Tear Production After Unilateral Removal of the Main Lacrimal Gland in Squirrel Monkeys

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Objective: To study the effects of lacrimal gland removal on basal and reflex tear production and on the ocular surface in the squirrel monkey.

Methods: Unilateral main lacrimal gland removal in 6 squirrel monkeys was followed by Schirmer testing, slit-lamp examination with fluorescein, and collection of basal and reflex (stimulated) tears for analysis of tear protein spectra between 0 and 20 kd, as well as histological evaluation.

Results: Schirmer test results showed an 80% decrease in basal tears and a 90% decrease in reflex tears during week 1, and a 32.2% and 33.3% decrease, respectively, at week 20 after surgery, compared with the contralateral control side. However, no gross abnormalities or fluorescein staining were seen in 5 of the 6 monkeys, and the conjunctival surfaces remained normal. The main and accessory lacrimal glands appeared to secrete similar types of proteins. No histological changes were seen in corneal, conjunctival, or eyelid tissues 20 weeks after surgery.

Conclusions: Tears from accessory lacrimal glands were sufficient to maintain a stable tear layer on the cornea, suggesting that so-called basal tear flow is made up of fluid from both main and accessory lacrimal glands and that decreased tear production by the main lacrimal gland is not a causative factor in keratoconjunctivitis sicca.

Clinical Relevance: This study shows that total removal of the main lacrimal gland does not in itself lead to keratoconjunctivitis sicca. However, the nature of neural control of the accessory glands is not yet clear.


Tear secretion is required for protection of the ocular surface and for the maintenance of the optical properties of the cornea. The main or orbital lacrimal gland is generally acknowledged to be the principal source for the aqueous component of tears and for many of the proteins that protect the surface from bacterial attack. It has been suggested that decreases in aqueous tear secretion, decreases in certain constituents of tears such as lysozyme and lactoferrin, and changes in growth factor production are the result of lacrimal apparatus dysfunction and may be involved in ocular surface disease.

The activity of the lacrimal secretory system can be broadly divided into the production of basal and reflex secretions. It is difficult to determine the relative contributions of the main and accessory lacrimal glands to the basal and reflex tears. A traditional concept is that the accessory lacrimal glands are the source of basal tears, whereas reflex secretion may include contributions from the main and accessory lacrimal glands, but this idea has been challenged. More recently, it has been suggested that all aqueous tear secretion is the result of stimulation. Previous studies indicated that activation of the several pathways leading to the production of reflex tears begins with activation of the sensory nerves in the cornea. By subsequent activation of central nervous system neurons, various conditions, including corneal dehydration, cooling, or trauma, could initiate a discharge of the secretomotor neurons in the pterygopalatine ganglion. This neural activity would, in turn, lead to lacrimal gland activation and the release of variable amounts of tear fluid onto the ocular surface. Routes of communication between the accessory lacrimal glands and the ocular surface are unclear. Tear characteristics such as osmolarity, volume, production, turnover, evaporation, and lipid viscosity are thought to be important for a healthy eye. Changes in these factors have been found to correlate strongly with the development of dry eyes. It is well known that a char-
MATERIALS AND METHODS

Six squirrel monkeys, 2 male and 4 female (weight = 1.0-1.5 kg), were used. All experimental procedures were conducted in accordance with the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Research in Vision and Ophthalmology.

Slitlamp examinations were performed before surgery. Only animals with a normal anterior segment in both eyes were included in the study. Also before surgery, fluorescein tests, rose bengal staining, and Schirmer tests were performed in both eyes, and tears were collected from both eyes for protein profile analysis, as will be described.

For surgery, the animals were anesthetized by an intramuscular injection of 0.8 mL of ketamine hydrochloride (Fort Dodge Laboratories Inc, Fort Dodge, Iowa). Proparacaine hydrochloride was used to provide local anesthesia of the skin. The skin around the right eye was shaved and wiped with 85% alcohol. A 7-mm vertical skin incision was made on the temporal side of the eye, through skin and underlying fascia. The temporal muscle was moved laterally away from the orbit to visualize the lacrimal gland. The duct, vessels, and innervation were found to be in contact with the orbit through a temporal foramen in the lateral wall of the orbit. The site was identified in each animal, and the lacrimal gland was removed at that site. The incision was sutured with 6-0 nylon sutures (Ethicon; Ethicon Inc, Somerville, NJ), and a 1-mL intramuscular injection of gentamicin sulfate, 40 mg/mL, was given. The sutures were removed 7 days after surgery. Healing was uneventful in all animals. The left eyes were used as controls.

After surgery, slitlamp examinations of both eyes were performed every 3 days for the first 2 weeks and then weekly for 20 weeks. Fluorescein testing was done weekly. A fluorescein strip (Alcon Laboratories Inc, Fort Worth, Tex) was wetted with isotonic sodium chloride solution and gently applied to the upper conjunctiva. After the animal was allowed to blink several times to distribute the fluorescein on the cornea, slitlamp examination was performed with a cobalt blue filter to visualize areas of fluorescein staining.

Rose bengal staining was performed every 2 weeks after surgery. The intensity of staining of the medial and lateral bulbar conjunctiva and the cornea was graded; the maximum grade for each section was 3, for a total maximum of 9 per eye.

Schirmer tear tests were done every 2 weeks. To measure basal tears, a Schirmer tear test paper strip (Iolab Corp, Claremont, Calif) was inserted in the lower fornix of the eye for 5 minutes, after which the strip was removed from the eye and the length of the wetted area of the strip was measured. To measure reflex tears, the superior and inferior bulbar conjunctivae were stimulated by gentle rubbing with a cotton-tipped swab. Special care was taken to avoid touching the cornea to prevent any possible trauma. The Schirmer tear test strip was placed immediately after this procedure, and the length of the wetted area of the strip was measured after 5 minutes.

For protein profile analysis, tears were collected weekly. The lower eyelid was pulled down gently, and a 10-μL fire-polished micropipette was allowed to touch the tear pool in the lower fornix. Care was taken not to touch the conjunctiva or cornea, which could cause reflex tearing. Tears were collected in amounts of 1 to 2 μL and frozen at −70°C. Tear samples were thawed, mixed (1:100) with a matrix solution containing an active component of α-cyano-4-hydroxy-cinnamic acid (Sigma-Aldrich Corp, St Louis, Mo), and analyzed by a mass spectrometer (Voyager Biospectrometry Workstation Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometer; PerSeptive Biosystems Inc, Framingham, Mass). The molecular mass range used in this tear analysis was 1000 to 22 000 d.

At the end of the experiment (20 weeks after surgery), the squirrel monkeys were euthanatized and the eyelids, corneas, and conjunctivae with accessory lacrimal glands were removed, immersed in fixative, and processed for plastic embedding for light and electron microscopy. The immersion fixative was a mixture of 1% paraformaldehyde and 2% glutaraldehyde in 0.1-M cacodylate buffer, pH 7.3. Thick sections were cut and stained with toluidine blue and basic fuchsin for orientation and photography. Thin sections were cut on an ultramicrotome (LKB, Bromma, Sweden), stained with uranyl acetate and lead citrate (Ultron stain; LKB), and observed by a transmission electron microscope (model 10 CA; Zeiss, Oberkochen, Germany).

Acral dystrophic keratoconjunctivitis sicca is reduced lacrimal gland secretion, and there has been associated damage to the ocular surface. In clinical studies, decreased lacrimal gland secretion is often attributed to changes related to aging or pathological features.

Dry eye syndromes, which are characterized by keratoconjunctival epithelial lesions and are thought to be caused in large part by reduced secretion of tear fluid, are roughly classified into 2 categories: those associated with Sjögren syndrome and others. Others include degenerative processes, tumors, orbital amyloidosis, and pseudolymphoma. In such cases, inadequate ocular lubrication may occur as a result of the loss of the secretory acinar units due to periacinar and periductal connective tissue hyperplasia and destruction of acinar and tubuloductal structures. Dry eye can be a major complication in about half of the patients who undergo surgery involving removal of the lacrimal gland, even when there are no signs of dry eye before surgery, the lacrimal gland is not involved in the malignant process, or both. A similar problem occurs in cases of radiotherapy, when incidental irradiation of the lacrimal gland is required. In all of these conditions, if dry eye develops, it is usually thought to be caused by tear loss after removal or dysfunction of the main lacrimal gland. The state of the accessory lacrimal gland is usually unknown in these patients, and it is unclear whether both secretory tissues are needed to prevent dry eye.

In the present study, the relative contributions of the main and accessory lacrimal glands to the tear fluid were assessed in the squirrel monkey. In attempting to determine the relative importance of these 2 secretory tissues, we took advantage of an unusual anatomical formation in the squirrel monkey: to provide room for the globe, in the course of evolution the main lacrimal gland has migrated to a temporal location outside the bony...
orbit. Thus, unilateral main lacrimal gland removal was performed to determine whether the accessory lacrimal glands could provide sufficient tears to maintain the ocular surface with no secretory contribution from the main lacrimal gland. Tear flow was measured, the ocular surface was examined, and analysis of tear spectra was performed to look for changes in the protein composition of the tears in eyes with and without a tear fluid contribution from the main lacrimal gland.

RESULTS

Slitlamp examination and fluorescein test results revealed no corneal abnormalities in any of the eyes before surgery. As the eyes were not directly involved in the surgical procedure, no ocular inflammation was observed the next day. Healing of the exorbital surgical wounds was uneventful.

No fluorescein staining was seen in 5 of the 6 eyes on the operated-on side or in any of the contralateral control eyes at any time after surgery. A barely noticeable central corneal epithelial erosion was observed 3 days after surgery in the eye on the operated-on side in 1 female squirrel monkey. According to the attending veterinarian, this female squirrel monkey was thought to be the oldest member of the study group. The results of fluorescein testing revealed a green oval about 1.5 × 2.0 mm. The defect healed by day 10, leaving an anterior stromal haze equivalent to 1 (mild haze not affecting refraction) on a 5-point scale. No other clinical or anatomical signs of keratoconjunctivitis sicca were found in this animal for the remainder of the study. No other corneal pathological features were detected in the contralateral control eye of this monkey or in any of the eyes of the other 5 squirrel monkeys (Figure 1).

Evaluation of rose bengal staining in the 5 younger monkeys produced an average grade of 1.4 in eyes on the operated-on side and 1.2 in the contralateral control eyes, of a total possible grade of 9. The operated-on sides showed no corneal staining and only slight staining of the nasal bulbar conjunctiva; staining patterns in the contralateral control eyes were similar (Figure 2, top). There were no changes in the staining pattern during the study.

The older female monkey that developed the transient epithelial defect had a grade of 3.5 in the second week after surgery (Figure 2, bottom) and 4.0 in the 12th week after surgery in the eye on the operated-on side compared with 1.8 in the control eye. Staining in the operated-on eye had a linear shape and was located in the inferior cornea, the lateral bulbar conjunctiva, and the nasal conjunctiva.

Schirmer test results showed that, on the operated-on side, the average amount of basal tears decreased markedly immediately after surgery and then increased gradually thereafter during the study without, however, ever reaching the levels seen in the contralateral control eyes (Table). An 80% decrease (SD, 9.4%) in mean basal tears in the operated-on eyes (n = 6) was found during the first week after surgery compared with the contralateral control eyes (n = 6); the decrease was 65.4% (SD, 6.7%) in weeks 2 to 4, 48.9% (SD, 5.6%) in weeks 5 to 10, 33.3% (SD, 3.1%) in weeks 11 to 15, and 32.2% (SD, 3.2%) in weeks 16 to 20. All differences were significant (P<.01). Basal tear production in the eye on the operated-on side of the older female monkey with the damaged corneal epithelium was similar to the average basal tear production on the operated-on sides of the other 5 monkeys.

Reflex tears were also dramatically decreased on the operated-on sides immediately after the surgery com-
Changes in Basal and Reflex Tear Values After Main Lacrimal Gland Removal*

<table>
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<tr>
<th>Eye</th>
<th>Tears</th>
<th>0-1</th>
<th>2-4</th>
<th>5-10</th>
<th>11-15</th>
<th>16-20</th>
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<tr>
<td>Operated-on</td>
<td>Basal</td>
<td>1.1 ± 1.2</td>
<td>1.8 ± 1.2</td>
<td>3.2 ± 1.4</td>
<td>3.6 ± 1.6</td>
<td>4.2 ± 1.6</td>
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<tr>
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<td>Reflex│ 1.6 ± 1.2</td>
<td>2.6 ± 1.2</td>
<td>5.0 ± 1.4</td>
<td>8.8 ± 1.6</td>
<td>11.2 ± 1.6</td>
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<td>Basal</td>
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<td>5.2 ± 1.2</td>
<td>6.2 ± 1.4</td>
<td>5.4 ± 1.6</td>
<td>6.2 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>Reflex</td>
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<td>17.3 ± 1.4</td>
<td>18.2 ± 1.6</td>
<td>16.8 ± 1.6</td>
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</table>

*Correlation coefficients for the change in tear production were significant only for the operated-on eyes (basal tear production, r = 0.66, P < .05; reflex tear production, r = 0.86, P < .05).
†Data are given as Schirmer tear test values, mean ± SD millimeters of wetting, of all eyes sampled during the given interval after surgery.

**Figure 3.** Matrix-assisted laser desorption–ionization time-of-flight mass spectrometric analysis of tears collected from eyes on the operated-on and contralateral control sides on day 14 after surgery. The range of molecular weights analyzed was from 1000 to 22 000 d. m/z indicates mass/charge.

**Figure 4.** Light micrograph of the plastic-embedded cornea from an operated-on side (main lacrimal gland removed). The anatomical structure is normal (toluidine blue, original magnification ×40).

pared with the contralateral unoperated-on control sides, and gradually increased thereafter. The decrease in reflex tears was 90.4% (SD, 10.4%) in the first week after surgery, 84.4% (SD, 10.7%) in weeks 2 to 4, 71.1% (SD, 6.9%) in weeks 5 to 10, 51.6% (SD, 18.3%) in weeks 11 to 15, and 33.3% (SD, 6.6%) in weeks 16 to 20. All differences were significant (P < .01). The amounts of basal and reflex tears produced by the contralateral control eyes did not change during the study (Table).

Comparison of matrix-assisted laser desorption–ionization time-of-flight mass spectra of tears from the operated-on side with tears from the contralateral control sides showed no major differences in tear protein profiles before surgery and on days 2, 7, and 14 after surgery. As shown in Figure 3, each peak representing a protein on the spectrograph of tears from eyes on the operated-on side is matched by a peak of similar molecular weight on the spectrograph of tears from the contralateral control eyes.

The results of light microscopy and transmission electron microscopy showed no differences in anatomical structure between corneas (Figure 4), conjunctivae (Figure 5), or eyelids from the operated-on and control sides at the end of the study. No inflammatory infiltration was found.

Tissue from main lacrimal glands removed at the beginning of the study showed the usual acinar structure (Figure 6 and Figure 7). In contrast, the accessory lacrimal glands of Krause (located in the conjunctival fornix area) removed from the operated-on side at the end of the study consisted of a collection of separate cellular units, each with an opening to the conjunctival surface (Figure 8). Cellular structures of the lobular forms of main and accessory lacrimal glands were similar, and large numbers of secretory granules were present in the main and accessory lacrimal glands. Myoepithelial cells, which are common in the main lacrimal gland, were not observed in the accessory lacrimal glands. A small number of accessory glands of Wolfring were found near the upper border of the tarsal plate. In the squirrel monkey, these glands appear to be notably smaller in size and more rudimentary compared with the glands of Krause. Analysis of the strand of tissue penetrating the temporal orbital foramen revealed the duct of the main lacrimal gland, along with the major vessels and nerves. Further dissection under an operating microscope showed that the termination of the structure connected the lacrimal gland to the conjunctiva of the ocular surface. By comparison, the accessory glands were found to be only a few cell layers from the conjunctival surface, obviating the need for an extensive ductal arrangement.

**COMMENT**

Important for the maintenance of the normal optical and tissue functions of the ocular surface is the presence of tears, most of which are thought to be produced by the main lacrimal gland. In this study, the main lacrimal gland
was removed unilaterally in squirrel monkeys to eliminate the contribution of this gland to tear secretion.

This approach has been used by many researchers, but in most cases in animal models whose secretory tissues are different from those of primates. The experimental conditions and surgical techniques have varied widely, including removal or cauterization of part of the conjunctiva, removal of the nictitating membrane, removal of the harderian gland, use of different medications, and variations in environmental conditions such as the humidity in animal care facilities.

The choice of animal model plays an important role. In dogs, the conjunctival accessory lacrimal glands appear to contribute little to the aqueous portion of the tear film; thus, removal of the main lacrimal gland leads to immediate development of the clinical symptoms of keratoconjunctivitis sicca. In cats, the conjunctival accessory lacrimal glands also do not provide much tear flow, but the accessory lacrimal gland apparatus on the nictitating membrane is apparently sufficient to prevent the appearance of dry eye symptoms. In rabbits and mice, removing the main lacrimal gland and the nictitating membrane does not necessarily cause clinical symptoms because the conjunctival accessory lacrimal glands provide an adequate tear supply. For comparison with humans, however, the nonhuman primate model is much more useful because it is similar to the human situation in the anatomical structure of the lacrimal gland and eyelids and the absence of a nictitating membrane and harderian glands. In addition, in the squirrel monkey, the main lacrimal gland is located outside the orbit, making the surgical approach easier and safer because the orbit and globe are not involved.

Slitlamp examinations with fluorescein and Schirmer tests are commonly used in ophthalmologic clinics for...
the diagnosis of ocular surface changes associated with insufficient tears. However, the Schirmer test has some limitations in its measurement of basal tear flow and reflex tear flow produced by gentle mechanical stimulation of the corneal surface. In some studies, a local anesthetic is used when basal tear flow is measured, although it is known that the lacrimal gland responds to sensory stimulation of the ocular surface with an increase in tear secretion mediated through a multisynaptic pathway. It is not yet clear whether the accessory gland is under the same type of neural control as the main lacrimal gland. With that in mind, we chose not to use local anesthesia for tear flow measurement or for tear collection to prevent possible changes in neural sensitivity of the corneal surface, the tear protein profile, the amount of tears distributed during blinking, and the blinking interval, any of which could lead to drying of the corneal surface.

**The only monkey** that developed corneal pathological features was examined by the attending veterinarian and was found to be an aged female. This finding was interesting as dry eye is known to be more common in the older female population. Although this study was not designed to investigate the relation between age and sex and tear production, we did note that the average amounts of tears collected from the operated-on side in this monkey were similar to the average amounts of tears collected from the operated-on sides of the other monkeys, so the thickness of the aqueous layer may not have been a primary factor in the development of the epithelial defect. We also found, however, that the rose bengal staining scores in the control eye of this animal were higher than the scores for the control eyes of the other animals, which suggests that this particular monkey may have been more susceptible to the development of dry eye symptoms.

Measurements of tear flow on the operated-on and control sides showed that basal tear flow is most likely a combination of contributions from the main and accessory lacrimal glands, whereas reflex tears consist mainly of secretion from the main lacrimal gland, with a smaller contribution from other sources. Although removal of the main lacrimal gland markedly decreased basal tear production in these monkeys, sufficient tears were produced to prevent dry eye–like syndrome. The marked decrease in reflex tear production was probably attributable to the small size of the accessory lacrimal glands, which could not provide the normal excess tearing response to stimulation or to different modes of innervation for the 2 types of glands. The increase in basal and reflex tear flow observed over time after surgery could have been caused by increased secretory activity, proliferation of the accessory lacrimal glands, or both; however, this remains to be determined.

Rose bengal staining of the cornea and conjunctiva is one of the most reliable tests for the diagnosis and evaluation of the severity of ocular surface damage in those with keratoconjunctivitis sicca. The fact that the average score (1.4) for rose bengal staining in the eyes on the operated-on sides (except for the older female monkey with the epithelial defect) was close to the average score (1.2) in the control eyes on the unoperated-on sides supports the suggestion that these monkeys did not have dry eyes. The high rose bengal test score (1.8) in the control eye of the affected monkey, together with the clinical findings, supports the diagnosis of keratoconjunctivitis sicca in this animal.

Matrix-assisted laser desorption–ionization time-of-flight mass spectrometry was found to be valuable for tear analysis. Protein analysis can be determined in each animal separately, particularly in comparison with sodium dodecyl sulfate gel chromatography and immunoelectrophoresis, as it provides a detailed and precise molecular spectrum and the sensitivity is in the range of $10^{-13}$ to $10^{-15}$ mol. In addition, our interest included not only previously known proteins but also unidentified proteins that may be present in tears. The limitation of this technique is a difficulty with quantitation that is inherent to the instrument; in addition, higher-molecular-weight proteins cannot be evaluated except in their multiply protonated forms. However, we were more concerned with the appearance of proteins in the molecular weight range from 1000 to 22 000, as this weight range contains growth factors and a multiply protonated form of lactoferrin, at about 16 000, whose presence or absence could be important in the development of keratoconjunctivitis sicca. The number of peaks in the tear spectra from the control eyes was similar to the number of peaks in the tear spectra from the eyes on the operated-on sides. This means that the proteins secreted into the tears by the main lacrimal gland and the accessory lacrimal glands are similar and, furthermore, that the accessory lacrimal glands continue to produce these proteins in eyes from which the main lacrimal gland has been removed.

There are many factors, including tear breakup time, dysfunction of meibomian glands, and others, that lead to the development of keratoconjunctivitis sicca. Dysfunction of the main lacrimal gland is probably often associated with dysfunction of the accessory lacrimal glands. However, the accessory lacrimal glands have the resources to provide the tears necessary to partly or fully compensate for loss of the main lacrimal gland. After removal of the main lacrimal gland in our study, drying of the ocular surface and a long-term inflammatory state did not develop.

In summary, tear production by the accessory lacrimal glands is sufficient to maintain a stable tear layer on the ocular surface in most cases, and the tear proteins from the accessory lacrimal glands are apparently able to maintain normal functioning. These findings demonstrate the functional ability of the accessory lacrimal glands to successfully provide an immediate and relatively constant tear source. These gland systems are redundant but also work in tandem in the maintenance of the normal conditions and functions of the ocular surface.

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