Human Tears: Osmotic Characteristics

William J. Benjamin and Richard M. Hill

Using a freezing point depression method, requiring volumes of only 200 nanoliters, the osmolalities of 324 tear samples collected at consecutive 10-minute intervals throughout 8.5-hour periods from each of six healthy young adults were measured. The population mean was found to be 318 mOsm/kg, with a population median of 315 mOsm/kg. Individual subject means ranged from 310 to 334 mOsm/kg. Short-term oscillations were observed for all subjects, with a net positive rate toward hypertonicity for the population of 1.43 mOsm/kg·hr as the day progressed. Two subjects did demonstrate mild net rates of decrease, however. All eyes in the study were asymptomatic, suggesting a broader range of tear osmotic pressure among normals than previously suspected. Invest Ophthalmol Vis Sci 24:1624–1626, 1983

Diurnal studies of human tear osmolality have been reported previously.1 Those observations, however, were based on sample volumes of several microliters, a substantial portion of the tear fluid normally present in the eye. Such depletion carried with it the risk of reflex response during the course of collection, and dictated extended intervals between samples to assure restabilization of the tear volume and quality.

A more recent processing method has now reduced the sample volume requirement to just a small fraction of a microliter (200 nanoliters), an amount causing negligible depletion or disturbances of the tear pool, thus allowing much more frequent collection.2 Such low volume sampling already has been applied to the study of certain tear-associated disorders, such as keratoconjunctivitis sicca.3–5

The objectives here, based on a large number of nanoliter samples, were to reexamine the diurnal characteristics of tear osmolality for a population of young, healthy, asymptomatic adults.

Materials and Methods. Using a precision controlled stage, the freezing point depression values of 200-nanoliter sample volumes could be measured and their osmolalities determined to within 1% accuracy. Each tear sample was paired and simultaneously analyzed with a 290 mOsm/kg standard solution for concurrent calibration and individual sample correction, should instrument drift occur. In all, 324 tear samples and 324 parallel control volumes were processed, their measurements being made in a windowless room with stabilized conditions averaging 21 C room temperature and 58% relative humidity.

Tear samples were obtained at 10-minute intervals over an 8.5-hour period from the same eye of each of six young, healthy adults (average age: 25 years, range: 23 to 29 years) by the same examiner throughout.* With the cornea averted, the sample was collected from the midpoint of the lower tear prism, using a finely drawn glass capillary. Collection always was done within one meter of the cold stage, so that transfer time between the eye and the measurement chamber rarely exceeded 5 seconds.

Results. Figure 1 shows the osmolality distribution of all 324 tear samples from this population. The population data were mildly skewed in the hypertonic direction and were positively kurtosed as well. One subject did show a mild negative skew however.

Figure 2 illustrates the osmotic changes observed at consecutive 10-minute intervals for: top, the subject displaying the most stable (smallest standard deviation) time course; middle, the subject displaying the least stable (largest standard deviation) time course; and, bottom, the 10-minute averages for the entire subject population, over durations of 8.5 hours.

Summarized in Table 1 are the measures of central tendency, ranges, and average rates for each of the six subjects studied, and for the combined data of the population as well.

Discussion. The very small sample volume requirement of the freezing point depression method employed here provided two distinctive advantages over procedures used in earlier diurnal studies of human tear osmotic pressure: (1) adequate samples could be obtained rapidly and reliably from all subjects without risk of reflex tear contamination; and (2) long sequences of frequent sample collection were readily feasible without depleting the normal tear volume of the eye significantly.

Here, sequences of 54 tear samples were collected at consecutive 10-minute intervals from one eye of each subject over an 8.5-hour period. All subjects remained within the same confines throughout the study, so that differences apart from the environment could be observed more readily.

Although risk of sample evaporation during transfer between the eye and the analytical chamber has been shown to be negligible (about 1% of hypertonicity being induced by processing delays of up to 3.5 hours),6 all samples were processed, nevertheless, immediately. The freezing stage and sample chambers also permitted

*This study was approved by The Ohio State University Human Subjects Committee. Informed consent was obtained for the procedures used.
Fig. 1. Frequency distribution of osmolality found for 324 tear samples collected at 10-minute intervals over an 8.5-hour period from the same eye of each of six subjects.

Measurement of a known standard simultaneously with each tear sample, in effect representing a recalibration of the system as each specimen was evaluated and an immediate means of correction should drift occur in the instrumentation.

The mean tear osmolality found for all 324 samples from this population was 318 mOsm/kg, or just slightly higher than the mean of 310 mOsm/kg reported earlier for a similar series of subjects (but based on sample volumes some 15 to 25 times larger). Because of the substantial depletion of the tear pool on each collection in that earlier study, more frequent sampling (than about once per hour) was not possible, and the risk of reflex tear induction was considerably greater. Increased (reflex) tear production has indeed been demonstrated to result in a decrease in osmolality, and may be a factor in accounting for the lower mean osmolality found in that earlier study.

Substantial individuality appeared among the six subjects studied here, the range between the two most extreme subject means being 24 mOsm/kg (310 compared with 334 mOsm/kg), a difference found to be statistically significant \( P < 0.01 \).

The osmotic shifts with time among subjects were often distinctive as well. Although there was an overall

Table 1. Subject and population summaries of human tear osmolality

<table>
<thead>
<tr>
<th>Subject</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample N</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>324</td>
</tr>
<tr>
<td>Average (mOsm/kg)</td>
<td>312</td>
<td>334</td>
<td>320</td>
<td>317</td>
<td>315</td>
<td>310</td>
<td>318</td>
</tr>
<tr>
<td>Standard Deviation (mOsm/kg)</td>
<td>310</td>
<td>335</td>
<td>315</td>
<td>314</td>
<td>314</td>
<td>306</td>
<td>315</td>
</tr>
<tr>
<td>Median (mOsm/kg)</td>
<td>9.69</td>
<td>-0.13</td>
<td>0.54</td>
<td>1.07</td>
<td>0.45</td>
<td>1.70</td>
<td>0.83</td>
</tr>
<tr>
<td>Skew Coefficient</td>
<td>+3.67</td>
<td>+2.39</td>
<td>+3.56</td>
<td>+4.26</td>
<td>+2.73</td>
<td>+8.45</td>
<td>+4.31</td>
</tr>
<tr>
<td>Kurtosis Coefficient</td>
<td>261-385</td>
<td>266-397</td>
<td>231-438</td>
<td>257-446</td>
<td>268-372</td>
<td>266-420</td>
<td>231-446</td>
</tr>
<tr>
<td>Range (mOsm/kg)</td>
<td>Average rate: (mOsm/kg-hr(^{-1}))</td>
<td>-0.94</td>
<td>+2.57</td>
<td>+4.09</td>
<td>-0.58</td>
<td>+0.75</td>
<td>+2.64</td>
</tr>
</tbody>
</table>
trend during the day toward increased hypertonicity (eg, a population average of 309 mOsm/kg for the period of 8:00 to 8:50 A.M. as compared with 324 mOsm/kg for the period of 3:00 to 3:50 P.M.), two subjects (33%) among the six did show a negligible, or an even mildly hypotonic net change. Short-term instabilities among subjects were also distinctive, ranging from the low amplitude and cyclically fairly regular pattern as seen for Subject 1 (in Fig. 2), to the higher amplitude and less predictable pattern of Subject 3. While the relative standard deviation was only 7% for Subject 1, it was nearly double that value, or 13% for Subject 3.

A threshold of 312 mOsm/kg has been suggested earlier as the lower osmotic limit for keratoconjunctivitis sicca patients. Although that value is below the mean of 318 mOsm/kg found for the group of healthy, asymptomatic subjects studied here, these two values are not necessarily in conflict. Rather, it appears that there may be greater variations among normals than previously suspected, and that a few may intrude somewhat into the hypertonic zone within which all keratoconjunctivitis sicca patients are contained. As the literature indicates, some keratoconjunctivitis sicca patients may be symptomatic because of an inability to properly wet their epithelial surfaces, even though adequate aqueous tear secretion is present. Such cases might then very well show signs and symptoms characteristic of keratoconjunctivitis sicca, even with tear osmolalities as low as the 312 mOsm/kg threshold suggested previously.

**The Effect of K-582, A New Antifungal Agent, on Experimental Candida Keratitis**

Shigeaki Ohno, David J. Fuersr, Masao Okumoro, Gunther Grabner, and Gilbe't Smolin

K-582, a new basic peptide antibiotic, was tested in rabbits with experimental Candida keratitis. It was shown that the K-582-treated group showed statistically highly significant therapeutic effects on days 2 and 3, as compared with the control group (day 2: P < 0.001; day 3: P < 0.001). The culture study showed that the average number of colonies was 1,573.1 in the controls and 463.3 in the treated group, and the difference was highly significant statistically (P < 0.001). No ocular or systemic toxic effects were observed with this drug. K-582 is a promising new drug for the treatment of Candida keratitis. Invest Ophthalmol Vis Sci 24:1626-1629, 1983

Corneal infections caused by fungi generally have a poor visual prognosis. Although keratomycosis was a rare disease before 1951, it has been occurring with increasing frequency within the past three decades. In particular, Candida species and Fusarium species predominate as the causal fungi of this disease. A number of antifungal drugs have been tried in the past for the treatment of keratomycosis; however, the discouraging clinical results and the undesirable side effects limit their usefulness.

K-582 (Myroridin K) is a new basic peptide antibiotic produced by Metarhizium anisopliae strain 582M which was isolated from a soil sample collected in Sendai, Japan, by Kondo et al. K-582 is a white powder that is soluble in water and methanol. Determination of in vitro antimicrobial activity showed that K-582 is effective against Candida albicans in the concentration of 0.2 μg/ml. There have been no studies of its effect on ocular diseases.