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The Ocular Surface in Keratoconjunctivitis Sicca

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Summary
The ocular surface is altered in kerato-conjunctivitis sicca, a disorder of aqueous tear production. Many of the factors leading to these surface changes are now more clearly understood and are discussed in this paper.

The Epithelium of the Normal Ocular Surface

The surface epithelial cells of the cornea and conjunctiva exhibit delicate microvilli. In the conjunctiva they are coated with a glycocalyx which is in continuity with glycoprotein secreted into subsurface vesicles.[1,2] This glycocalyx interacts with tear mucin histochemically stainable at the surface of the eye, whose precise disposition *in vivo* is not yet known.

Goblet cell distribution is not uniform across the globe and tarsus. Cell density is greater infranasally on the globe and is greatest on the caruncle. Cell density peaks shortly after birth and plateaus between the fourth and eighth decade.[3] Goblet cells are absent from the limbus.

Inflammatory cells are present in the normal conjunctival epithelium and substantia propria, with neutrophils and lymphocytes in the epithelium and with neutrophils, lymphocytes, plasma cells and mast cells in the substantia propria.[4] Normal tears collected by micropipettes from the marginal strip are usually free of inflammatory cells; tears sucked from the bulbar surface do contain granulocytes.[5,6,7]

Vitamin A is essential to the maintenance of the normal architecture of the surface epithelia of the eye. As in other tissue it is assumed in the conjunctiva, a vascular tissue, that retinol is transferred from plasma retinol binding protein (pRBP) to a cellular retinol binding protein (cRBP). The corneal epithelium is denied a direct vascular source and it is likely that this is supplied by the tears, which contain less than 0.4–10.6 ng/ml.[8] Recently it has been proposed that tear-specific prealbumin may play the role as a carrier for retinol in the tears, similar to that of RBP in the plasma.[9] Tseng *et al.* in 1985 have suggested on the basis of transdifferentiation experiments that the degree of vascularisation of the ocular surface influences goblet cell density by modulating the delivery of retinol.[10] If rabbit cornea including the limbus, is denuded of epithelium it is resurfaced by conjunctival epithelial cells which at first retain their biochemical and morphological features, including goblet cells. After a matter of weeks however they transdifferentiate and take on the appearance of corneal epithelial cells; the goblet cells are lost.[11] This process of transdifferentiation can be prevented by topical treatment with retinoids[12,13] or if the cornea which is resurfaced is vascularised (and therefore, it is presumed, provided with a source of retinol).

Tseng *et al.* have also suggested that scarring may diminish conjunctival vascularity in various forms of dry eye and contribute to the accompanying squamous metaplasia of the ocular surface.[10] The alternative view has been proposed, that hyperosmolarity (*vide infra*) may be the cause of metaplasia, includ-

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ing goblet cell loss.[14,15] This may be true but it must be noted that in animal models in which the lacrimal excretory duct is occluded, this would let tear retinol as well as lead to hyperosmolarity.

Tear Mucin

The goblet cells are assumed to be the major source of tear mucin (i.e. mucous glycoprotein). There is immunoidentity between at least one mucin fraction of the tears and a component of conjunctival goblet cell secretion.[16] No such immunoidentity was found between tear mucin and lacrimal secretory acini. Huang *et al.* in 1987 have confirmed this in the rabbit using antibody against the core protein of a mucin fraction.[17]

Viscosity of the Tears

Mucus is responsible for the high viscosity of the tears and their non-Newtonian or pseudoplastic behaviour, that is, their viscosity is shear-dependent and they exhibit a fall in viscosity, at high shear rates ('shear thinning').[18] This property is thought to confer the advantage of low viscosity during a blink or saccade and stability of the tear film when the eye is stationary, for example, during fixation.[19,20]

During reflex tearing in normal subjects tear viscosity falls, which supports the view that the lacrimal gland is at least not the major source of tear mucin. The paper by Allen *et al.* (1972) identified the presence of glycoprotein within storage granules of human lacrimal gland. However, although this was taken to be mucus glycoprotein at the time, the histochemical techniques used are not able to distinguish between mucus and other types of glycoprotein.[21]

Wetting of the Ocular Surface

Holly and Lemp in 1971 proposed that the corneal surface was non-wettable (hydrophobic) *in vivo* and that tear mucus served the role of rendering the epithelium hydrophilic.[22] This conclusion was based on studies in which surface mucus was removed by gentle wiping or by chemical treatment. The corneal surface was found in these circumstances to become hydrophobic and the addition of mucus restored the normal hydrophilic properties of the corneal surface by lowering the surface tension. Recent studies by Cope *et al.* (1986) attempting to reproduce the conditions of these experiments, have shown that physical and chemical treatment of the corneal epithelium causes significant surface damage as demonstrated by scanning electron microscopy (SEM).[23] Thus the hydrophobic behaviour of the cornea under these conditions may not reflect the native state of the epithelium in the absence of mucin. Preliminary studies by Tiffany have suggested that the native surface tension of the corneal epithelium, after removal of mucin by mild saline washing is low, that is, it is a wettable, hydrophilic surface.[24]

Holly has also proposed that dry spots would be initiated in dry eye by contamination of the epithelial surface by meibomian lipid. Mucin is envisaged as interacting with such lipid to prevent dry spots forming in this way. On these grounds mucin deficiency would encourage tear film instability.[25]

If tear mucin is not essential to render the corneal or indeed the conjunctival surface wettable, studies which correlate a loss of goblet cells with a reduction in tear stability must be interpreted with caution. If it is naturally wettable, then early tear break up in the presence of reduced goblet cell destiny may still imply the occurrence of a surface change in KCS which renders it hydrophobic; the 'lipid trapping' role for mucin may prove to be more tenable than a primary role in maintaining a hydrophilic epithelium (see below). Another possibility would be that loss of tear mucin reduces stability by lowering tear viscosity, since Benedetto *et al.* in 1975 demonstrated a relationship between viscosity of polymers used as tear substitutes and the thickness of the film formed.[26]

Changes in the Tears and Ocular Surface in Kerato-conjunctivitis Sicca

Infiltration of the lacrimal gland with round cells occurs in KCS and is assoicated with acinar atrophy[27] and reduced secretion of lacrimal fluid[28]. This is the primary event from which most if not all changes at the ocular surface follow.

Many of the features of KCS are quantifiable (Table I), but only a small number of

Table I

A. Aqueous deficiency
- ★ Flow
- ★ Volume
- ★ Osmolarity

B. Mucin deficiency
- ★ Goblet cell loss
- ★ Lowered viscosity
- ★ Increased surface tension
- ★ Altered tear stability
- ★ Altered mucus ferning

C. Lipid deficiency
- ★ Fatty acid changes
- ★ Interference microscopy
- ★ Meibomian morphology

D. Chemical changes
- ★ e.g. Lysozyme, lactoferrin, ceruloplasmin

E. Surface cellular damage
- ★ e.g. Fluorescein, Rose Bengal staining

F. Inflammation
- ★ Polymorphonuclear leucocytes
- ★ Prostaglandins
- ★ Superoxide

assessable features have been assessed as diagnostic tests.

It can be seen from the study of Van Bijsterveld (1969) that the Schirmer test has a high sensitivity (85%) and specificity (83%) using a value of <6 mm wetting as a cut off in the diagnosis of KCS.[29] Farris *et al.* in 1983 not unexpectedly found a much lower sensitivity using a cut off of 3.5 mm wetting or less (Table II).[30]

Decreased flow is associated with decreased tear volume[28,31] and a decreased height of the tear meniscus[32,33] The meniscus height is not correlated with the Schirmer value in normal subjects. Only 7% of the normal subjects show a meniscus height of less than 0.1 mm.[34] Continued evaporation from a preocular tear film of reduced volume leads to hyperconcentration of the tears. A rise in tear osmolarity was anticipated by von Bahr in 1941 and later confirmed by Mastmann *et al.* in 1961 and Mishima *et al.* in 1971.[35,36,28]

Gilbard *et al.,* (1978) using a micropipetting technique and a depression of freezing point method to determine osmolarity, found the osmolarity of normal tears to be 302±6.3 mOsm/L while that in a group of patients with KCS was considerably higher, 343±32.3 mOsm/L.[37] Farris *et al.* in 1983 using a cut off value of >312 mOsm/L have found that the test has a sensitivity of 76% and a specificity of 84%.[30] Further studies by this group have shown hyperosmolarity in the range encountered in KCS to be damaging to rabbit corneal epithelial cells *in vitro* and hyperosmolarity has been suggested as the basis of ocular surface damage in KCS.[38,39] We have confirmed a rise in marginal strip osmolarity in KCS.[5] Damage to the ocular surface is detected clinically by staining with fluorescein, which demonstrates corneal staining most effectively, or by Bengal Rose which demonstrates conjunctival surface damage better than corneal epithelial damage. However, Bengal Rose is poorly tolerated by KCS patients because of its low pH and the inability to wash away the compound with time following instillation. Fluorescein can be used to show conjunctival damage more effectively if the fluorescein is viewed

Table II *Diagnostic tests of dry eye*

Test	*Cut off*	*Sensitivity (per cent)*	*Specificity (per cent)*	
Schirmer[1]	<3mm	10	100	Flow
Schirmer[2]	<6mm	85	83	
Rose Bengal[1]	<4	58	100	Damage
Rose Bengal[2]	<4	95	96	
Basal tear vol.[1]	104	59	77	Volume
Osmolarity[1]	312mOsm/L	76	84	Evaporation
Nibut[3]	<10sec	83	85	Stability
Lysozyme: mg[1]	110	67	67	
Lysozyme: ([2]Dia)	<21mm	99	99	

[1] Farris *et al.* 1983; [2] Bijsterveld 1969; [3] Mengher *et al.* 1986.

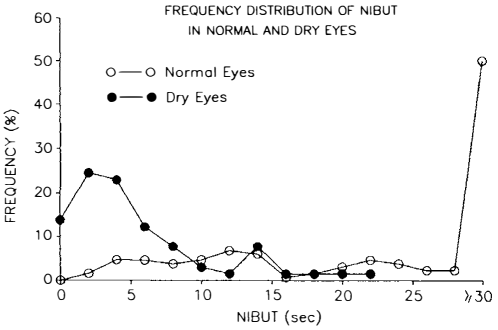

Fig. 1. *Frequency distribution (%) of non-invasive tear film break-up time (sec) in normal and dry eyes. (Mengher 1989)*

through a yellow filter and a reasonable correlation is obtained if conjunctival staining assessed by Bengal Rose is compared with that demonstrated by fluorescein viewed in this way.[40]

Van Bijsterveld in 1969 devised a scoring system for Bengal Rose staining, with a score of 0–3 for each interpalpebral conjunctival zone and for the cornea itself, to give a maximum aggregate of nine.[29] Using a cut off value of >3.5 the test had a sensitivity of 95% and a specificity of 96%. Farris *et al.* in 1983 in a similar study found a sensitivity of 58% and specificity of 100%.[100]

Van Bijsterveld further explored the diagnostic value of measuring tear lysozyme, recognising that lysozyme levels fell early in KCS and Sjögren's syndrome. Using a cut off value of 21.5mm lysis, this test was found to have the greatest ability to discriminate between normals and dry eye patients, with a sensitivity of 99% and a specificity of 99%.[29] The value obtained by Farris *et al.*[30] is shown in Table II. Lactoferrin, another protein of lacrimal gland origin, also shows reduced tear levels in KCS.[41] Mackie and Seal in 1984 have demonstrated the diagnostic value of this measurement as well as that of lysozyme when values are compared with age matched norms.[42]

Assessment of tear film stability by tear film break-up after the instillation of fluorescein, was introduced by Norn[43] and by Lemp *et al.*[44] and has become known by the name of break up time (BUT). Lemp and Hamill in 1973 advocated a cut off value of <10 seconds in the diagnosis of mucus deficiency.[45] Although the BUT test has been criticised on the basis of its variability in normal subjects[46] its repeatability in KCS patients does not appear to have been studied and the test is still held by us to be of value in patients with dry eye.

In 1983, in conjunction with Tonge and Gilbert of Smith and Nephew Pharmaceutical Research we devised a non-invasive tear break up test for clinical use in which break-up of a grid projected on the surface of the pre-ocular tear film is observed by specular reflection.[47] We confirmed that tear stability varies widely in normal subjects but found that variation is greatly reduced in dry eye patients. When a cut off of <10 seconds was used to discriminate normals from those with dry eye, the sensitivity of the test was 82% and the specificity 86%.[48] (Fig. 1).

There are many events which occur at the ocular surface in KCS and which have been quantified, but for which sensitivity and specificity information is lacking.

(1) There is an increase in tear proteins of plasma origin, such as albumin[5] and caeruloplasmin, due to an increase in capillary permeability presumably of conjunctival vessels (Fig. 2) although it is not excluded that permeability of lacrimal gland vessels is affected also. Since conjunctival capillaries are fenestrated and would be expected to leak protein anyway, it must also be considered whether conjunctival epithelial permeability increases also.

(2) There is an increased desquamation of

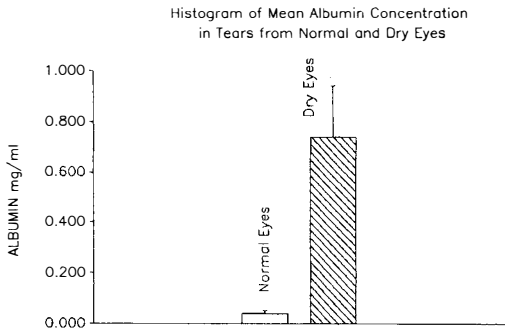

Fig. 2. *Histogram of the concentration of albumin (mean±s.e.m) in tears from normal and dry eyes. (Mengher 1989)*

Table III *White cell invasion of the tear fluid*

KCS	*Eye*	*PMN/μl*	*Epith/μl*	*Grade of bulb conj. injection*	*Remarks*
1	L.E.	12,555	45	+++	Corneal ulcer
2	L.E.	2,385	270	+++	
3	L.E.	600	105	+	Young epith cells
4	R.E.	605	55	+	Young epith cells
5	L.E.	55	770	+	Young epith cells
6	R.E.	55	1,155	+	Young epith cells
7	R.E.	45	195	+	Young epith cells
8	L.E.	15	930	+	Young epith cells

epithelial cells into the conjunctival sac, related to the occurrence of squamous metaplasia and the increased stratification and separation of the epithelial cell layers noted for instance in the conjunctiva.[49,6]

(3) White blood cells, chiefly granulocytes, appear in the tears in KCS, sometimes in large numbers (Table III).[5]

(4) Tear viscosity falls in KCS and the tears appear to become more Newtonian in behaviour, that is their viscosity is less dependent on shear rate (Fig. 3).[5] If this is correct, it would imply a diminished mucus content in the tears. However, because of the extended period in dry eye patients over which tears must be collected in sufficient volume to conduct viscosity measurements, the possibility of reflex dilution does arise. Confirmation must await the development of micromethods of viscosity measurement.

(5) Tear surface tension, measured by two microvolume techniques, either a hanging drop method[5] or a capillary method[50] is found to rise in KCS, again suggesting that tear mucus content is reduced (Fig. 4). This is supported by a negative correlation between tear viscosity and NIBUT (Fig. 5).

Since Norn,[51] in other studies found an increased mucus production in KCS, it must be inferred that mucus, apparently present in such excess, is qualitatively different from that which confers viscous properties on the normal tears.

Surface Morphology

(6) Changes in the corneal surface have recently been detected by specular microscopy. Lemp and Gold in 1985 reported a decrease in corneal epithelial cell diameter and suggested that it might reflect an increased epithelial cell turnover.[52]

(7) In the conjunctiva the changes which occur are embodied in the term squamous metaplasia,[53] with goblet cell loss[54,55,56,57,58] enlargement of non-goblet epithelial cells[56,53] increased cellular stratification,[49] keratinisation[60] and loss of surface microvilli.[49]

(a) Ralph in 1975 found a normal value of 8.84±4.66 goblet cells/mm of conjunctiva, based on biopsies in the inferior nasal fornix.[55] The density of goblet cells was significantly less in KCS (2.11±0.78; $P = 0.001$), Stevens—Johnson syndrome (0.81 ± 0.75), Ocular pemphigoid (0.33 ± 0.37) and in acute alkali burns (0.00).[55]

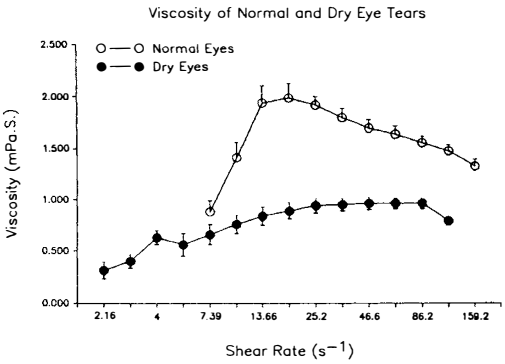

Fig. 3. *Shows the viscosity of normal and dry eye tears over a range of shear rates. Each point represents a mean±s.e.m. (Mengher 1989)*

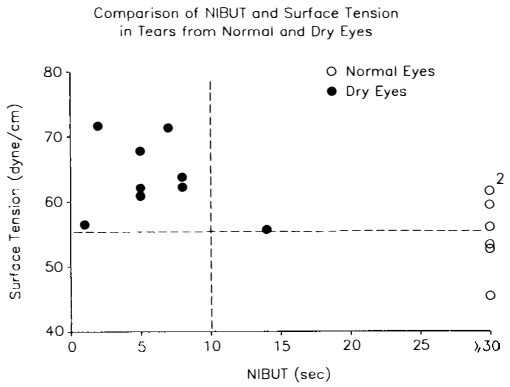

Fig. 4. *Comparison of the non-invasive tear film break-up time and tear surface tension in normal and dry eyes. (Mengher 1989)*

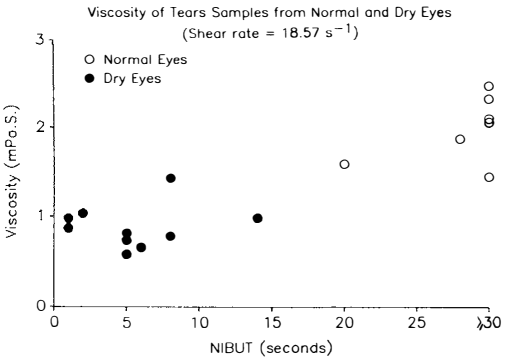

Fig. 5. *Comparison of non-invasive tear film break-up time and tear viscosity in normal and dry eyes at a specified shear rate of 18.57 s^{-1}. (Mengher 1989)*

(b) Egbert *et al.* in 1977 reported their technique of impression cytology for the study of conjunctival epithelial morphology. In this technique, a strip of cellulose acetate paper is pressed against the ocular surface, fixed and stained with such stains as H&E / PAS or PAS/ Papanicolaou to demonstrate goblet cells and epithelial cells.[61]

Nelson,[62] has standardised the technique using an ophthalmodynamometer to control the force of application and has devised a grading system involving the measurement of average epithelial cell area and goblet cell density using a calibrated microscope graticule. In grades 0–1, mean individual cell area is less than 1000 μm^2 and the cells are round or slightly polygonal, with a nucleus to cytoplasm ratio of 1:2 to 1:3. Goblet cell density is greater than 350 cells/$mm.^2$ In grades 2 and 3 there is progressive enlargement of the epithelial cells (mean individual cell area: MICA) to >1000 μm^2 and the cells are polygonal. Goblet cell density falls to <100 cells/$mm.^2$ Grades 2 and 3 are regarded as abnormal and are found increasingly in KCS with the higher grades said to be associated with more severe disease (Table III). The grade does not however, correlate with either Schirmer test or Bengal Rose staining.

Nelson and Wright,[57] confirmed the loss of goblet cells in KCS, Stevens–Johnson syndrome and pemphigoid reported by Ralph in 1975.[55] They emphasise that in KCS uncomplicated by blepharitis or drug toxicity the fall in goblet cell density in the interpalpebral conjunctiva (77%) precedes that in the inferior tarsal conjunctiva (60%) whereas in pemphigoid and Stevens–Johnson syndrome there is major goblet cell loss in both zones.

Tseng *et al.*[10] have postulated that the fall in goblet cell density in KCS may be due to a diminution of conjunctival vascularity due to scarring, and a consequent reduction in the delivery of vitamin A to the conjunctiva. It is difficult to accept this as a mechanism in KCS since

Table IV *Grading impression cytology*

Grade	0	1	2	3
Cell shape	Small, round			Polygonal
NUC/CYT ratio	1 : 2	1 : 3	1 : 4–1 : 5	>1 : 6
Mean indiv. area	<1,000 μm^2			>1,000 μm^2
Goblet cell density/mm^2	>500	>350–500	>100–350	<100

After Nelson and Wright 1984.

clinically visible scarring would be unusual. Impression cytology has been used effectively in the study of xerophthalmia. Using the more extended grading scheme (0–5) devised by Tseng *et al.*[10] Wittpenn *et al.*[63] in a study of children in Madurai, India, found that all children with early xerophthalmia showed complete goblet cell loss and the appearance of enlarged, partially keratinised cells. Biopsies from treated and normal children showed normal goblet and non-goblet epithelial cells. In a subsequent study in Indonesia, taking the lowest grade from an individual eye, it was found that a grade of 0 or 1 was consistent with a serum retinol level of greater than or equal to 0.70 mol/L (20g/dL) while a grade of 2–5 signified a retinol level below this, which was regarded as abnormal. In a study of 75 pre-school children with mild xerophthalmia and 74 neighbourhood, age-matched clinically normal controls, impression cytology was closely correlated with baseline serum vitamin A levels. Subclinical vitamin A deficiency was detected in 23% (14 out of 60) of the control population: treatment of both groups with vitamin A capsules resulted in improvement in the cytology grade in 95% of those with originally abnormal grades.[64]

The value of impression cytology in diagnosing vitamin A deficiency, particularly in its subclinical form arises not only from its implications for blindness but also from the observation that vitamin A deficiency is associated with an increased morbidity and mortality.[65,66,67,68] Vitamin A supplementation of xerophthalmic, but also non-xerophthalmic children from the same region reduces mortality, suggesting that benefit is conferred on those with subclinical as well as clinically evident disease.

Table V *Snake-like chromatin*

	Per cent	*(n)*
Normals	1.3	300
1° and 2° Sjögrens	57.1	14
KCS	40.0	30
Contact Lens	39.6	48

After Kruse *et al.* 1986.

Table VI *Natural history of KCS*

1. Lacrimal inflammation and atrophy leads to—
2. Reduced tear flow, volume, thickness, lubrication
3. Increased tear osmolarity and altered salt concentration, causes—
4. Ocular surface damage with squamous metaplasia, directly or secondary to vitamin A deficiency
5. Inflammation supervenes with hyperaemia, tear white cell invasion and Meibomitis
6. Secondary interactions exacerbate these events

(8) An intriguing conjunctival sign demonstrated by impression cytology in KCS is the 'snake-like chromatin' pattern observed by Marner[69,70] in 25% of patients with suspected Sjögren's syndrome and 50% of patients with chronic KCS. The pattern consists of a sinous condensation of the nuclear chromatin and is found preferentially over the upper bulbar conjunctiva.[70] The presence of 'snake-like chromatin' pattern correlated with the severity of the disease as measured by BUT, Bengal Rose staining and Schirmer's test.

The pattern is however, not specific to KCS, and is found for example in allergic conjunctivitis[69] and in contact lens wearers, but rarely in normal subjects (five out of 300).[71] It has been suggested that it represents a non-specific response to trauma. Curiously it has not been demonstrated in cells removed by conjunctival scraping[69] (Table V).

Conclusions

Based on our current understanding of the disease, the natural history of KCS may be hypothesised as follows (Table VI):

Reduced tear flow leads to reduced tear volume and an increased osmolarity of the tears. Increased osmolarity damages the ocular surface, detected as punctate surface staining. A fall in goblet cell density may be related to hyperosmolarity or to a reduced delivery of vitamin A (retinol) to the conjunctiva via conjunctival vessels. But an additional possibility is that tear retinol levels or

tear carrier protein levels are reduced due directly to lacrimal gland damage.

In keeping with the reduced goblet cell density there is an altered physical behaviour of the tears with a reduction in viscosity, rise in surface tension and loss of tear stability as measured by BUT and NIBUT. The surface of conjunctival epithelial cells increase in area while those of the cornea decrease. It is not possible to explain both events by a single mechanism, but possibly there is a reduced limbal contribution to corneal epithelial cells.

Damage to the ocular surface stimulates a white cell response and white cells may release mediators (PGE2) and active oxygen species (superoxide) which may themselves damage the ocular surface. The ability of the tears to buffer these effects may be reduced by a loss of scavenger molecules. So far no information is available as to whether the primary change occurring in ocular surface epithelium is similar to those affectng the lacrimal gland itself.

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