ENZYMOLOGY OF THE TEARS

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To my father

István Berta, M.D.

who turned my mind onto medical sciences, and who so successfully planted his ambitions in his three children.

AUTHOR

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Dr. Berta graduated summa cum laude and received his M.D. degree in 1979 from the University Medical School of Debrecen, Hungary. He obtained his C.Sc. degree (a Ph.D. equivalent scientific degree of the Hungarian Academy of Sciences) in 1987 based on a thesis entitled, *Diagnostic Tear Protein Determinations*.

As a student he worked as a part time university lecturer, taught general biology for medical students, and did research work in the Department of Biophysics. He was one of the organizers and a leader of the Students' Scientific Society at the University Medical School of Debrecen. Since 1979 he has been working at and has held different positions at the Department of Ophthalmology, University Medical School of Debrecen. After 4 years of residency and a qualifying examination in 1984 in General Ophthalmology he specialized in anterior segment surgery of the eye. Besides his medical practice and teaching activity he has been the head of the General Diagnostic and Research Laboratory since 1984. In 1989 he did a research fellowship in the Wilmer Eye Institute at The Johns Hopkins University, Baltimore, MD (USA). In 1990 he worked for a year as a visiting professor at the Dry Eye Institute in Lubbock, TX (USA).

Dr. Berta is a member of the Hungarian Ophthalmolgical Society, European Society of Lacrimology, International Society for Eye Research, and the European Association for Eye Research. He is a founding member of the International Society of Dakryology. He was the secretary of the Organizing Committee of the "First Meeting of the International Society of Dakryology" held in 1987 in Budapest, Hungary. He is an elected member of the Advisory Board of the International Society of Dakryology. He was a member of the Scientific Committee of the "Sixth International Symposium on the Lacrimal System" held in 1990 in Singapore.

He received the Weszprémi Award and the Pro Universitate Award of the Medical University of Debrecen, Hungary, the Lacrima Award of the Dry Eye Institute, Lubbock, Texas (USA); the Papolczy Award of the Papolczy Founadation, Budapest, Hungary; twice the First Prize of the Hungarian Ophthalmological Society, Budapest, Hungary; and the Chibret Travel Fellowship Award of Chibret International, Zurich, Switzerland.

He presented more than 70 papers at scientific congresses, and is the author of 45 papers published in international journals and of 2 book chapters published in the United States. His major scientific interest, besides tear research and the diagnosis and therapy of dry eyes, includes corneal transplantation, ophthalmic laser therapy, and the radiotherapy of intraocular tumors.

PREFACE

The total protein concentration of tears is about 10% of that of the plasma. Up to 80 polypeptide components have been detected in tears by two-dimensional electrophoresis. The number of identified proteins is above 30 and nearly half of them are enzymes. One of these, lysozyme accounts for approximately 30% of the total protein concentration; the others are present in varying amounts. Not only are the number and the quantity of enzymes in the tear fluid high but they are the most active, therefore, probably functionally the most important tear proteins. Some of the tear enzymes are secreted by the lacrimal glands; others are produced by or released from the epithelial cells of the cornea and the conjunctiva; still others originate from the plasma and appear in tears only in cases with increased permeability of the conjunctival vessels.

Lysozyme is the most studied tear enzyme, and is also the most studied enzyme in general. We know much about lysozyme, including its conformation, the exact mechanism of its enzymatic action, and many other characteristics, but its specific role (or roles) in tears is still poorly understood. 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 cause why the ability to keep such high concentration in tears has not been lost in the process of the evolution of species.

The activity of lactate dehydrogenase (LDH) in the tears is higher than that in the plasma. In tears, unlike in plasma, LDH isoenzymes built up mainly of M (muscle)-type subunits are predominant, suggesting the local production of this enzyme. Different diseases that affect corneal epithelium increase LDH activity and change LDH isoenzyme pattern in tears. The amount or rather the ratio of enzymes involved in the aerobic and anaerobic pathways of energy-producing mechanisms are thought to reflect the general metabolic status of corneal epithelial cells in health, disease, and contact lens wear.

Various tear enzymes are used in the diagnostics of genetically determined enzymopathies. The determination of β-hexosaminidase and α-galactosidase in tears is important in heterozygote screening in Tay-Sachs' and Fabry's diseases. In both diseases heterozygotes have about one half of the normal enzyme activity in tears, whereas it is virtually absent in homozygotes.

Proteolytic enzymes are some of the most important tear enzymes playing a decisive role in the pathogenesis of a number of corneal diseases, including bacterial ulcers, caustic injuries, herpetic keratitis, persistent or recurrent erosions, contact lens-associated lesions of the cornea, etc. The detection of collagenase in the tears of patients with corneal ulcers raised great enthusiasm and hope concerning the use of collagenase inhibitors in the treatment of these devastating diseases. The use of collagenase inhibitor eyedrops on humans provided only limited results, suggesting that other enzymes and enzyme inhibitors may be involved in the degradation and healing of the cornea.

Recently the plasminogen activator-plasmin system of tears has been shown to initiate a proteolytic cascade resulting in the activation of various proteases capable of degrading corneal tissue. A complex mechanism regulating the secretion, activation, and inhibition of different enzymes of this cascade is thought to be responsible for maintaining a homeostasis in the healthy eye. Any change in the balance of inhibitors and activators, some of which are also active enzymes, may result in the formation of tissue defect, the propagation of microorganisms in the otherwise compact corneal tissue, or the defective healing of some of the above-mentioned corneal diseases. The studies of plasminogen activators, plasmin, plasminogen activator inhibitors, and plasmin inhibitors are some of the most promising fields in the diagnosis and therapy of corneal diseases.

A large number of studies concerning various enzymes of the tear fluid have been performed and published; various authors studied tear enzymes from different aspects. I have attempted to review the literature and put together the small pieces of information available in ophthalmological and biochemical literature. While preparing this manuscript it was my intention to cover various fields of clinical and experimental tear research, to evalute different views, and to integrate the results produced by ophthalmologists and biochemists. I also did have the opportunity to summarize the results of our own clinical and experimental studies, express my personal opinions, and make suggestions for the solution of unsolved problems. The aim of this work is to provide clinical and biochemical information about tear enzymes both for ophthalmologists and for research scientists who are interested in the clinical and the experimental aspects of tear enzymology.

András Berta

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Chapter 1

ANATOMY AND PHYSIOLOGY OF THE LACRIMAL SYSTEM

I. INTRODUCTION

The exposed surface of the eyeball is covered by a thin layer of tears. This layer is formed and maintained by the lacrimal apparatus. A continuous tear film provides the cornea with a surface of high optical quality, serves as a lubricant ensuring the smooth movement of the lids during blinking, owing to its antibacterial properties protects the eye from infections, and helps to maintain the well being of the corneal and conjunctival epithelium by keeping the surface wet and providing oxygen and nutrients for the superficial epithelial cells.[1]

The lacrimal system is also capable of protecting the eye by a flushing and cleansing action resulting from an increased secretion of tears. The secretion rate of the lacrimal glands can increase by a hundredfold or more in a very short time due to a reflex started by mechanical or chemical irritation of the eye or the nasal mucosa. A sudden increase in tear secretion is highly effective in removing minor foreign bodies and flushing and diluting contaminants or noxious chemicals from the surface of the eye.[1]

The lacrimal apparatus consists of three parts: the secretory part, the distributory part, and the excretory part.

II. THE SECRETORY SYSTEM

Aqueous tears forming the overwhelming majority of the tear secretion are produced by the main and accessory lacrimal glands. Palpebral glands, producing lipids, and conjunctival goblet cells secreting mucus also contribute to the composition of tears.

The main lacrimal gland is an almond-shaped secretory gland located in the upper outer orbital region above the eyeball. The lacrimal gland consists of two parts: a large orbital or superior portion and a small palpebral or inferior portion, divided by the upper part of the orbital septum. The orbital part is lodged in the fossa glandulae lacrimalis of the frontal bone on the anterior and lateral part of the roof of the orbit. This part of the gland cannot be examined directly. In order to reach this portion during surgery one has to penetrate the skin of the upper lid, the orbicularis muscle, and the orbital septum. The palpebral portion of the lacrimal gland lies anterior to the lateral portion of the upper part of the orbital septum. When the patient's eye turns nasally and down, and the upper lid is everted, this part of the gland can be seen through the upper fornix of the conjunctiva.

Lipids forming a continuous layer on the surface of the aqueous tears are secreted by palpebral glands. The main source of lipid secretion are the

meibomian glands. They are located in the tarsus of the upper and lower lids. Their openings are located along the lid margins just behind the gray line. Their secretions supply the outer portion of the tear film, which prevents rapid tear evaporation and tear overflow, and provide tight eyelid closure. Some lipids are also secreted by the glands of Zeis, located at the palpebral margin of each eyelid, and by the glands of Moll, found at the roots of the eyelashes. Meibomian glands are the most important secretors of the tear lipids. The other palpebral glands help with their secretions, as similar glands elsewhere in the body to prevent the hair (eyelashes) from becoming dry and brittle.

Most mucous material covering the superficial epithelium under the layer of the aqueous tears originates from the conjunctival goblet cells. These are large one-cell mucous glands in the superficial layers of the conjunctiva. Their secretion is distributed over the ocular surface by lid motion. The epithelial cells of the conjunctiva and the cornea are also capable of secreting glycoproteins that can serve as the foundation of the mucous layer covering the epithelial surface. In adddition, small amounts of glycoproteins are secreted by the lacrimal glands, and under pathological conditions plasma glycoproteins passing the blood-tear barrier also contribute to the mucous content of tears.[2,3]

III. THE DISTRIBUTIONAL SYSTEM

The eyelids have many functions such as protecting the eye, regulating the light, and covering the eye during sleep. Its paramount functions are, however, to distribute the lacrimal fluid on the anterior surface of the eye, to regulate the evaporation, to expel superfluous quantities of tears, and to form a stable precorneal tear film. The eyelids are closed by the simultaneous contraction of the upper and lower parts of the orbicularis muscle. There is also a horizontal component of the lid movement in closing, and the lid is stretched as it moves medially when the eye is closed. The upper lid is raised by the levator palpebrae superioris muscle, and the lower lid is retracted by the inferior rectus muscle (capsulopalpebral part). The lid retractors have a reciprocal innervation with the protractors, the upper and lower palpebral part of the orbicularis muscle. When the eye is closed pressure against the globe increases. When the eye is open the pressure decreases.[4]

Every time the lids pass over the exposed surface of the eyeball, the lipid layer is compressed and the tearfilm-air interface is eliminated. The shear forces acting across the thin aqueous tear layer between the moving lid and the eyeball rejuvenate the mucous layer by removing lipid-laden mucus and redistributing mucus freshly expressed from the goblet cells.[1]

The tear volume in the normal open eye is approximately 8 µl. This volume consists of three parts: 1 µl the actual volume of the precorneal tear film, 3 µl in the tear menisci, and 4 µl in the fornices. The tear film is so thin that gravitation has no effect on it. Practically no hydraulic flow occurs in it over the ocular surface. The only flow that takes place is in the upper and lower tear menisci.[5]

IV. THE EXCRETORY SYSTEM

The lacrimal excretory system consists of the upper and lower lacrimal ducts (canaliculi), the tear sac, and the nasolacrimal (or lacrimonasal) duct. The excretory system is the sink of the lacrimal apparatus. Tears enter the drainage system through the lacrimal puncta, which are small round openings of the lacrimal ducts located on slight elevations (lacrimal papillae) of the lid margins near the medial canthus. Both puncta point backward and immerse in the lacrimal lake (lacus lacrimalis), a local thickening of the tear film around the lacrimal caruncle and near the semilunar plica. Normally the puncta are not visible unless the lid is pulled away from the globe or the lid margin is everted. If the puncta is visible without such a maneuver it is usually a sign of the eversion of the lacrimal punctum, or of the malposition of the lid or of the lid margin. Either of these situations prevents tears from entering the drainage system and usually causes epiphora. The puncta lead into the canaliculi. Each canaliculus consists of a vertical and a horizontal part. The vertical portion is about 2 mm, the horizontal about 8 mm long. The upper and lower canaliculi lead either directly or meet in a short common canaliculus into the lacrimal sac. The lacrimal ducts are located in the palpebral parts of the orbicularis muscle. The contraction of this muscle provides a peristaltic action propelling the lacrimal fluid through the excretory passages. A bunch of fibers belonging to the same muscle is attached to the upper part of the lacrimal sac pulling it on each contraction, creating a negative pressure inside the sac. The suction created by this mechanism also contributes to the propagation of tears through the lacrimal ducts into the lacrimal sac. From the sac, the tears drain into the inferior nasal meatus. This latter movement is brought about by the gravitation and may be assisted by increased pressure within the sac.

A. Basal Tear Secretion — Reflex Tear Secretion

According to Jones[6] the tear secretory system consists of basic secretors and reflex secretors. The former are the accessory lacrimal glands of Krause and Wolfring (lacrimal secretors); the goblet cells, and the glands of Manz (mucin secretors); the meibomian glands, the glands of Zeis and Moll (oil secretors); while the latter are the main lacrimal glands (orbital and palpebral portion). He proposed that the two systems function separately and are responsible for tear secretion under basically different conditions. In this concept in sleep, when the eyelids are closed, the basic secretors alone produce tears. During the waking hours reflex secretors secrete various amounts of tears to provide enough hydration.[7]

The idea of basic and reflex tear production was described in one of Jones' early papers.[6] In this paper he refers to the results of another investigator de Roeth (de Rötth), Sr.[8] de Roeth measured tear secretion with the Schirmer test without and with stimulation in dry eye patients[8] and in normal subjects.[9] de Roeth did not provide data directly proving the existence of basic tear flow. No such data can be found in Jones' original paper either, except for a

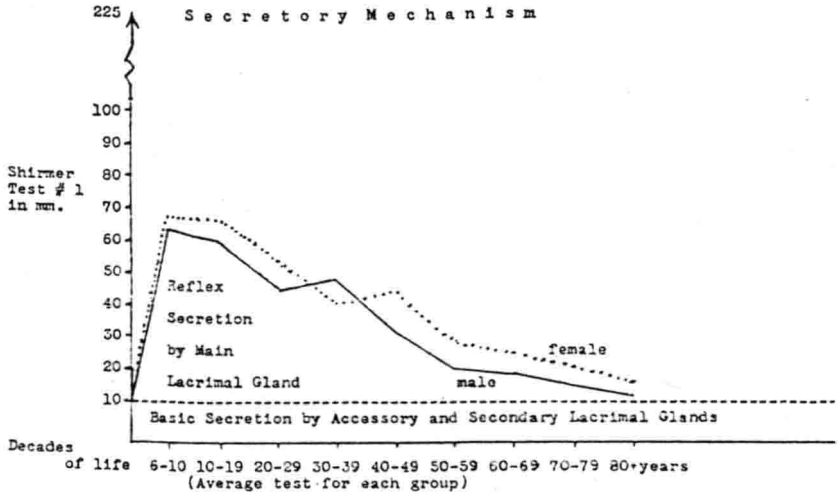

FIGURE 1. Reflex and basic secretion of tears in millimeters pf wetting in Shirmer No. 1 test (based on a study of 827 persons with normal eyes by A. deRoeth, Sr., 1953). (From Jones, L. T., *Arch. Ophthalmol.*, 66, 137, 1961. With permission.)

horizontal dotted line drawn under the curves plotted from the data of de Roeth (Figure 1).

In spite of the dearth of data supporting the idea of basically different basal and reflex tear secretory systems and secretory mechanisms, Jones' concept became widely accepted. Much effort was devoted to study basal tear flow and the composition of basal tears trying to minimize the stimuli caused by the measurements or by tear sample collection. No direct data proving the existence of separate basal and reflex tear secretion have been published until now; it has become clear, however, that the composition and the osmolarity of tears changes with increasing secretion rates.[10-13]

Jones' idea inspired a number of clinical and experimental studies and had a clearly beneficial effect on the development of dakryology. Other opinions and explanations, however, were also published[14-16] arguing that both the main lacrimal glands and the rest of the tear secretory glands (accessory lacrimal glands, meibomian glands, palpebral glands, conjunctival glands) function under the influence of continuously acting stimuli. The effects of the regulating neuroendocrine system, and those of external stimuli, though are different under various circumstances, never can be completely eliminated. Neither of these glands function in a "black box", and it can hardly be accepted that any of these glands are completely turned off when the eye is closed. Various physical and psychic stimuli most likely reach all tear-secreting glands in sleep, too. The proper function of all tear glands seems to be necessary for the maintainance of the three-layered tear film and for the sufficient hydration of the ocular surface both when the eye is closed and when the eye is open.

This latter view may also be supported by the fact that, in diseases and in pathological states where the so-called basic secretors are missing or not functioning, no obvious signs of dryness develop in sleep; on the contrary, all disabling symptoms are present during the day when the "reflex secretors" are functioning. In diseases with decreased lacrimal gland secretion like Sjögren syndrome, on the other hand, the lack of sufficient hydration during the night most likely play a role in the development of epithelial lesions due to the temporary adhesions of the surface of the cornea and the inner surface of the upper lid.[17]

Based on the concept of the two basically different secretory systems and separate secretory mechanisms, Jones[7] also developed a test to measure basic tear secretion. The basic tear secretion test is a modification of the original Schirmer test, and is performed after the topical anesthesia of the conjunctiva and "gentle drying" the lower cul de sac with a cotton applicator. To decide whether such a test is suitable to measure basic tear flow eliminating the effects of external stimuli is beyond the scope of this book. This test certainly gives lower values for tear secretion than the original Shirmer test.

B. Normal Tear Secretion and the Turnover of Tears

Schirmer estimated the normal tear flow to be 0.6 to 0.8 µl/min, by measuring the time required to produce epiphora in patients with obstruction of the lacrimal puncta. He also developed a test, bearing his name, to measure tear secretion by placing a filter paper strip in the eye with a 5-mm long bent portion placed between the globe and the lower eyelid, and evaluating the length of the wetted part of the paper in 5 min.[18]

Kirschner measured the rate of tear secretion by instilling fluorescein into the conjunctival sac, and evaluating the change in the fluorescence of the tear samples collected periodically from the eye. He found values for tear flow close to those reported by Schirmer.[19]

Mishima et al.,[5] using a quantitative fluorophotometer, measured the concentration of fluorescein in and the disappearance of this dye from the lower tear meniscus. The average tear flow with this method was 1.2 µl/min, with a range from 0.5 to 2.2 µl/min. Sorensen[20] measured tear flow rate to be 0.6 µl/min using a technetium 99 tracer and a gamma counter. Both Mishima and Sorenson emphasized that measurements of tear flow should be made without causing reflex lacrimation, trying to exclude one of the important drawbacks of the Schirmer test. Mishima and colleagues have shown, however, that the instillation of 1 µl fluorescein solution also causes an increase in tear secretion, lasting up to 5 min in some eyes.[5]

Lamberts et al.[21] reported the results of a study of normal tear secretion performed with calibrated Schirmer strips. They found a tear flow of 2.51 ± 1.18 µl/min in unanesthetized eyes and 1.52 ± 1.00 µl/min in anesthetized eyes.

The estimated turnover rate of tears determined by Mishima et al.[5] with *in situ* fluorophotometry was 16%/min. Puffer et al.[22] using the same method found a mean value of 15% for the tear elimination coefficient in normal subjects.