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REVIEW

Tear Proteins in Health, Disease, and Contact Lens Wear

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Abstract—Although well known as manifestations of sorrow, emotions, frustration, and blackmail, tears have a more prosaic and important function as a lubricant and as a blood substitute for the cornea. Tears transport oxygen and carbon dioxide and play a central role in the cellular economy of the ocular surface and conjunctiva. In addition to proteins, tears contain lipids and glycoproteins, which increase the wetting effect of the aqueous component and delay evaporation. The total protein concentration of tears is about 10% of that of the plasma. About 80 proteins and polypeptide components have been detected by electrophoresis. Among 30 proteins identified in tears, about 50% are enzymes. Some of the tear enzymes are secreted by the lacrimal glands; others are produced by or released from epithelial cells of the cornea and the conjunctiva. Finally, a few enzymes originate from plasma and appear in tears only in cases with increased permeability of the conjunctival vessels. The aim of this review is to provide clinical and biochemical information about tear enzymes both for ophthalmologists and for biochemists interested in clinical and experimental tear enzymology.

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The lacrimal system protects the eye by a flushing and cleaning action resulting from increased secretion of tears. The opaque elements of the eye are supplied with nutrients via a vascular system. The transparent components (cornea, the anterior chamber aqueous, the lens, and the vitreous) are supplied by an aqueous fluid. The aqueous fluid supplies the deep layers of the eye, but the superficial layers are dependent the on tear fluid. Tears play some very important functions as a lubricant and as a blood substitute for the cornea. Their biochemistry is similar to the biochemistry of blood serum in many aspects. Tears are made of three important layers, the largest proportion of them being an aqueous layer that contains proteins, lipids, and glycoproteins. The lipid layer of the tears increases the wetting effect of the aqueous component and delays evaporation. The biochemistry of the tear film could, therefore, be very important for its function.

Abbreviations: CK) creatine kinase; HSV) herpes simplex virus; LDH) lactate dehydrogenase; MDH) malate dehydrogenase; PA) plasminogen activator; PAI) plasminogen activator inhibitor; PK) pyruvate kinase.

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TEAR FLOW

"Tears, idle tears, I know not what they mean." When Tennyson wrote this line he was referring to the fluid that flows from the eye and the nose when one weeps for sorrow, pain, anger, joy, frustration, blackmail, or sympathy. These are "psychogenic tears", which are only one of four categories of tears shed by man. Tears seem to be unique to man; in spite of the term: "crocodile tears" there is no evidence that any other animal weeps in response to emotional stress. The tears that flow from the eye when its surface is excessively exposed to light, cold, wind, foreign bodies, or irritating gasses and liquids are called "reflex tears". Reflex tears occur in man, domestic animals, and perhaps all terrestrial and amphibian vertebrates with the probable exception of snakes. Every animal that has a backbone and spends all or part of its life out of water sheds a third type of tears; "continuous tears". A fourth type of tearing is induced when such substance as war gases are administrated not to the eye but elsewhere in the system, ultimately reaching the tear glands by the way of the blood stream. These "secretagogues" are distinct from the irritant substance contained in many tear gases (and perhaps in onions) that induce reflex tearing.

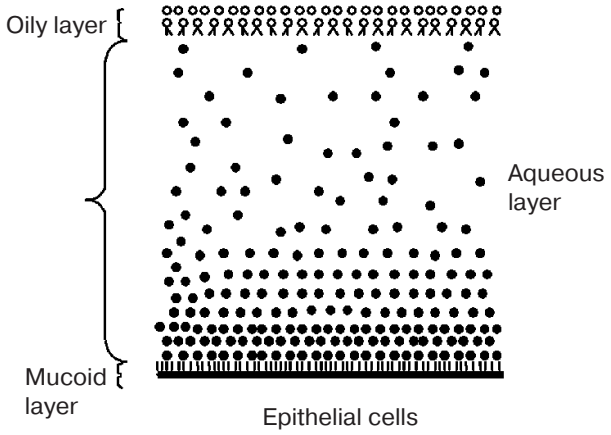
Although well known as manifestations of sorrow, emotions, frustration, and blackmail, tears have a more prosaic and important function as a lubricant and as a blood substitute for the cornea. They are not just bitter salt water, as the poets would have us believe. They contain in addition proteins, lipids, and glycoproteins, which increase the wetting effect of the aqueous component and delay evaporation. The mechanical action of tears in protecting the external eye has been recognized. Tears coat and lubricate the ocular surface, maintain a distortion-free optical surface, and remove foreign and cellular bodies and detritus from the external surface of the eye. Tears transport oxygen and carbon dioxide and play a central role in the cellular economy of the ocular surface and conjunctiva.

Tears are produced by the lacrimal and accessory lacrimal glands, entering the eye via the superior cul-de-sac. A portion of the tear fluid flows over the eye and around the cul-de-sac during the blinking action of the lids. Any remaining tear fluid drains away via small holes in the eyelids called puncta and along minute canals to collect in a tear sac. Normally tear fluid flow is about 1 μl/min, with about 7 μl of the tear fluid in the eye at one time [1]. The cul-de-sac can hold about 24 μl maximum with excess tearing. The turnover period for the tears is about 5 to 6 min.

PRECORNEAL TEAR FILM

The precorneal film is probably the most regularly arranged fluid to be found within the body. The thickness of the film has been variously estimated as 6 μm [2], 10 μm [3], and just below 10 μm [4]. Even though the film is very thin, it is composed of three distinct layers. It attains its thickness from the moment after blinking, and slight thinning of the film as a result of the evaporation or mixing of eye drops may be observed [5]. The division of the aqueous layer into two separate layers, however, is less accepted, and the mucoid layer is in fact a part of the aqueous layer.

The superficial lipid layer is principally derived from the tarsal (meibomian) glands. The aqueous phase is wholly derived from the main and accessory lacrimal glands, while the mucus layer is derived from the conjunctival goblet cells. The function of the lipid layer is the reduction of evaporation from the aqueous phase [6]. It is, however, suggested that the non-polar nature of the surface layer is an important factor in preventing surface contamination of the film with highly polar skin lipids. The aqueous phase of the tears contains a wide variety of organic and inorganic substances. In addition to the principal inorganic ions, as many as 60 proteins, mostly enzymes, are present in the tears, together with a variety of biopolymers, glycoproteins, glucose, and urea. The figure shows the three layers of tear film and the relative thickness of each layer.



The three-layer structure of human tear film showing its relative thickness

TEAR VOLUME

The tear volume has been estimated to be 7 ± 0.2 μl [7], with a production rate of 1.2 μl/min. Tear production rates reduce with age, declining from an average of 2 μl/min in fifteen-year-olds to less than 1 μl/min at sixty-five years of age [8]. The tear film is not evenly distributed over the ocular surfaces, but forms a distinct meniscus at the lid margins; the marginal tear strips, or lakes. The volume of tears in this area is approximately 3 μl, while the volume covering the cornea is about 1 μl and a further 3 to 4 μl are distributed in an even manner over the conjunctiva [7].

Although it is possible to ascribe differing volumes to the tear film located in differing parts of the anterior surface of the eye, the tears are not static [9]. The act of blinking has a substantial effect upon the film and the marginal strips. As the upper lid moves downwards, the superficial lipid layer is compressed. As it thickens, it begins to exhibit interference colors. When the eye opens, at first the lipids spread in the form of a monolayer against the upper eyelid. In this spreading process, the limiting factor is the motion of the eyelid. The spreading of the excess lipid follows, and in about 1 sec the duplex (multimolecular) lipid layer is formed. The spreading lipid drags some aqueous tears with it, thereby thickening the tear film. The magnitude of this effect is controlled by the size and the shape of the tear meniscus; a local thinning adjacent to the meniscus takes place, which effectively prevents further fluid flow from the meniscus to the tear film.

TEAR FILM pH AND BUFFERING CAPACITY

Due to the active transport of Na^+ and Cl^- in the cornea, the maintenance of corneal transparency depends on the pH [10]. Knowing the pH value in the

three-layered pre-corneal fluid is the key to understanding corneal physiology [11]. Using semi-micro- and micro-glass pH electrodes, Fischer [12] reported the mean pH value of pre-corneal lacrimal fluid of the human eye to be 7.6 ± 0.4. The pH value shifts toward the alkaline range the longer the lids remain open. The alkalization is a consequence of the equilibration of the bicarbonate in the lacrimal film with the CO_2 in the surrounding air.

Although the complete arrays of the buffering capacity mechanisms that act in the tears have not been determined, the bicarbonate system seems to be very important. It has been shown that the more shift of tear pH toward alkaline (upward) the weaker the buffering capacity of the tears [13].

BIOCHEMISTRY OF THE TEAR FILM

Tear film possess a complicated chemical structure, which contains many proteins, lipids, and a number of inorganic substances.

Lipid Phase

The composition of human tear lipids was probably first described in 1897 as cholesterol, fatty acids, and fat [14]. However, the large size and number of meibomian glands suggest that the eye has a considerable requirement for fatty materials. Lipids are required to prevent wetting of the skin of the lids adjacent to the eye and to contain the tears. Some lipids are spread over the tear film surface, forming the outermost layer of the film and reducing evaporation. This spreading action may be aided by the particular nature of the fatty acids found in lipids, which form an unusual group of high molecular weight compounds. It has been suggested that considerable variations in lipid composition exist between different individuals [15]. However, there are considerable technical difficulties in the analysis of the very small samples that can be obtained from subjects and the close similarities between post-mortem samples of human and bovine fluid suggest that the requirement for lipid on the eyelids of humans and animals is similar. Table 1 shows the lipid composition for bovine and human meibomian samples [16]. It has been shown that the delivery of meibomian oil depends highly on the temperature of the eyelid, and in the case of higher temperature the rate of delivery is higher [17].

Aqueous Phase

The aqueous phase of tears forms the major component of the film, comprising about 98% of its total thickness. It is a complex dilute solution of both inorganic electrolytes and low and high molecular weight organic substances.

Electrolytes. The main cation found in the aqueous phase of the tear film is sodium, and its concentration is similar to that found in serum. Potassium is another principal cation found in tears, but its concentration is about 3-6 times higher than its concentration in serum. Calcium and magnesium cations are found in small quantities in tears. The two principal anions in tears are chloride and bicarbonate ions, and their concentrations are very similar to those found in serum (Table 2) [18].

Organic substances. *Glucose.* Glucose is present in tears only in very low concentrations. Raised glucose lev-

Table 1. Comparative lipid composition for bovine and human meibomian samples (% by weight)

Compound	Bovine	Man
Cholesterol esters	31.7	29.5
Weak acid esters	31.2	35.0
Material in the diester region	11.4	8.4
Triacylglycerol	1.6	4.0
Material in the post-triacylglycerol region	2.8	3.2
Free cholesterol	3.0	1.8
Free fatty acids	5.1	2.1
Polar lipids	13.3	16.0

Table 2. Comparison between average concentrations (mM) of human tear and serum electrolytes

Ion	Tears	Serum
Na^+	80-170	140
K^+	6-42	4.5
Ca^{2+}	0.3-2.0	2.5
Mg^{2+}	0.3-1.1	0.9
Cl^-	106-135	100
HCO_3^-	26	30

els may occur with diabetes, but these values are attributable to the raised tissue fluid levels rather than raised tear levels.

Amino acids. The free amino acids present in tears have not been fully identified, but may be present at concentrations that are three to four times the serum levels.

Urea. The concentration of urea in tears is similar to that of plasma (20-40%). This suggests an unrestricted passage of urea across the blood/tear barrier of the lacrimal gland.

Tear proteins and enzymes. The aqueous phase of tears contains a remarkably complex mixture of both locally produced and serum derived proteins. Using the cross immunoelectrophoresis technique, Gachon et al. [19] have identified at least 60 protein components, some of which are immunologically indistinguishable from serum homologs, while others are clearly distinguishable and of specific tear origin. Table 3 shows the average concentration of total and some of the most studied tear proteins. A more detailed list of identified tear proteins is presented in Table 4. Although a complex mixture of proteins has been identified in tears, the most important tear proteins are lysozyme, lactoferrin, albumin, tear specific pre-albumin, and globulins [20].

Table 4. Some of the most important proteins identified in human tears [20]

1. Principal tear proteins	
Secretory IgA	
Lysozyme	
Lactoferrin	
Albumin	
Tear specific pre-albumin	
2. Other proteins	
IgG	β-Lysin (?)
IgM	Transferrin
IgD	Antichymotrypsin
IgE	Antitrypsin
Complement component C_3	Prostaglandins
Complement component C_4	Zinc αII glycoprotein
Histamine	Ceruloplasmin (?)
3. Enzymes from the lacrimal gland (in addition to lysozyme)	
α-Galactosidase	Glutamate pyruvate
β-Hexosaminidase	transaminase
β-Glucuronidase	Glucose-6-phosphate
Acid phosphatase	dehydrogenase
Alkaline phosphatase	Sorbitol dehydrogenase
Amylase	Glutamate dehydrogenase
Hexokinase	Glutamate oxaloacetate transaminase
4. Corneal and conjunctival enzymes	
Lactate dehydrogenase	
Malate dehydrogenase	
Pyruvate kinase	
Isocitrate dehydrogenase	
Aldolase	

Mucous Phase

The major source of mucus is the conjunctival goblet cells. The essential function of mucoid layer over both corneal and conjunctival surfaces is to render this hydrophobic surface hydrophilic. In addition, mucus plays a role in removing lipid and debris from the surface of the anterior eye [21]. The effect of the surface bound mucous is to increase the surface tension to a level at which surface wetting will occur (about $42 \cdot 10^{-5}$ N/cm).

Table 3. Average concentrations (mg/ml) of some important tear proteins

Protein component	Average concentration
Total protein	7.51
Lysozyme	2.36
Albumin	1.30
Tear specific pre-albumin	1.23
Lactoferrin	1.84
IgA	0.30
IgG	0.126
IgM	0.00086
IgE	0.0001

Mucus is made up of high molecular weight glycoproteins. Each polypeptide chain has, at about every tenth residue, a carbohydrate chain, and each chain is about ten saccharide units long. The principal mucin complex GP1 ($M_r > 2 \cdot 10^6$) and its subunit GP3M ($M_r \sim 2 \cdot 10^5$) have been detected in an un-reduced saline extract of mucus. Reduction of disulfide bonds in the extract gave rise to GP3 ($M_r > 1.3 \cdot 10^6$), but a considerable variation between people has been observed.

The ocular mucus is composed of mucin-type glycoproteins, many of the tear components found in the aqueous phase being also found in mucus. The presence of some components such as IgA, lysozyme, and lactoferrin in the mucin layer can provide bacteriostatic properties of the mucin.

TEAR ENZYMES

The enzymes make up the largest and most highly specialized class of proteins. Specific cells in each tissue contain enzymes appropriate to its function, and they are largely determined by the nature of chemical reactions occurring there [22]. They can, therefore, be used as tissue markers. Certain enzymes such as aspartate aminotransferase (also called glutamate-oxaloacetate transaminase) and lactate dehydrogenase (LDH) are present though in different amounts in various tissues—heart, liver, skeletal muscle, corneal and conjunctival epithelium, lacrimal gland, etc. In contrast, alanine aminotransferase (also called glutamate-pyruvate transaminase) is a good liver marker, as is creatine kinase (CK) for heart and skeletal muscles. This lack of absolute specificity can be compensated by the study of isoenzymes such as lactate dehydrogenase LDH-1 and creatine kinase-MB (CK-MB) (specific for myocardium), LDH-5 and CK-MM (specific for skeletal muscle), or the use of a combination of several enzyme assays providing an enzymatic profile.

Human tear possesses a potent defense system that limits the replication and even survival of bacteria. Tears have long been recognized to contain nonspecific immune mechanisms active against bacteria, including complement, lysozyme, defensins, and lactoferrin [23-29].

Tear Enzyme Classification

Tear enzymes and the enzymatic systems present in tears are generally classified into four main groups including lysozyme, collagenases, plasminogen activator–plasmin system, and metabolic enzymes.

Tear lysozyme. Lysozyme (EC 3.2.1.17) is the most important antibacterial tear enzyme that is also widely found in other animal and human tissues, body fluids, secretions, and exudates. Lysozyme hydrolyzes β-1,4-glycosidic bond in the polysaccharide cell wall structure of a variety of microorganisms [30-32]. Lysozyme was discovered in 1922 by the English bacteriologist Alexander Fleming [33], who demonstrated the presence of an antibacterial substance in human nasal secretion. He also found that human tears are rich in lysozyme and used tears as a source of lysozyme for further experiments. Lysozyme was first isolated from egg white [34] and its molecular weight [35], amino acid sequence, and tertiary structure [36-38] were identified. The isoelectric point of the enzyme is pH 10.5-11.0 [33] with the optimum pH at 9.2 [39]. Human and plant lysozyme have also been isolated, and they have shown some differences from egg white lysozyme [40, 41].

The level of lysozyme in tears was determined by Fleming in 1922 [33]. Using a dilution technique, he showed that the level of lysozyme in tears was up to thousand fold greater than in serum [42, 43]. The specific activity and ultraviolet spectrum of human tear lysozyme is different from egg lysozyme [44]. However, some similarities in structure and immunological characteristics have been detected between tear lysozyme and the lysozyme found in human serum and other secretions [45]. Investigations on the relationship between serum and tear lysozyme activities have revealed that the lacrimal gland not only collects from the blood, concentrates and excretes lysozyme into tears but also a *de novo* synthesis of lysozyme takes place in the gland followed by active secretion [46].

Tear lysozyme levels can been determined by various methods that are generally classified into three main categories as indicated in Table 5. It is emphasized that standardization of the determinations based on the measurement of enzyme activity is difficult due to the large differences in enzymatic action under different conditions and the presence of promoting and inhibiting factors in tears [47]. On the other hand, the immunological methods are highly influenced by the relative concentration of antigens and antibodies, the precipitation conditions, and the specificity of the antisera used in the reaction. The determination of enzyme concentration is accomplished either by the elution of the dye from the stained protein or by densitometric evaluation of stained gels under well defined conditions [48]. A quantitative method for the determination of tear lysozyme was developed in 1968 [49]. The method has been modified for determination of the enzyme in tear samples collected by different methods [50], and for quantitative determination of the lysozyme deposited into or leached out of spoiled contact lenses into storage solutions [51-57].

The effect of flow rate, sampling, and changes in vascular permeability on the tear levels of lysozyme and other tear proteins has been investigated [47]. It was shown that lysozyme concentrations and total tear proteins did not change within flow rates of 10 and

Table 5. Methods used for assessment of lysozyme in human tears

I. Methods based on the measurement of enzyme activity	Lysozyme titer determination, viscosity, agar diffusion test, lysoplate method, spectrophotometric and turbidimetric methods
II. Methods based on the determination of the enzyme as a protein	Radial immunodiffusion, electro immunodiffusion, radioimmunoassay, laser-nephelometry, immunoturbidimetry
III. Methods based on protein–dye interactions	Dye elusion test, electrophoretic determination followed by densitometry

50 μl/min. However, at very low flow rates (<10 μl/min) the level of total protein was significantly higher due to the effect of proteins deriving from the conjunctiva and the cornea.

Corneal collagens and tear collagenases. Collagen is a ubiquitous structural protein and occurs in varying amount in almost all tissues and organs of the human body. Collagen is able to form insoluble fibers responsible for the multiple structural role of the protein. Collagen molecules are originally synthesized in the form of procollagen. This precursor protein consists of three polypeptide chains coiled around each other in a right handed triple helical formation in the central core [58]. There are substantial non-helical extensions at the N- and C-terminals of the molecule with molecular weights of 15,000-20,000 and 33,000-35,000, respectively, the molecular weight of the whole chain being 145,000-180,000. When collagen is synthesized, both the amino and carboxy terminal regions of the newly synthesized collagen have extensions, which are removed enzymatically in the extracellular compartment before fibril assembly occurs. The procollagen amino terminal (NH) and carboxy terminal (COOH) prevent intracellular fibril formation and help regulate the process in the extracellular space following excretion.

At least six different types of collagen exist, differing in the type, ratio, and primary structure of their constituent α-chains. The composition and distribution in different organs and tissues of various types of collagen are summarized in Table 6. There is a certain though not very strict correlation between the composition of the collagen and the function of the connective tissue that contains it.

Table 6. Composition and tissue distribution of various types of collagen

Type	Composition	Distribution
I	$[\alpha\text{-}1(I)]_2$, α-2(I), or $[\alpha\text{-}1(I)]_3$	skin, tendon, bone, cornea, dentin, fascia
II	$[\alpha\text{-}1(II)]_3$	cartilage, vitreous body, intervertebral disk
III	$[\alpha\text{-}1(III)]_3$	blood vessels, uterus, fetal skin, reticular fibers
IV	$[\alpha\text{-}1(IV)]_2$, α-2(IV), $[\alpha\text{-}1(IV)]_3$	basement membranes, lens capsule, kidney glomeruli
V	$[\alpha\text{-}1(VI)]_2$, α-2(V)	placenta, skin, muscles, exoskeleton of fibroblasts
VI	short triple helix and globular domains	aorta, placenta, uterus, liver, kidney, skeletal muscles, cornea

Specific collagenases are enzymes capable of cleaving across the native collagen triple helix under physiological conditions. Mammalian and other vertebrate collagenases specifically cleave the α-1 chain of type I collagen at the Gly772–Ile773 peptide bond. Highly purified mammalian collagenases are unable to attack non-collagen proteins such as casein, bovine serum albumin, transferrin, fibrinogen, and hemoglobin. Collagenases isolated from different animals and human tissue sources cleave the native collagen molecule basically in the same way. However, the rate of different collagenases action on various types of collagen substrate is not identical.

Plasminogen activator–plasmin system of tears. Normal tear fluid contains plasminogen activators (PAs) but no active fibrinolytic enzyme (plasmin). However, *in vitro* experiments have revealed that fetal corneal explants released fibrinolytic agents when fibrin clots were present in the culture medium [59]. Investigations on tear components have concluded that normal tears contain only a small amount of PA and a large amount of inactive proactivator, which can be transformed by streptokinase to active PA [60]. PA activity has been studied during epithelial wound healing in vitamin A-deficient rat corneas [61]. The results of this study and many similar investigations have shown that both excessive and inadequate levels of PA activity may result in impaired epithelial wound healing. Elevated levels of PA in tears of patients with corneal and conjunctival inflammations have been demonstrated. Accumulating experimental and clinical data indicate that determination of PA, plasmin, plasmin inhibitor, and PA inhibitor has diagnostic value in a number of diseases including different types of corneal ulcers, persistent epithelial defects, recurrent erosions, contact lens associated lesions, herpetic keratitis, bullous keratopathy, keratoconjunctivitis sicca, and chemical burns of the cornea and conjunctiva. Tear levels may reflect the degree of cellular damage, indicate the progress of the healing process, and be used as prognostic indicators [62-64].

Metabolic enzymes in tears. Isozymes of *lactate dehydrogenase* (LDH) exist in different proportions in various tissues (Table 7). Changes in the serum levels of LDH isozymes can give information on the site and degree of cell destruction and on the tissue and organ involved in the pathogenic processes. The first observation on LDH in tears was the detection of very high activities of LDH measured in tear samples collected with Schirmer strips from the conjunctival sac of healthy individuals [65]. It has been demonstrated that unlike human serum, normal human tears contain isozymes LDH-5, LDH-4, and LDH-3 in large quantities [66-68]. These isozymes are built up mainly from M subunits and are called muscle-type isozymes responsible primarily for the anaerobic conversion of pyruvate to lactate. The same LDH isozyme pattern has been observed in the extracts of lacrimal gland and the tear fluid, suggesting that these enzymes are of lacrimal source [69].

Table 7. Percent of total activity of LDH isozymes in various body tissues

Tissue	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
Heart muscle	60	30	5	3	2
Kidney	28	34	21	11	6
Brain	28	32	19	16	5
Liver	0.2	0.8	1	4	94
Skeletal muscle	3	4	8	9	76
Skin	0	0	4	17	79
Lung	10	18	28	23	21
Spleen	5	15	31	31	18

In order to determine the possible origin of tear enzymes, Haeringen [70] measured the activities of LDH, *malate dehydrogenase* (MDH), and *pyruvate kinase* (PK) in successive specimens collected during stimulated lacrimation. The activities ranged between 10 and 25% of the initial values, those measured in the first collected samples. The activities of LDH, MDH, and PK changed parallel to each individual sample, suggesting a similar source of the three enzymes. The activities of amylase did not show a correlation with the enzyme of energy-producing metabolism. *Lysozyme* activities measured in the same tear samples were found to be rather constant and independent from the degree of lacrimation. The enzyme pattern of tear samples depends highly on the method of sampling [50, 71].

Other metabolic enzymes are present in normal tears in lower concentrations. *Peroxidase* in normal tears originates from lacrimal gland, not from conjunctiva. Its activity in normal human tears is 1000 U/liter, while in rats the activity of tear peroxidase is as high as $3.7 \cdot 10^5$ U/liter [72]. It has been found that the biological significance of peroxidase is associated with thiocyanate–hydrogen peroxide antimicrobial system, which is suggested to play a role in the control of the bacterial flora in the oral cavity and in the eye. This shows that the antibacterial effect of this system is independent from lysozyme, lactoferrin, and immunoglobulins [73-77]. Peroxidase production by the lacrimal gland is age related, and the change in peroxidase production can be used as an indicator of the age-related lacrimal gland atrophy. It has been shown that aged tissue upon stimulation with isoproterenol secretes significantly less protein and peroxidase than young tissue. This finding suggests that the decrease in tear secretion and tear proteins in aged lacrimal gland is related to different responses to physiological neurohormonal stimuli [78].

Catalase, the enzyme involved in the enzymatic conversion of hydrogen peroxide, has also been detected in the extracts of corneal epithelium and endothelium but not in the stroma. In corneal epithelium, catalase is associated with a subcellular fraction. The importance of catalase is appreciated when considering the cytotoxic effect of oxygen-derived free radicals and their possible role in tissue damage [79]. The physiologic function of catalase is the protection of tissues from the oxidative effect of hydrogen peroxide by breaking the H_2O_2 molecule into H_2O and O_2.

Proteinases are present in normal tears samples collected from various species. The origin of these enzymes has been suggested to be the lacrimal gland [69] and epithelial cells of cornea and conjunctiva [80, 81]. The activities of *cathepsin B*, *cathepsin C*, *cathepsin D*, *elastase*, and *urokinase* have been determined by spectrophotometric methods in normal human tears. It was shown that the presence and proportions of different proteases in normal human tears differ widely among each individual [82]. It was suggested from this study that various proteases affect the surface activity of aqueous tears by cleaving various tear proteins to produce low molecular weight substances that may act as tear surfactants.

TEAR ENZYMES IN EYE DISORDERS

The activity of various tear enzymes may change in response to different disorders that originate from eye disease, infections, and exposure of the eye to chemicals such as drugs, air pollutions, smoking, and contact lenses [83-85].

Bacterial and Fungal Keratitis

Keratitis is a term used to describe various corneal inflammations, accompanied by loss of luster and transparency and cellular infiltration of the cornea. Since the cornea is normally avascular, inflammatory cells originate from blood vessels of the limbal parts of the conjunctiva

and sclera and, therefore, reach the affected region of the cornea via the tear film. Microbial keratitis is caused by specific microorganisms leading to inflammatory reaction as a direct response to the invading microorganism. Several types of keratitis including superficial or deep, ulcerative or non-ulcerative, and microbial or non-microbial are known depending on morphological and pathological features of the disease. Most superficial keratitis is caused by external agents, viruses, or physical influences such as UV light or disorders of tear film. Deep keratites develop either due to the progression of superficial keratitis involving the stroma and are caused by bacteria or fungi or affect only the stroma and are caused by immunological reactions. Most cases of ulcerative keratitis begin with epithelial defect, which renders the cornea susceptible to infection. Microorganisms are not able to pass the intact epithelium, but readily invade the corneal stroma. Besides corneal injury and the presence of the infective agent, other factors (disturbed or missing defense mechanism) must also be present as not all infected corneal injuries lead to the development of a corneal ulcer. Active secretion of collagenase and plasminogen activators (PAs) has been reported in ulcerating corneas [86].

Corneal Ulceration

Polymorphonuclear leukocyte collagenase, serum albumin, α-1-antitrypsin, and α-2-macroglobulin have been shown to increase in the tears of patients with corneal ulceration [87]. It has been suggested that the measurements of tear fluid concentration of these enzymes in tears can be used as indicator of corneal healing.

Proteolytic activity are normally absent in freshly collected normal tears, while tears collected from patients suffering from various corneal diseases can produce measurable proteolytic activity that is due to the presence of plasmin in the tears of corneal ulcer patients [88]. Plasmin activity has also been demonstrated in the tears of patients suffering from various corneal diseases, of contact lens wearers, of patients with allergic conjunctivitis, and in the tears of normal individuals following the performance of Schirmer's test [89, 90].

The presence of collagenase in corneal ulcers suggests that application of some enzyme inhibitors can help healing the ulcers of cornea. However, clinical trials have not yet proven that collagenase inhibitors are as effective as suggested by results of *in vitro* experiments [91].

Fibronectin and Corneal Ulceration

Fibronectins are complex dimeric proteins composed of two similar but not identical subunits of 25 kD joined near their carboxyl terminal by disulfide bonds [92]. They are found in large amounts on the surface of injured tissues. Fibronectins are normally found in serum and are also present in various normal tissues in association with basement membranes. They form fibrillar arrays on the surface of different types of cells and on the surface of basal membranes; they interconnect cells with each other and with basal membranes [93]. They play an important role in the process of wound healing both in skin and in corneal injuries [94-96]. Extensive studies have shown that a provisional fibronectin–fibrinogen matrix can be detected on the bare surface and under the migrating epithelium of corneal wounds. This matrix gradually disappears as the epithelial defect closes. It is, therefore, suggested that fibronectin and fibrin migrate from the perilimbal conjunctival vessels through the tear film to the wound area [97, 98].

Collagenase and Wound Healing of the Cornea

Collagen constitutes 70% of the dry weight in corneal tissue, about 80-90% of which is type I collagen. This collagen is primarily located in the extracellular matrix of the corneal stroma [99]. Type III collagen is produced in the cornea in association with wound healing [100]. It is found that most of the collagen in the basement membrane of the corneal epithelium is type IV collagen.

The healing process of deep or penetrating corneal wounds consists of four steps, similar to wound healing in general. A fibril plug is formed in the wound and surrounding tissues are infiltrated first by inflammatory cells, then due to the action of chemotactic factors by keratocytes. Collagen is secreted by keratocytes showing signs of fibroblastic conversion. Formation of fibrils from the newly synthesized collagen molecules and appearance of collagen–collagen cross-links is characteristic of remodeling of fibrotic tissue.

Viral Herpetic Keratitis and Tear Enzymes

Herpes simplex virus (HSV) is the most frequent causative agent of human viral infections. Primary infection usually occurs in early childhood and the virus is able to survive in the sensory ganglia. The virus persists in a latent form and is triggered by a variety factors such as fever and stress. Oral (type 1) HSV is the predominant cause of herpetic keratitis, and, in rare cases, the disease is also caused by genital (type 2) HSV. Herpetic keratitis is the major cause of corneal blindness in most societies [101-103]. Patients suffering from primary or secondary systemic immunodeficiencies, leukemia, and AIDS and those receiving systemic immunosuppressive therapy are at high risk of severe systemic and ocular herpetic infections [104].

The activity of some proteinases may be altered in tears of patients with herpetic keratitis. It has been reported that of 25 patients with the disease, 67% showed plasmin activity in the range of 10 μg/ml [105]. It was suggested that the appearance of plasmin in tears of patients may be associated with the production and release of tissue type plasminogen activator (t-PA) from the affected epithelial cells. The presence of plasminogen activator inhibitor (PAI) activity in tears of patients with corneal epithelial diseases including herpetic keratitis has also been reported [106]. It must be emphasized that PAIs are not normal components of the tear fluid but can be detected in tears of patients with different inflammatory and traumatic lesions of the cornea. Based on the experimental data, it can be proposed that a multilevel regulatory system controlling the action of proteinases is present in tears of patients suffering from herpetic keratitis.

It is suggested that the determination of enzymes, activators, and inhibitors in tears may provide useful information concerning the progress of tissue destruction in herpetic keratitis and other viral infections of the cornea. Measurement of enzyme activity in tear samples may also be used in deciding whether inhibitor therapy might be necessary in the case of a patient suffering from one particular and stage of this multifaceted disease [107].

Allergic and Immunological Diseases and Tear Enzymes

Hypersensitivity reactions, which can occur in the eye, are harmful immunological mechanisms and there are four major types (types I-IV) of these reaction. Table 8 shows the types of hypersensitivity reactions occurring in the eye. Histamine is the most studied of the mediators involved in the development of symptoms of ocular allergy. Histamine is present in very low concentrations in normal tears, but elevated levels of histamine concentrations have been observed in allergic conjunctivitis and in vernal conjunctivitis [106]. Tryptase, a neutral endopeptidase, is an enzyme secreted by activated mast cells of allergic patients. Tryptase levels in tears and other body fluids can be used as an indicator of mast cell activation.

Table 8. Types of hypersensitivity reaction and their mediators

Type of reaction	Hypersensitivity reaction	Mediator
I	immediate	IgE
II	cytotoxic	IgG, IgM, and complement
III	immune complex	IgG, IgM, and complement
IV	delayed type hypersensitivity	mononuclear cells

TEAR PROTEINS AND CONTACT LENSES

Contact lenses have been used to correct errors of refraction, as protective devices for the eyes against undesirable fluids, gases or solids; as a mechanical aid in the treatment of several pathological eye conditions; for their cosmetic effect in neurotic conditions associated with eye defects; as a valuable aid to vision where the wearing of spectacles is impossible; as a vocational aid to vision, and as the only refractive device which will give useful vision in certain abnormal conditions.

Biocompatibility of Contact Lenses

In general, the most common problem with any kind of biomaterial is its biocompatibility. If the implanted biomaterial is not biocompatible with the environment, it will be rejected by the biological site. This may be manifested in many ways depending on the environment in which the biomaterial is used. For example, a biomaterial rejected by the blood can cause thrombosis, while a non-biocompatible biomaterial of contact lenses may deposit more tear components leading to sever infection and conjunctivitis.

The widespread use of hydrophilic contact lenses has demonstrated the problem of lens spoilage. Polymer deterioration and protein deposition will change the optical quality and permeability of the lenses and may reduce wearer tolerance [108]. The protein adsorbed is also a primary layer for the subsequent adsorption of other proteins and tear components. Most of the previous studies have concentrated on the proteinaceous deposits on the surface of the lens. The presence of proteins inside the hydrogel matrix, however, has received little attention.

Changes in Tear Proteins Due to Contact Lens Wear

Adsorption of tear protein onto contact lenses has long been known and there is much concern that their adsorption and, in some cases absorption, may seriously alter their function in the tear fluid [51-57]. It has been shown that the biological activity and stability of lysozyme, the most important tear protein, is highly affected due to its adsorption to various kinds of contact lenses [109, 110]. There are many factors influencing absorption of tear proteins into contact lenses including tear biochemistry, eye health conditions, contact lens material, pH, temperature, and the type of disinfection and care solutions used. Hydrogen peroxide is an effective disinfectant that is widely used in most care solutions for

hydrogel contact lenses [111]. It is suggested that residual H_2O_2 dissolved from the contact lens may also have an unfavorable effect on the cornea [112, 113].

Although very important from different aspects of ophthalmology, there are only a few articles concerning tear biochemistry and, especially, various enzymes of the tear fluid. In this review we have attempted to put together the small pieces of information available in biochemical and ophthalmologic literature. The number of identified proteins in human tears is around 30, lysozyme accounts for approximately 30% of the total protein. Tear enzymes are the most active and most frequent of total tear proteins. The change in various tear proteins and enzymes can be used in the diagnostic of many eye diseases as well as other internal or external disorders including genetically determined enzymopathies, harmful effects of smoking and air pollution, diabetes mellitus, and various allergies.

Lysozyme is the most known and well-studied enzyme in general and it is highly studied in tears. Lysozyme accounts for at least 70% of tear proteins absorbed into high water content hydrogel contact lenses. Lysozyme is an impact protein with low molecular weight that can penetrate into the matrix of contact lenses leading to many eye problems associated with the use of soft hydrogel contact lenses. It is a bacteriolytic substance, but it actually acts on a very limited number of bacteria that are mostly apathogenic saprophytes. The antibacterial effect of lysozyme might not be the only reason why the ability to keep such high concentration in tears has not been lost in the process of the evolution of species.

Proteolytic enzymes play a decisive role in pathogenesis of a number of corneal diseases. They are some of the most important tear enzymes, and even minor changes in their biological activity can be used for early diagnosis of a disease in the eye or other body sites.

The LDH activity in human tears is higher than that in the plasma. In tears, unlike in plasma, LDH isozymes built up mainly of M type subunits are predominant, suggesting local production of this enzyme. Various disorders that affect corneal epithelium cause an increase in LDH activity and change LDH isozyme pattern in tears.

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