Changes in rabbit lacrimal gland fluid osmolarity with flow rate. Jeffrey P. Gilbard and Darlene A. Dartt.

To determine whether the osmolarity of rabbit lacrimal gland fluid (LGF) changes with flow rate, microvolumes (approximately 0.2 μl) were collected directly from the cannulated glandular excretory duct of anesthetized rabbits. Low flow rates were obtained by collection of LGF 5 min after instillation of proparacaine; higher flow rates were obtained by stimulation with 0.45, 0.9, 3.8, or 15 μg of acetylcholine administered by local arterial injection. At low flow rates (less than 0.11 μl/min), LGF osmolarity was 334 ± 4 mOsm/L (n = 19). As flow rate increased to maximal rates (13.0 to 19.1 μl/min), LGF osmolarity decreased to a value of 299 ± 2 mOsm/L (n = 7). In keratoconjunctivitis sicca, increase in LGF osmolarity, as well as tear film evaporation, may contribute to elevated tear film osmolarity. (Invest Ophthalmol Vis Sci 23:804-806, 1982.)

In keratoconjunctivitis sicca the rate of tear production is abnormally decreased and tear film osmolarity is abnormally elevated.1, 2 Evaporation in association with decreased tear film turnover has been assumed to be responsible for the elevation of osmolarity.3 Botelho et al.4, 5 studied lacrimal gland fluid (LGF) collected directly from the cannulated excretory duct of the lacrimal gland of rabbits and found that the concentration of sodium, chloride, and calcium rose as the rate of secretion decreased. The present study examines whether the osmolarity of rabbit LGF, collected directly from the cannulated excretory duct, increases with decreasing flow rates, independent of the effect of evaporation.

Materials and methods. Three male New Zealand rabbits weighing 2.2 ± 0.1 kg (all values expressed as mean ± S.E.M.) were anesthetized with intravenous sodium pentobarbital (35 mg/kg initial dose, subsequently sustained when necessary with 7 to 9 mg/kg), paralyzed with 0.5 to 1.0 mg d-tubocurarine at 15 min intervals, and ventilated through a tracheostomy tube with an animal ventilator (Harvard 662). Rabbits were monitored by continuous recording of femoral artery pressure on an ink-writing polygraph (Beckman R 511A). The lacrimal gland excretory duct of the right eye of each rabbit was cannulated with a tapered glass tube (0.3 to 0.5 mm tip outer diameter, 6 mm length) for collection of LGF. In rabbits 2 and 3 the volume of this cannula measured 1.6 μl. Samples were collected directly from the cannula with L-shaped glass micropipettes (~0.2 mm tip outer diameter) that had originally been developed for tear sampling.1

To obtain low LGF flow rates, ~60 μl of 0.5% proparacaine HCl were instilled onto the ocular surface.6 To collect LGF, the cannula was first emptied in situ with the L-shaped micropipette. LGF was then allowed to fill the cannula to a volume adequate for osmolarity measurements (approximately 0.2 μl). Filling periods in rabbit 1 varied from 2 min 15 sec to 3 min; in rabbits 2 and 3 they were consistently 2 min 15 sec. At the end of the filling period, the accumulated LGF was collected with an L-shaped micropipette and transferred to an oil-filled storage tube.1 In rabbits 1 and 2 the volume collected within each filling period was estimated, and in rabbit 3 the volume was determined by marking the LGF meniscus on the L-shaped micropipette and subsequently refilling the pipette with distilled water and determining volume by weight. Average flow rate was obtained by dividing volume by collection time.

To obtain higher flow rates, various doses of acetylcholine CI (ACh) were injected into the lacrimal gland arterial supply.7 Prior to stimulation, the cannula was permitted to fill. Upon stimulation, the volume of LGF produced in a 30 sec period was collected into a preweighed L-shaped micropipette. Volume was determined from weight, and this LGF was then discarded. Flow rate was calculated as described for low flow rates. For osmolarity measurements, a separate L-shaped micropipette was used to take an LGF sample from the distal portion of the cannula immediately after the 30 sec period. To correct for evaporation during the 2 min 15 sec to 3 min filling period at low flow rates, samples of LGF (approximately 0.2 μl) obtained at stimulated flow rates were held in the L-shaped micropipettes for periods matched to the filling period required at low flow rates. Samples were then loaded into oil-filled storage tubes.

To ascertain the effect of evaporation during the 2 min 15 sec to 3 min period, ACh dose-matched control samples were loaded into oil-filled storage tubes immediately after removal from the cannula. To correct for the effect of sample size, we attempted to take samples of the same size at both
high and low flow rates. In rabbit 3 the volume of all samples taken was marked on the L-shaped micropipette and volume was subsequently determined from weight by means of distilled water. Mean sample volume at low flow rates was identical to mean sample volume at high flow rates (0.21 ± 0.01 μl).

Initially each rabbit was stimulated with 15 μg ACh to confirm successful cannulation. This fluid was discarded. We collected samples as follows: (1) three low-flow-rate samples, (2) samples after stimulation with 15, 15, 3.8, 0.9, and 0.45 μg ACh, respectively, (3) three low-flow-rate samples, and (4) samples after stimulation with 0.45, 0.9, 3.8, 15, and 15 μg ACh, respectively. The second 15 μg stimulation in each sequence was a dose-matched control for evaporation. To diminish the effect of external stimuli, all samples were collected 5 min after instillation of proparacaine.

Osmolarity sampling was measured by freezing-point depression using a nanoliter osmometer (Clifton Technical Physics) within 12 hr after collection.

**Results.** The osmolarity of LGF samples obtained after stimulation with 15 μg ACh averaged 308 mOsm/L for the six samples for which storage was delayed and 304 mOsm/L for the six samples that were stored immediately (Table I). We concluded that evaporation produced an average elevation of 4 mOsm/L in both the low-flow-rate samples and the stimulated samples for which storage was delayed. All osmolarity values given are corrected for this evaporative effect.

In rabbit 3 the low flow rates ranged from 0.08 to 0.11 μl/min and averaged 0.09 ± 0.01 μl/min (n = 6). This was comparable to the flow rates obtained from estimates of collected LGF volume in rabbits 1 and 2. At low flow rates, LGF osmolarity was 334 ± 4 mOsm/L (n = 19). As flow rate was increased by ACh stimulation, osmolarity of LGF decreased as follows: 329 ± 6 mOsm/L (n = 5) at flow rates from 3.6 to 4.5 μl/min, 311 ± 4 mOsm/L (n = 6) at flow rates from 6.1 to 7.5 μl/min, 310 ± 4 mOsm/L (n = 7) at flow rates from 9.2 to 11.0 μl/min, and 299 ± 2 mOsm/L (n = 7) at flow rates from 13.0 to 19.1 μl/min (Fig. 1). LGF osmolarity at the lowest flow rates was significantly higher than that at maximal flow rates (p < 0.001).

**Discussion.** Our results indicate that rabbit LGF osmolarity increases as flow rate decreases. Previous studies have shown that acinar cells of most exocrine glands, including the rat exobital lacrimal gland, secrete a primary fluid that is plasmalike with respect to electrolyte concentration and remains unchanged and apparently isotonic at all flow rates. 8, 9 In contrast, the concentration of sodium and chloride in the final fluid collected from the excretory duct of the rabbit lacrimal gland is plasmalike at high flow rates but increases to approximately four times that of plasma at low flow rates. 4 From these findings, Botelho and Martinez 4 postulated that at low flow rates, water was reabsorbed in the ducts distal to the acinus. Such a mechanism may account for the relationship between flow rate and osmolarity observed in the present study.

Botelho et al. 6 found rates of lacrimal gland fluid secretion in the unanesthetized eyes of rabbits under general anesthesia to average about 0.8 μl/min, with most of their measurements falling below 2.5 μl/min. In the current study, LGF was found to be hyperosmotic at rates in this range. We have measured the osmolarity of tear samples collected from the lateral inferior marginal tear strip of the unanesthetized eye of an awake rabbit over the course of 2 weeks. Tear film osmolarity, in contrast to LGF osmolarity, was essentially isotonic, averaging 302 ± 4 (S.D.) mOsm/L (n = 6) (unpublished data).

Consistent with the difference between the osmolarity of tears and LGF is the difference in sodium and chloride in these two fluids. Sodium and chloride remain plasmalike in concentration in tear fluid collected at all flow rates from the inferior cul-de-sac. In contrast, the concentration of these ions in LGF collected from the cannulated duct increases as flow rates decrease, exceeding their concentration in plasma. 4 We suspect that the isotonicity of rabbit tear fluid is due to transport processes across the surfaces of the conjunctiva and cornea, which alter the LGF composition. Furthermore, we cannot rule out the possibility of

<table>
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<tr>
<th>Rabbit</th>
<th>ACh dose (μg)</th>
<th>Storage delayed*</th>
<th>Stored immediately</th>
<th>Evaporative effect (mOsm/L)</th>
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<tr>
<td>Average</td>
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<td>329</td>
<td>304</td>
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*Storage was delayed between 2 min 30 sec and 3 min 15 sec, depending on filling and loading period required for low-flow-rate sample collection in each rabbit.

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### Table I. Effect of evaporation on LGF sample osmolarity

- **Rabbit**: 1, 2, 3
- **ACh dose (μg)**: 15, 15, 15
- **Storage delayed**: 304, 300, 300
- **Stored immediately**: 300, 296, 302
- **Evaporative effect (mOsm/L)**: 4, 4, 1

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8. Consistent with the difference between the osmolarity of tears and LGF is the difference in sodium and chloride in these two fluids. Sodium and chloride remain plasmalike in concentration in tear fluid collected at all flow rates from the inferior cul-de-sac. In contrast, the concentration of these ions in LGF collected from the cannulated duct increases as flow rates decrease, exceeding their concentration in plasma. 4 We suspect that the isotonicity of rabbit tear fluid is due to transport processes across the surfaces of the conjunctiva and cornea, which alter the LGF composition. Furthermore, we cannot rule out the possibility of
dilution by potentially nonhyperosmotic fluid secreted by accessory lacrimal glands.

The relationship between LGF osmolarity and flow rate in the rabbit suggests that in keratoconjunctivitis sicca, increases in LGF osmolarity, as well as tear film evaporation, may contribute to elevated tear film osmolarity.

Fig. 1. LGF osmolarity vs. flow rate. △, Data from rabbit 1; ■, data from rabbit 2; ◼, data from rabbit 3. Vertical and horizontal crossbars represent mean ± S.E.M. for LGF osmolarity and flow rate, respectively.

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**Key words**: lacrimal gland, lacrimal gland fluid, lacrimal gland fluid osmolarity, tears, keratoconjunctivitis sicca, cholinergic stimulation

**REFERENCES**


