

Tear Film Osmolarity and Ocular Surface Disease in Two Rabbit Models For Keratoconjunctivitis Sicca

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We report the natural history of keratoconjunctivitis sicca (KCS) in two rabbit models. The first one (full KCS model) was created by closing the lacrimal gland excretory duct, and removing the nictitating membrane and harderian gland. We created the second one (lacrimal gland duct only [LGDO]-KCS model) by closing the lacrimal gland excretory duct. Although tear film osmolarity was abnormally high in both models, it was higher in the full KCS model. Decreases in corneal epithelial glycogen and in conjunctival goblet cell density, and morphological abnormalities of the conjunctiva correlated with increases in tear film osmolarity and duration of disease. Invest Ophthalmol Vis Sci 29:374-378, 1988

We recently reported a new rabbit model for keratoconjunctivitis sicca (KCS), created by cauterizing the lacrimal gland excretory duct and surgically removing the nictitating membrane and harderian gland.¹ In this model tear film osmolarity was elevated by postoperative day 1, and corneal epithelial glycogen and conjunctival goblet cell density decreased progressively and remained decreased for the first 8 weeks postoperatively. In a separate animal model, closure of the lacrimal excretory duct elevated tear film osmolarity.¹

We report here tear film osmolarity and ocular surface changes in two rabbit models for keratoconjunctivitis sicca between 8 and 20 weeks postoperatively. In one model the lacrimal gland excretory duct was closed, and the nictitating membrane and harderian gland were removed; in the second model the lacrimal gland excretory duct was closed.

Materials and Methods

Our rabbit models for KCS were created as previously described.¹ In the first one designated the "full KCS model," the lacrimal gland excretory duct was closed with cautery, and the nictitating membrane and harderian gland were removed surgically. In the second one designated the "lacrimal gland duct only (LGDO) model," the lacrimal gland excretory

duct was closed by cautery, and the nictitating membrane and harderian gland were left undisturbed. All procedures using animals conformed to the ARVO Resolution on the Use of Animals in Research.

We operated on one eye in each animal, and the contralateral eyes served as the controls. The experimental group for the "full KCS model" consisted of eight rabbits, four of which were sacrificed at 12 weeks postoperatively and the remainder at 20 weeks postoperatively. The experimental group for the "LGDO model" also consisted of eight rabbits that were sacrificed according to the above schedule.

For the full KCS model, slit lamp examinations, tear osmolarity measurements,²⁻⁴ and Schirmer tests with proparacaine were performed usually every other week between 8 and 20 weeks postoperatively. For the LGDO model, tear osmolarity measurements were performed usually weekly or every other week between 8 and 20 weeks postoperatively.

The rabbits were sacrificed by an overdose of intravenous pentobarbital at 12 and 20 weeks postoperatively. Conjunctival goblet cell density¹ and corneal epithelial glycogen⁵ were measured as previously described. In all ocular surface morphology studies, eyes at the time of death were fixed immediately with Karnovsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in cacodylate buffer). Tissue samples were then processed for light microscopy and scanning and transmission electron microscopy using routine techniques.

Results

Earlier studies found large differences in goblet cell densities among normal rabbits but none between contralateral normal eyes. For this reason loss of goblet cell density is expressed as percent remaining compared with contralateral normal controls.

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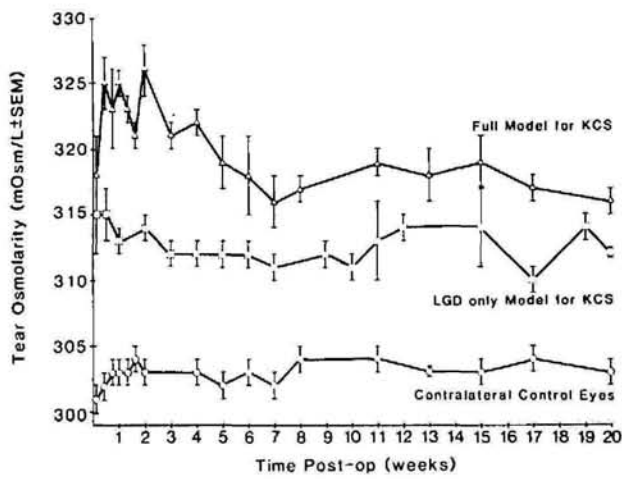


Fig. 1. Tear osmolarity in full keratoconjunctivitis sicca (KCS) model and lacrimal gland duct only (LGDO) KCS model up to 20 weeks postoperatively.

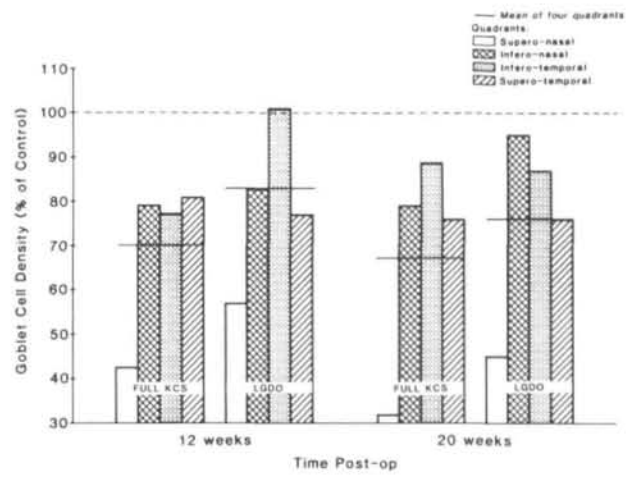


Fig. 2. Goblet cell density in full keratoconjunctivitis sicca (KCS) model and lacrimal gland duct only (LGDO) KCS model at 12 and 20 weeks postoperatively.

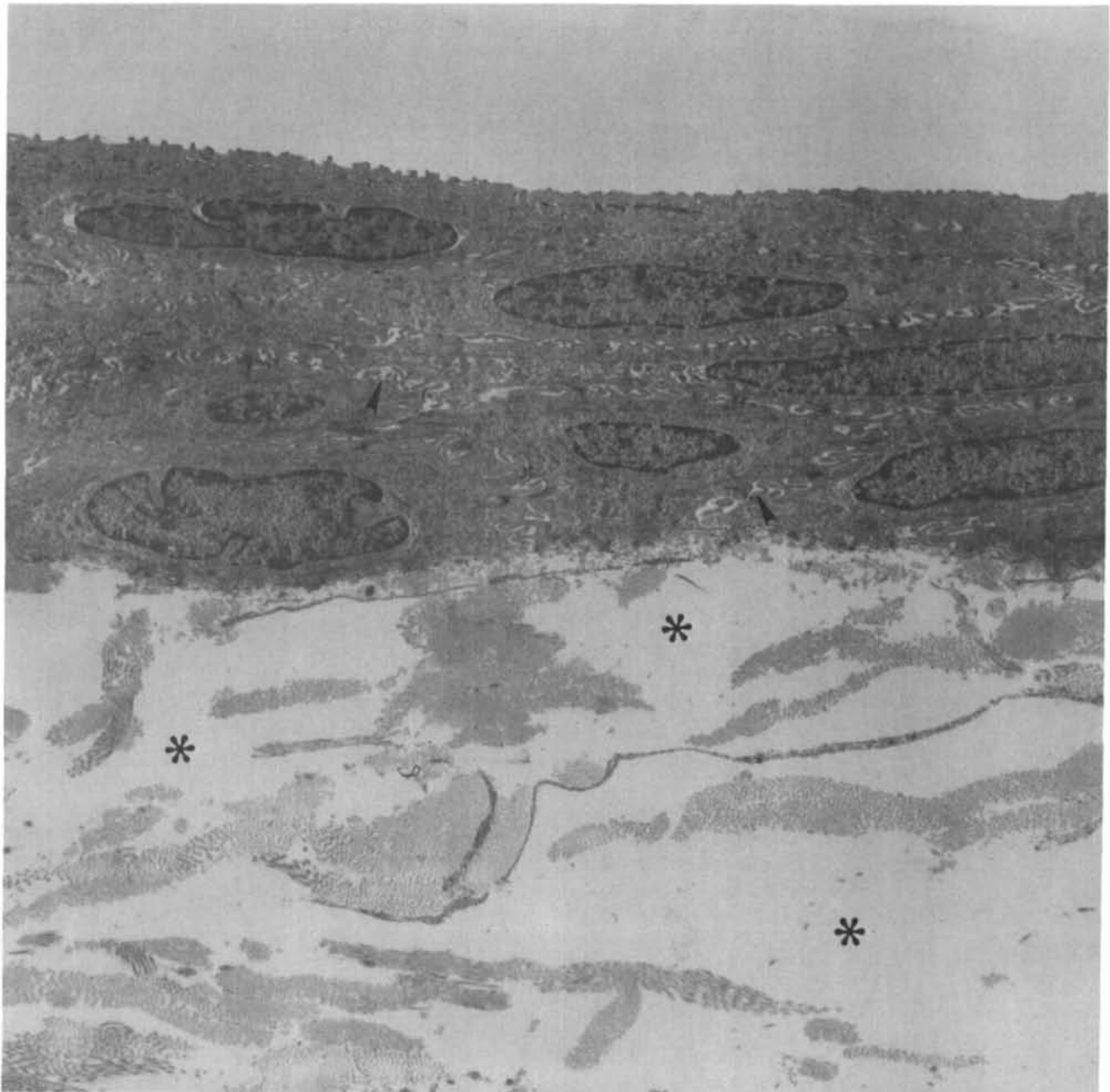


Fig. 3. Inferior bulbar conjunctiva of lacrimal gland duct only (LGDO) KCS model 20 weeks postoperatively. Edema is evident throughout conjunctival stroma (asterisks). There is also mild intercellular edema in deeper epithelial layers (arrowheads). Original magnification $\times 6,000$.

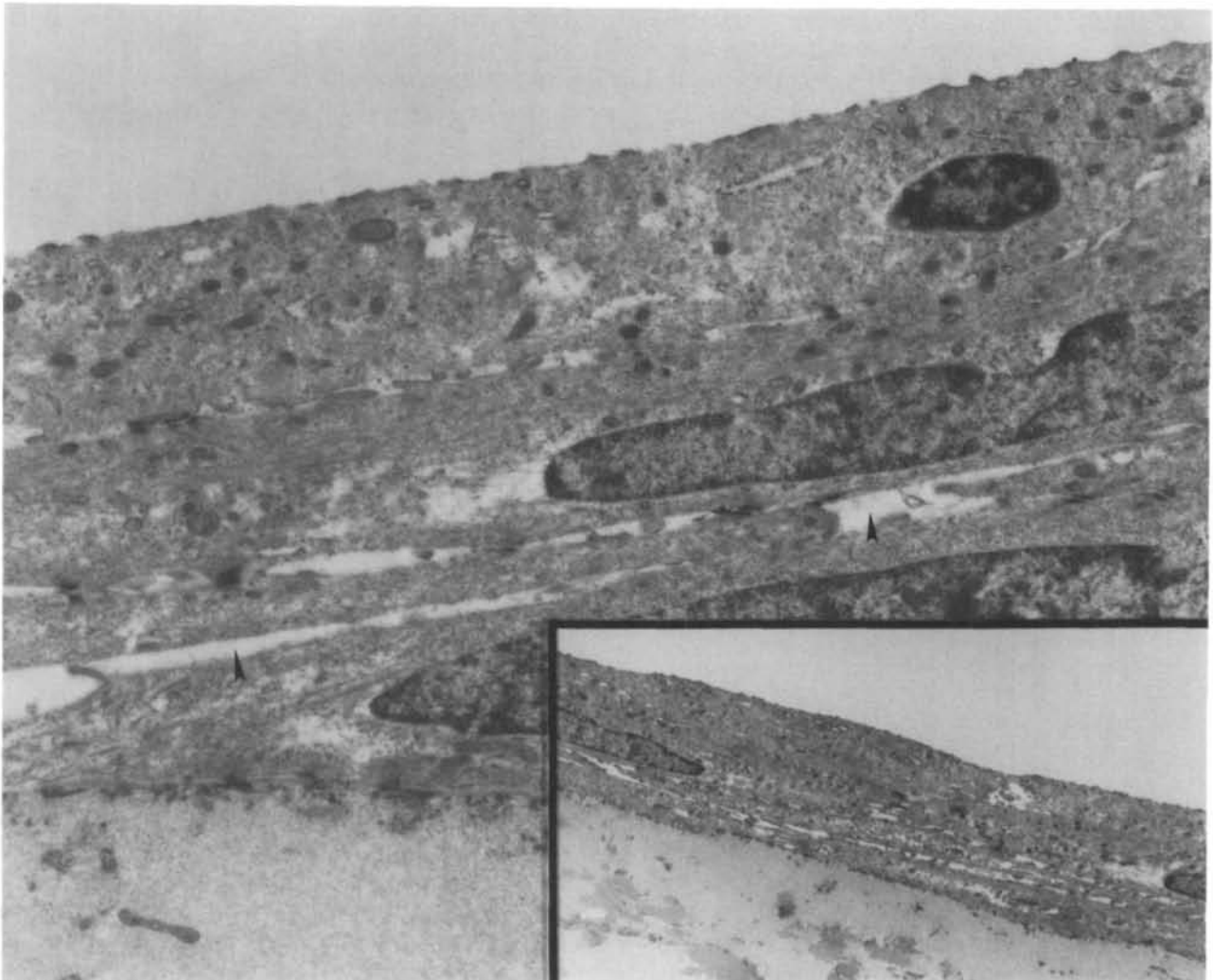


Fig. 4. Inferior bulbar conjunctiva of full keratoconjunctivitis sicca (KCS) model 12 weeks postoperatively. Intercellular edema (arrowheads) is more prominent than in Figure 3, and decreased cytoplasmic density indicates intracellular edema (original magnification $\times 17,750$). Inset: Different area of inferior bulbar conjunctiva from same rabbit shows intercellular edema but more normal cytoplasmic density (original magnification $\times 4,000$).

Up to 20 weeks postoperatively, slit lamp examination did not reveal any differences between control eyes and those with the full KCS model, aside from the absence of the nictitating membrane in operated eyes.

In eyes with the lacrimal gland excretory duct occluded and the nictitating membrane and Harderian glands removed, Schirmer tests with proparacaine averaged 8.0 ± 0.3 mm (SEM) vs. 8.4 ± 0.4 mm (SEM) in contralateral control eyes ($n = 28$, not significant).

Between 8 and 20 weeks postoperatively, tear film osmolarity averaged 318 ± 1.0 mOsm/L (SEM) ($n = 30$) in the full KCS model, 313 ± 1.4 mOsm/L (SEM) ($n = 24$) in the LGDO model, and 304 ± 0.5 mOsm/L ($n = 30$) in the control eyes for the full KCS model only. Tear film osmolarity in the full KCS

model was significantly higher than that in the LGDO model ($P < 0.01$), and osmolarity values in the full KCS and LGDO models were both significantly higher than those in control eyes ($P < 0.01$ and $P < 0.01$, respectively) (Fig. 1).

Conjunctival goblet cell density was decreased at 12 and 20 weeks in both the full KCS and LGDO models. This decrease was proportional to the increase in tear film osmolarity in these models. Specifically, the full KCS model that had a greater average increase in tear film osmolarity than the LGDO model also had a greater average decrease in conjunctival goblet cell density as indicated by flat mount. In both the full KCS and LGDO models, goblet cell density was decreased, particularly in the superonasal quadrant in all rabbits studied (Fig. 2).

The decrease in corneal epithelial glycogen in our



Fig. 5. Inferior bulbar conjunctiva of full keratoconjunctivitis sicca (KCS) model 20 weeks postoperatively. Intracellular edema is severe. Note discontinuities in anterior cell membrane (arrowheads) (original magnification $\times 26,000$). Inset: Inferior bulbar conjunctiva from same rabbit appears normal (SEM, original magnification $\times 1,000$).

two models was similarly proportional to tear film osmolarity. In the full KCS model, glycogen levels decreased an average of 28.2% and 33.1% at 12 and 20 weeks, respectively; in the LGDO model, glycogen levels decreased an average of 14.9% and 20.2% at 12 and 20 weeks, respectively.

Corneal morphology appeared normal in both models up to 20 weeks postoperatively by light microscopy and scanning and transmission electron microscopy. In the conjunctiva of both models stromal (Fig. 3) and intercellular (Fig. 4) edema intensified with time but was most prominent in the full KCS model at 20 weeks. The intercellular edema was confined to the basal epithelial layers, and even at 20 weeks postoperatively in the full model, the changes were not detected by electron microscopy in every field. Similarly, the more superficial conjunctival epi-

thelial cells had intracellular edema, manifested by decreased cytoplasmic density. This tendency also became more pronounced with time but was most prominent in the full KCS model at 20 weeks postoperatively (Fig. 5). Cells with decreased cytoplasmic density demonstrated decreased cell-surface microvillae. Rabbits with more advanced disease had occasional conjunctival areas where superficial cells had been lost.

Discussion

We reported previously that closure of the lacrimal gland excretory duct and removal of the nictitating membrane and Harderian gland elevated tear film osmolarity and decreased corneal epithelial glycogen levels and conjunctival goblet cell density.¹ Our new study demonstrated that the decrease in corneal epi-

thelial glycogen was progressive, and the increase in tear film osmolarity and decrease in goblet cell density persisted for 20 weeks postoperatively. Although we demonstrated previously that closure of the lacrimal gland excretory duct elevated tear film osmolarity,¹ the current study showed that closure of this duct also decreased corneal epithelial glycogen levels and conjunctival goblet cell density progressively. Between 12 and 20 weeks postoperatively in both rabbit models for KCS, the decreases in glycogen values and in goblet cell density as well as morphological changes were proportional to elevations in tear film osmolarity and duration from time of surgery. The changes that we observed by light and electron microscopy have been reported in morphological studies of human KCS.^{6,7} The conjunctival stromal and epithelial intercellular edema in our KCS models may have been caused by the increased osmotic gradient between the serum and tear film in these rabbits, and a resultant increase in fluid transport from the fenestrated conjunctival vessels across the epithelium and into the tear film.⁸

Although corneal epithelial glycogen levels continued to decline with time in our study, corneal morphology remained normal at 20 weeks postoperatively. We interpret the progressive decrease in corneal glycogen as an indication of cellular stress, or a lack of delivery of nutrients to the cells.

The current data indicate that increases in tear film osmolarity correlate directly with the severity of ocular surface disease, which progresses with time in both of our rabbit models for KCS.

Key words: goblet cells, keratoconjunctivitis sicca, ocular surface, tears

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