paul-Ramsey Medical Center in Minnesota. He and his team analyzed two types of tears: the emotional ones (crying when emotionally upset and stressed) and the ones arising from irritants (such as crying from onions). They found that emotional tears contained more of the protein-based hormones, prolactin, adrenocorticotrophic hormone, and leucine enkephalin (natural painkiller), all of which are produced by our body when under stress. It seems as if the body is getting rid of these chemicals through tears. The levels of the major tear proteins are known to decline with age [30–32], at the same time the volume of tears also tends to decline, while levels of added serum proteins (albumin, caeruloplasmin, etc.) remain constant or increase. Ageing therefore parallels to some extent the onset of a fairly mild dry eye, even if it cannot adequately be diagnosed as such. During sleep, secretion of all major proteins and water is inhibited, but sIgA release continues, producing a highly concentrated solution in the limited fluid. Polymorphonuclear leucocytes migrate into the conjunctiva sac, bringing a variety of destructive and remodelling enzymes and effectively creating a subclinical inflammatory state [33]. Lipid is delivered from the meibomian glands to the lid margin and spreads on the tear film in a thin layer whose thickness can be assessed by its interference colors. This is thought to promote tear film stability by reducing evaporation from the open eye. Increase in thickness of the layer by more forcible blinking has been shown to promote stability [34].

## Mucins

Mucins are a class of very large glycoproteins containing more than 50% carbohydrate, consisting of a linear polypeptide core with regions containing many serine and threonine residues as attachment points for oligosaccharide side chains. Other regions of the polypeptide are unglycosylated but contain cysteine residues capable of forming linkages to other molecules to build up polymeric molecules and gels.

The principal gel-forming conjunctival mucin (MUC5AC) is produced by goblet cells, but the products of other mucin genes are also found, including MUC1 and MUC4 bound to the free surface of corneal epithelium and identified as the glycocalyx [29]. At one time, it was believed that mucin (largely MUC5AC) dissolved in the tears was responsible for both the surface tension and viscosity, on the grounds that similar behaviour was seen in mucin solutions of about 5 mg/ml. Recent reports show that the mucin levels in normal tears are both much lower than this and very variable, mostly in the range 0–100 mg/ml, [35] so it now seems unlikely that mucin is a major contributor to these physical properties, although some mucin/protein or mucin/lipid interactions cannot as yet be ruled out. There is comparatively little information on changes in levels of these components in any eye diseases except for various forms of dry eye.

## Biochemical Mechanisms of Tear Secretion

The secretors of tears (tear film) are the Meibomian gland, lacrimal gland, and conjunctival goblet cells. The most studied of these sources of the tear film are the lacrimal glands, which are the largest of these organs in mammals and are easily accessible. In rodents, it has been shown that the extraorbital lacrimal gland is found under the skin on the lateral side of the face near the ear. In the rabbit, the gland is located within the orbit but is relatively easy to remove and is larger in size. Most physiological studies have used glands from the mouse, rat, or rabbit to examine the control and mechanisms of secretion by this epithelium. This work is important in part because dysfunction of the lacrimal gland can lead to dry eye, which is a painful and potentially blinding condition. The lacrimal gland epithelium also is an elegant secretory tissue of multiple functions with complex control systems that can serve as a model for other secretory epithelia [36]. The lacrimal gland plays a major role in the secretion and production of tear fluid components essential for eye maintenance and function. Absent or inadequate tear fluid secretion by lacrimal acinar cells can be the consequence of cell stress, infection, or cell death [37]. Damage or dysfunction of the lacrimal gland's capacity for secretion can eventually create disturbances of the ocular surfaces including immunological syndromes including dry eye disease, secondary infections, or Sjögren's syndrome [38,39]. In view of that, it is important to understand the secretion process and the key elements required in the lacrimal gland. The lacrimal gland is a polarized secretory tissue that secretes proteins, water, and electrolytes. This secretion is necessary to maintain the health of the ocular surface and is regulated by parasympathetic and sympathetic nerves [40]. Binding of the parasympathetic neurotransmitter vasoactive intestinal peptide (VIP) to its receptor induces activation of a cAMP-dependent pathway [41] through the  $G_{\alpha s}$  (G protein subunit), which in turn stimulates adenylyl cyclase (AC) [42]. Activating AC with VIP or the AC activator forskolin or preventing cAMP breakdown by inhibiting the cyclic nucleotide phosphodiesterase (PDE) causes an increase in intracellular cAMP levels, which in turn activates PKA [43,44] that activation of PKA mediates VIP-induced protein secretion was demonstrated using two PKA inhibitors, protein kinase inhibitor (PKI) and H-89. VIP also increases the intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) in the lacrimal gland [37]. Another parasympathetic neurotransmitter, acetylcholine, activates M3 muscarinic receptors located on the basolateral membranes of acinar cells [45,46]. The activated M3 receptor then interacts with a  $G_q/11\alpha$  G protein subtype to activate phospholipase C- $\beta$  (PLC- $\beta$ ) to break down phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol 1,4,5-trisphosphate (1,4,5-IP3) and diacylglycerol (DAG) [47]. The 1,4,5-IP3 binds to IP3 receptors located on intracellular  $\text{Ca}^{2+}$  stores in the endoplasmic reticulum and increases the  $[\text{Ca}^{2+}]_i$ . DAG



activates a family of enzymes known as protein kinase C (PKC). The lacrimal gland contains PKC- $\alpha$ , - $\delta$ , - $\epsilon$ , and - $\gamma$  and protein kinase D (PKD). Both increasing the  $[Ca^{2+}]_i$  and activation of PKC- $\alpha$ , - $\delta$ , and - $\epsilon$  and - $\gamma$  play integral roles in protein secretion stimulated by cholinergic agonists. Activation of M3 receptors also stimulates the non-receptor tyrosine kinases Pyk2 and Src that subsequently stimulate the Ras/Raf, mitogen activated protein kinase kinase (MEK), and p44/p42 MAPK pathway. Interestingly, it has been shown that activation of the p44/p42 MAPK pathway attenuates cholinergic agonist-stimulated protein secretion in the lacrimal gland [48]. Norepinephrine, a sympathetic neurotransmitter, activates both  $\alpha$ - and  $\beta$ -adrenergic receptors. Activation of adrenergic receptors by isoproterenol stimulates the cAMP signaling pathway to induce protein secretion [49]. Activation of  $\alpha$ 1D-adrenergic receptors in the lacrimal gland by phenylephrine stimulates production of nitric oxide (NO). NO activates guanylate cyclase to increase guanosine 3',5'-cyclic monophosphate (cGMP) to induce secretion [50].  $\alpha$ 1D-Adrenergic receptors also activate the p44/p42 MAPK pathway by increasing epidermal growth factor (EGF) ectodomain shedding to transactivate the EGF receptor (EGFR) to stimulate p44/p42 MAPK activity. Similarly to its effect on cholinergic agonist-induced secretion, activation of MAPK inhibits protein secretion stimulated by  $\alpha$ 1D-adrenergic agonists [51,52].

### The roles of the lacrimal gland acinar cells in the secretion of tears

The lacrimal gland is a polarized secretory tissue that secretes proteins, water, and electrolytes which are the components that make up the lacrimal fluid (tears). This secretion is necessary to maintain the health of the ocular surface and is regulated by the parasympathetic and the sympathetic nerves that innervate the lacrimal gland. These nerves come from the autonomic ganglia. Studies on the lacrimal gland elucidated that it secretes protein, transports immunoglobulin A (IgA) and secretion of water.

### Protein Secretion

A number of proteins are synthesized and secreted by the lacrimal gland acinar cells. The secretion of these proteins is regulated by parasympathetic and sympathetic nerves (neurons). These neurons (nerves), that is, parasympathetic and sympathetic nerves produce parasympathetic neurotransmitters and sympathetic neurotransmitters respectively. These neurotransmitters stimulate secretion of protein synthesis in the lacrimal gland acinar cells. The acinar cells, therefore, have receptors for these neurotransmitters {(acetylcholine), (muscarinic M3), VIP (types I and II), and norepinephrine ( $\alpha_1$  and  $\beta$ )} coupled to G-proteins that in turn regulates the activity of several second messenger systems [46].

### Secretion of Water

One of the major secretory "products" of the lacrimal gland is water. This water is moved from the interstitial spaces of the gland into the lumen of the gland where it is mixed with the other secretory products. This water movement is accomplished by osmosis, which depends on the movement of particles (ions) from the acinar cells into the lumen. Therefore, most studies have examined the process of water movement indirectly by characterizing the membrane channels through which ions move in and out of the acinar cells. Similar to the salivary gland, the lacrimal gland has a distinction between the acinar cells that produce the bulk of the fluid and protein and the duct cells that modify the ionic composition of the fluid by retaining  $Na^+$ . However, most physiological studies are not able to differentiate between these two cell

types and most consider that these mechanisms take place in all the cells. The acinar cell surface membrane is differentiated into basolateral and apical domain, which are separated by the junctional complex. The apical domain is thought to contain water channels (aquaporin 5), which facilitate the movement of water across the epithelium. In addition,  $Cl^-$  and  $K^+$  channels are present to allow the movement of solute across the epithelium. The basolateral membranes contain large numbers of  $Na^+$  pumps, the  $Na^+-K^+$  ATPase, which actively move  $K^+$  into the cell and  $Na^+$  out of the cell, maintaining the usual gradients that are seen in all cells. It is this gradient (more  $Na^+$  outside and  $K^+$  inside) that provides the motive force for the movement of ions and water across the epithelium. The basolateral membrane contains aquaporin4 (AQP4) which transport water from the interstitial space into the epithelial cells, from where it is been transported into the lumen through APQ5 by the motive force created of  $Na^+$  pump [36].

### Diseases and Disorders of Tears

Dry eye is a disorder of the tear film due to tear deficiency or excessive tear evaporation which causes damage to the interpalpebral ocular surface (i.e. exposed eye surface) and is associated with symptoms of ocular discomfort. This definition of dry eyes was adopted by the National Eye Institute [53]. The eye becomes dry either because there is not enough tears being produced or because there is abnormally high rate of evaporation of tears. In the light of new knowledge about the roles of tear hyper osmolality and ocular surface inflammation in dry eye and the effects of dry eye on visual function, [54,55] adopted a revised definition of dry eyes. According to report of the International Dry Eye Workshop [54] definition, dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tears film instability with potential damage to the ocular surface. It is accompanied by increased osmolality of the tear film and inflammation of the ocular surface. Dry eye is recognized as a disturbance of the Lacrimal Functional Unit (LFU), an integrated system comprising the lacrimal glands, ocular surface (cornea, conjunctiva and Meibomian glands) and lids, and the sensory and motor nerves that connect them. According to the report of the International Dry Eye Workshop [54], prevalence of dry eye ranges from 5%-30% in people aged 50 years and older. Prevalence of moderate- to-severe dry eye lies closer to the lower estimate of the range, whereas prevalence of mild dry eyes lies closer to the higher estimate of the range. It is estimated that about 3.2 million women and 1.7 million men, for a total of 4.9 million Americans 50 years and older have dry eye. Tens of millions more have mild dry eyes that may be notable only when some adverse contributing factor is present, such as low humidity or contact lens wear.

### Classification of Dry Eye Disease

Although the 1995 NEI/Industry Dry Eye Workshop classification has served as a useful and durable scheme for over a decade, it does not reflect newer knowledge on pathophysiological mechanisms, effects on vision, and the clinical value of an assessment of disease severity. To address this, DEWS based the revised classification scheme on the updated Triple Classification published in 2005 and the report of the Delphi Panel published in 2006. A three-part classification system was developed. The first part is "etiopathogenic" and illustrates the multiple causes of dry eye. The second is "mechanistic" and shows how each cause of dry eye may act through a common pathway, and that any form of dry eye can interact with and exacerbate other forms of dry eye as part of a vicious circle. The third is a scheme based on the "severity

of dry eye disease”, which is expected to provide a rational basis for therapy.

## Major Etiological Causes of Dry Eye Disease; Etiopathogenic Classification of Dry Eye Disease

According to National Eye Institute [54] and Report of the International Dry Eye Workshop [54] workshop report, term “dry eye” is synonymous with the term “keratoconjunctivitis sicca” (KCS), a condition often influenced by environmental factors. The term ‘environment’ is used broadly to include physiological conditions particularly to an individual (the *milieu interieur*), as well as the external conditions that they encounter (the *milieu exterieur*). The recommended scheme retains the two major classes of dry eye used in the 1995 NEI/Industry Dry Eye [53,54] adopted a similar workshop classification-aqueous tear-deficient dry eye (ADDE) and evaporative dry eye (EDE). ADDE, as its name implies, is primarily due to a failure of lacrimal tear secretion, although a failure of water secretion by the conjunctiva can also be a contributing cause. ADDE has two major subclasses, Sjögren Syndrome Dry Eye (SSDE) and non-SSDE. SSDE is an exocrinopathy in which the lacrimal and salivary glands, as well as other organs, are targeted by an autoimmune disease. Primary Sjögren Syndrome consists of this systemic autoimmune disease in the absence of another discrete autoimmune disease. Secondary Sjögren Syndrome consists of primary Sjögren Syndrome features together with an overt autoimmune connective disease, most commonly rheumatoid arthritis. Non-SSDE is a form of ADDE due to lacrimal dysfunction, where systemic autoimmune features of SSDE have been excluded. It most commonly presents as age-related dry eye (ARDE), a form that is caused by lacrimal deficiency and to which the term KCS has sometimes been applied in the past. Non-SSDE may also result from obstruction of the lacrimal glands due to cicatrizing conjunctivitis, reflex hyposecretion due to sensory or motor block, and the use of systemic drugs including antihistamines, beta-blockers, antispasmodics and diuretics.

## The Causative Mechanisms of Dry Eye

In general terms, dry eye is caused by a disturbance of the lacrimal function unit (LFU), an integrated system comprising the lacrimal glands, ocular surface and lids, and the sensory and motor nerves that connect them. This functional unit controls the major components of the tear film in a regulated fashion and responds to environmental, endocrinological and cortical influences. Its overall function is to preserve the integrity of the tear film, the transparency of the cornea, and the quality of the image projected onto the retina. While disease or damage to any component of the LFU can result in dry eye, the core mechanisms of dry eye are driven by tear hyperosmolarity and tear film instability. In this section the report shows how the several etiopathogenic subclasses of dry eye activate these core mechanisms, and that disease initiated in one major subgroup may coexist with or even lead to events that cause dry eye by another major mechanism. Based upon a scheme proposed by Christophe Baudoin, M.D. and reformatted by Anthony Bron, FRCP, this depiction of core mechanisms operative in dry eye disease facilitates understanding the complexity of the disease. Tear hyperosmolarity is regarded as a central mechanism causing ocular surface inflammation, damage, and symptoms, as well as the initiation of compensatory events in dry eye [55]. Tear hyperosmolarity arises as a result of water evaporation from the exposed ocular surface, in situations of a low aqueous tear flow and/or as a result of excessive evaporation. Hyperosmolarity causes damage to the surface epithelium by activating a cascade of inflammatory events and the release of inflammatory mediators into the tears. Epithelial

damage involves cell death by apoptosis, a loss of goblet cells, and a reduction in mucus secretion, and leads to tear film instability. This instability exacerbates ocular surface hyperosmolarity, thereby creating a vicious circle. Tear film instability can also be initiated without the prior occurrence of tear hyperosmolarity by several etiologies, including xerophthalmia, ocular allergy, topical preservative use and contact lens wear. The epithelial injury caused by dry eye stimulates corneal nerve endings, leading to symptoms of discomfort, increased blinking and, potentially, compensatory reflex lacrimal tear secretion. Alteration of normal tear and ocular surface mucins by elevated tear osmolarity contributes to symptoms by increasing frictional resistance between the lids and the globe [55].

In the initial stages of dry eye, it is considered that ocular irritation results in reflex stimulation of the lacrimal gland. However, with time, inflammation accompanying chronic secretory dysfunction and a decrease in corneal sensation eventually compromises the reflex response and results in even greater tear film instability. Regardless of which individual risk factor or group of factors initiates the disease process, the final common expression involves tear hyperosmolarity and tears instability leading to ocular surface damage. Since both aqueous tear deficiency and increased evaporative tear loss occur in most cases of dry eye disease and are linked by common pathogenetic mechanisms, expert clinicians are increasingly basing treatment decisions on an assessment of severity rather than discrete deficiencies [55]. The group believed that a classification of disease based on severity would be of considerable value in clinical practice, particularly in terms of guiding therapeutic decisions. This third component of our classification system was based on the severity grading scheme included in the Delphi Panel report. By establishing these definitions and classification of dry eye disease, we believe clinicians will be better able to determine the level of DED, as well as the best treatment course for their patients.

## Recommendation

There have been some attempts to treat dry eye with the omega-6 essential fatty acid gamma linolenic acid (GLA) found in black currant seed oil, evening primrose oil and borage oil. There are two published studies that concluded GLA was not effective in treating dry eye. Moreover, there are risks in long-term GLA and omega-6 supplementation related to the accumulation of arachidonic acid (inflammation, thrombosis and immunosuppression).

Long-chain and short-chain omega-3s each help dry eye. But as it has been shown, the long-chain omega-3s from fish oil and the short-chain omega-3s from flaxseed oil in Thera Tears Nutrition work together synergistically to work more effectively, consistently and across a broader range of patients than either oil alone. Together they decrease inflammation, and augment both the oil and water layers of the tear film, while providing the health benefits of a balanced spectrum of omega-3s. Additional studies are now underway with this flaxseed-fish oil-vitamin E blended to fully evaluate the magnitude of its efficacy in treating these dry-eye and meibomitis patients.

It is however suggested that individuals should eat a lot of green leafy vegetables, avoid sugar and/or artificial sweeteners, avoid coffee and smoking, avoid hydrogenated and trans fatty acids containing foods (margarine, most chips, fried foods) as these fats interfere with the proper metabolism of essential fatty acids and are indirect cause of dry eye syndrome. Also, the general populace should couple their meals with (flaxseed oil and fish oil) containing essential fatty acids, g-linolenic acid,  $\alpha$ -linolenic acid as they decrease inflammation,

apoptosis and stimulates tear secretion by producing PGE1(anti-inflammatory) which acts on E-prostanoid receptors EP2 and EP4 to activate adenylate cyclase, increasing cAMP and stimulating aqueous tear secretion. In addition, 8-10 glasses of water should be taken per 24 hours, diet should be supplemented with good multivitamins, the use of equipment known as humidifier at home and at workplace should always be put on to keep the surrounding air from drying out. Also, regular blinking of the eyes at computer usage enhances the release of oils from the meibomian gland into the tear film. Therapeutic drugs such as restasis (cyclosporine agents), tetracycline (doxycycline & minocycline), azasite (azithromycin) and oral omega-3 fatty acids are encouraged by patients suffering from severe dry-eye because these therapies are anti-inflammatory and they prevent MGD.

## Conclusion

Adequate tears secretion is an indispensable tool needed for the maintenance of a healthy ocular surface due to the vital functions of tears and that any alteration in the tear volume secretion could result in various ocular eye diseases. Adequate tear secretion should be enhanced by obviating societal and age-dependent influence especially the male folks, which could in one way or the other suppress the emotional tear secretion via the sympathetic nerves. Medications such as  $\beta$ -adrenergic blockers, anti-histamines, anti-cholinergic, sedatives, anti-depressants and analgesics paracetamol known to inhibit tear production should be avoided as much as possible while drugs (restasis), food nutrients and supplements to enhance tear secretion and suppress inflammation of dry eye syndrome should be produced for adequate human consumption. Remarkably, crying it out would go a long way in maintaining a healthy ocular surface.

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