Graefe's Arch Clin Exp Ophthalmol (1986) 224:583–586

Graefe's Archive
for Clinical and Experimental
Ophthalmology

Tear osmotic differences across the ocular surface

W.J. Benjamin* and R.M. Hill
College of Optometry, The Ohio State University, 338 West I0th Avenue, Columbus, OH 43210, USA

Abstract. We used a freezing point depression method to determine the osmolalities of 502 non-stimulated 200-nanoliter tear samples. The samples were collected hourly at 9 times on each of 6 days. Four reference (tear prism) sites on the ocular surface were used: mid-superior, mid-inferior, and the tear prisms at the medial and lateral canthi. While 256 of the samples were from a young (25 year old) male volunteer having no complaints of dry eyes, the remaining 246 samples were taken from a second male volunteer of the same age range, but who had occasional complaints of dry eyes. The mean osmolalities for all sites and all times for both subjects were found to be 315 and 331 mOsm/kg, respectively, and were significantly different ($P=0.0004$). Two of the six time-averaged intersite comparisons investigated here were found to be significantly different for each of the subjects, with the inferior tear prism and the medial canthus being the only shared pairs present. The magnitudes and patterns of these osmotic site differences were found to shift for both subjects over time, although this was more prominent and with a greater hypertonic bias for the subject with dry eye complaints. While these data do demonstrate statistically different osmolalities across the ocular surfaces of the two subjects examined in this report, these findings must be considered a preliminary view of broader population patterns yet to be studied.

Introduction

The occurrence of short- and long-term shifts of human tear osmolality based on both large (microliter, integrated) samples (Terry and Hill 1978) and small (nanoliter, site discrete) single-site samples (Benjamin and Hill 1983) have now been demonstrated. However, it is not clear whether it is reasonable to assume that all sites on the ocular surface shift in a homogenous pattern, i.e., simultaneously and over the same osmotic range with time.

Different path lengths between glandular sources and the punctal drainage, varying velocities and dwell periods of particles moving across the ocular surface and in the tear prisms, and distinctive circulation patterns traced by dye tracks are all suggestive of local osmotic non-homogeneities across the tear film over time.

* *Present address*: College of Optometry, University of Houston, 4913 Calhoun, Houston, TX 77004, USA

Offprint requests to: Richard M. Hill, Ph.D.

The purposes of our present study were: (1) to test for the presence of local tear osmotic differences (spatial non-homogeneities) across the ocular surface and (2), if present, to monitor over time the magnitudes and relative stabilities of such differences among four specific reference sites.

Materials and methods

Using a precision-controlled stage (Gilbard and Farris 1979), the freezing point depression values of 200-nl volume samples could be measured to within an accuracy of 1%. All measurements were made by the same operator in a windowless room with stabilized conditions averaging 21° C room temperature and 58% relative humidity. Sample collections were done by very fine heat-drawn glass capillaries just lightly touched to the tear film. Collection always took place within 1 m of the freezing stage, thereby keeping transfer time from the eye to the stage chamber to less than 10 s. In those few instances where a freezing depression endpoint could not be reliably identified (due, for example, to microdebris caught in the tear fluid), such samples (10 of 512) were excluded from the data base.

In all, 502 tear samples from four reference sites on the ocular surface (mid-superior tear prism; mid-inferior tear prism; medial canthus; lateral canthus) were analyzed. Collections were made from all four reference sites at each of nine waking hour times, this schedule being repeated for each subject on six different days. Just over half of these samples (256) were contributed by a young, healthy male (subject A) who had no tear-related complaints, while the balance of the samples (246) were contributed by another young healthy male (subject B) who had occasional complaints of ocular dryness.

Results

Tables 1 and 2, respectively, summarize the site-time data bases assembled for subject A who had no tear related complaints, and for subject B who had occasional complaints of ocular dryness. Tables 3 and 4 summarize for each of these subjects the tests of significant difference among the four reference sites at each of the nine sampling times. Tests among the site averages (i.e., with all hours combined for each site) are also included in these tables.

Tables 5 and 6 summarize for each subject the tests of significant difference among the hours associated with

Table 1. Subject A: tear osmolalities (mOsm/kg) at four ocular surface sites sampled hourly

Sampling site		Sampling time									Site averages
		8:00	9:00	10:00	11:00	12:00	1:00	2:00	3:00	4:00	
Lateral canthus	Mean:	316	309	314	322	295	284	296	307	318	307
	SEM:	14.1	17.7	8.2	22.2	10.8	13.5	12.6	19.4	24.1	5.4
	n:	9	8	8	6	6	7	7	6	8	65
Medial canthus	Mean:	318	304	314	282	299	299	300	337	318	308
	SEM:	18.9	12.4	11.0	11.5	9.2	16.2	12.3	12.1	16.9	4.8
	n:	9	8	9	6	6	7	8	6	8	67
Superior tear prism	Mean:	329	357	341	309	348	326	326	307	301	328
	SEM:	14.0	16.8	20.4	8.6	25.5	27.8	23.1	7.5	18.0	6.3
	n:	8	9	8	7	4	5	7	5	8	61
Inferior tear prism	Mean:	315	343	335	325	314	313	307	308	319	320
	SEM:	15.0	20.9	27.0	11.4	20.0	22.7	13.2	14.2	7.7	5.6
	n:	7	8	6	7	5	7	8	7	8	63
Hour averages	Mean:	319	329	325	310	311	304	307	315	314	315
	SEM:	7.6	9.1	8.2	7.3	8.4	9.7	7.7	7.3	8.5	2.8
	n:	33	33	31	26	21	26	30	24	32	256

SEM = standard error of the mean; *n* = number of samples contributing to the mean

Table 2. Subject B: tear osmolalities (mOsm/kg) at four ocular surface sites sampled hourly

Sampling site		Sampling time									Site averages
		8:00	9:00	10:00	11:00	12:00	1:00	2:00	3:00	4:00	
Lateral canthus	Mean:	312	327	300	300	322	314	311	347	347	317
	SEM:	15.7	14.8	8.2	9.9	19.2	16.5	12.0	25.2	22.2	5.2
	n:	9	8	10	7	7	6	8	5	6	66
Medial canthus	Mean:	314	364	323	320	343	374	306	321	339	333
	SEM:	15.5	18.5	14.0	12.5	19.2	32.9	13.3	22.7	12.4	6.3
	n:	8	8	10	7	7	6	7	6	5	64
Superior tear prism	Mean:	328	334	316	304	306	360	328	354	364	331
	SEM:	26.0	26.8	10.4	13.6	7.3	28.5	17.8	12.6	19.7	6.5
	n:	8	6	10	6	5	4	7	5	7	58
Inferior tear prism	Mean:	348	337	343	326	321	373	334	366	334	343
	SEM:	29.3	14.8	19.0	25.5	18.9	38.0	26.9	20.7	12.2	8.0
	n:	10	7	10	5	4	5	6	5	6	58
Hour averages	Mean:	326	341	323	312	324	354	319	346	347	331
	SEM:	11.4	9.2	7.2	7.3	8.9	14.9	8.5	10.5	8.7	3.3
	n:	35	29	40	25	23	21	28	21	24	246

SEM = standard error of the mean; *n* = number of samples contributing to the mean

each site, as well as for the hour averages for all four sites combined. Tables 7 and 8 give a intersubject comparisons for each site (with all hours combined) average, and for each hour (with all sites combined) average, respectively. When the tear osmotic mean of all time-site samples from subject A ($n=256$) was compared with that for subject B ($n=246$), the two were found to be significantly different ($P<0.0004$).

Discussion

The average tear osmolality of 315 mOsm/kg ($n=256$) found for our young healthy subject with no tear-related complaints was just slightly less that the 318 mOsm/kg reported previously by Benjamin and Hill (1983) for a population of six complaint-free subjects. However, the *median* value for this previously studied population was 315 mOsm/kg.

In contrast to the single site collection program used in Benjamin and Hill's (1983) study, four different reference site surrounding the cornea were sampled in our present study. The superior and inferior tear prisms of subject A were found to be relatively hypertonic, averaging 328 and 320 mOsm/kg, respectively, over all hours sampled. In contrast, the lateral and medial canthal regions were found to have somewhat lower time-averaged values (307 and 308 mOsm/kg, respectively), as seen in Table 1. The inferior prism-time-averaged value (and the only site of collection) in the 1983 study was 318 mOsm/kg and compares very closely with the inferior tear prism-time-averaged value found for our complaint-free subject (320 mOsm/kg).

As indicated by the hourly and the time-averaged values

Table 3. Subject A: simultaneous differences in tear osmolality found among four ocular surface sites when measured at various hours of the day

Time	Site 1[a]	versus	Site 2[a]	Level of significant difference (P)*
0900	STP		LC	0.065
0900	STP		MC	0.026
1100	STP		MC	0.074
1100	ITP		MC	0.021
1200	STP		LC	0.059
1200	STP		MC	0.065
1500	STP		MC	0.079
For all hours[b]	STP		LC	0.015
For all hours	ITP		LC	0.099
For all hours	STP		MC	0.015

[a] STP = superior tear prism; LC = lateral canthus; ITP = inferior tear prism; MC = medial canthus
[b] Values for all samples taken at all times (hourly from 0800 to 1600 on each of six different days) from a given site
* All other site-hour combinations for this subject were not significantly different at the $P < 0.10$ level

Table 4. Subject B: simultaneous differences in tear osmolalities found among four ocular surface sites when measured at various hours of the day

Time	Site 1[a]	versus	Site 2[a]	Level of significant difference (P)*
1000	ITP		LC	0.049
For all hours[b]	MC		LC	0.064
For all hours	STP		LC	0.069
For all hours	ITP		LC	0.007

[a] STP = superior tear prism; LC = lateral canthus; ITP = inferior tear prism; MC = medial canthus
[b] Values for all samples taken at all times (hourly from 0800 to 1600 on each of six different days) from a given site
* All other site-hour combinations for this subject were not significantly different at the $P < 0.10$ level

Table 5. Subject A: significant differences in tear osmolalities found among nine sampling times over the day when measured at a given ocular surface site

Site[a]	Time 1	versus	Time 2	Level of significant difference (P)*
LC	1000		1300	0.073
MC	0900		1500	0.091
MC	1000		1100	0.070
MC	1100		1500	0.008
MC	1200		1500	0.030
MC	1300		1500	0.099
MC	1400		1500	0.058
STP	0900		1100	0.036
STP	0900		1500	0.056
STP	0900		1600	0.038
For all sites[b]	0900		1300	0.064
For all sites	0900		1400	0.069

[a] STP = superior tear prism; LC = lateral canthus; ITP = inferior tear prism; MC = medial canthus
[b] Values for all samples taken at all sites (STP, ITP, LC, and MC) on each of six different days at a given hour
* All other site-time combinations for this subject were not significantly different at the $P < 0.10$ level

from subject A summarized in Table 3, significant differences in osmotic value occurred, and were even found to simultaneously occur between several sites. These site differences could also be seen to shift with time, being seldom maintained for more than 1 h and never for more than 2. As shown in Table 5, the tear osmolality at a given site could be found to shift significantly as well, this occurring most commonly in the medial canthus region.

In the case of subject B, patterns of significant osmotic differences were most evident when viewed on a time-averaged basis, as shown in Table 4. The lateral canthus region in this pattern was found to be most consistently different from other sites, being relatively hypotonic to the others in every instance. As shown in Table 6, each site observed in patient B, with the exception of the inferior tear prism, was found to have several significantly different osmotic values at different hours of the day. Significant shifts were seen for the whole eye as well (all sites averaged). This often occurred from hour to hour, but was found much more commonly in the earlier than in the later hours of the day.

Parallel comparisons of the time-averaged site means (far right columns of Tables 1 and 2) revealed that those for subject B were more hypertonic in every case than for patient A, and sufficiently so in two cases (the medial canthal and inferior prism) to be significantly different statistically, as shown in Table 7. Hourly means for all sites averaged were in some instances found to be significantly different as well, as shown in Table 8, being more commonly so for in the later hours of the day, however. The mean osmotic values for all sites and times for these two subjects, as indicated in both Tables 7 and 8, were found to be significantly different ($P < 0.0004$), with subject B who had occasional complaints of ocular dryness being on the average 16 mOsm/kg more hypertonic than subject A.

What then might be the underlying mechanisms of osmotic site differences across the ocular surface? The condition of the oily surface layer of the tears has been shown to affect the evaporation rate (Mishima and Maurice 1961; Iwata et al. 1969; Lemp et al. 1970), and might therefore be expected to affect the osmotic status of the aqueous layer immediately below as well. With proper lighting, nonuniformities of the tear oily layer can be observed even under common clinical conditions (McDonald 1969; McDonald and Brubaker 1971). In addition Hamano et al. (1982) have suggested a number of qualitative relationships between the oily layer appearance, and particular conditions (e.g., keratoconjunctivits sicca) based on interference microscopy. More recently, Guillon (unpublished work) has extended that technique, applying it to the construction of detailed quantitative maps of oily layer variations. Secondary osmotic effect due to such tear film variations might then be expected to be the rule rather than the exception.

Table 6. Subject B: significant differences in tear osmolalities found among nine sampling times over the day when measured at a given ocular surface site

Site[a]	Time 1	versus	Time 2	Level of significant difference (P)*
LC	1000		1500	0.042
LC	1000		1600	0.034
LC	1100		1500	0.078
LC	1100		1600	0.066
MC	0800		0900	0.055
MC	0800		1300	0.096
MC	0900		1000	0.089
MC	0900		1100	0.075
MC	0900		1400	0.027
MC	1300		1400	0.067
STP	1000		1300	0.086
STP	1000		1500	0.043
STP	1000		1600	0.033
STP	1100		1300	0.084
STP	1100		1500	0.027
STP	1100		1600	0.036
STP	1200		1300	0.079
STP	1200		1400	0.011
STP	1200		1500	0.039
For all sites[b]	0900		1000	0.075
For all sites	0900		1100	0.018
For all sites	0900		1400	0.084
For all sites	1000		1300	0.024
For all sites	1000		1500	0.044
For all sites	1000		1600	0.023
For all sites	1100		1300	0.011
For all sites	1100		1500	0.009
For all sites	1100		1600	0.003
For all sites	1200		1300	0.089
For all sites	1200		1600	0.077
For all sites	1300		1400	0.036
For all sites	1400		1500	0.050
For all sites	1400		1600	0.027

[a] STP = superior tear prism; LC = lateral canthus; ITP = inferior tear prism; MC = medial canthus

[b] Values for all samples taken at all sites (STP, ITP, LC, and MC) on each of six different days at a given hour

* All other site-time combinations for this subject were not significantly different at the $P<0.10$ level

Table 7. Subject A versus subject B: comparisons of osmolalities at the same ocular surface site[a] of each subject

Subject A site[a]	versus	Subject B site	Level of significant difference (P)*
MC		MC	0.002
ITP		ITP	0.019
All sites at all times		All sites at all times	0.0004

[a] All samples from a given site being combined (i.e. for all times from 0800 through 1600 on all six collection days)

[b] STP = superior tear prism; LC = lateral canthus; ITP = inferior tear prism; MC = medial canthus

* Neither of the other site pairs, i.e., subject A, LC vs subject B, LC or subject A, STP vs subject B, STP were found to be significantly different at the $P<0.10$ level

Table 8. Subject A versus subject B: comparisons of osmolalities at the same sampling time[a] for each subject

Subject A sampling time	versus	Subject B sampling time[b]	Level of significant difference (P)*
1300		1300	0.006
1500		1500	0.017
1600		1600	0.011
All times at all sites		All times at all sites	0.0004

[a] All samples from a given hour were combined (i.e., for all sites and all six collection days)

[b] For all nine collection times (i.e., hourly from 0800 through 1600), from all four collection sites (superior tear prism, inferior tear prism, lateral canthus and medial canthus), and for all six collection days

* None of the other hour pairs (i.e., between 0800 and 1600) for these subjects were significantly different at the $P<0.10$ level

In turn, their presence then opens a wide range of clinical inquiries for future study; for example, could such site variations be indicative of an incipient dry eye, and a basis for transient dry eye sensations; are they attendant to Bitot-like spot formations; are they sufficient to affect the site rates of absorption for topical drugs and possibly even local tissue susceptibility to organisms? Mechanically, such local osmotic differences may also underlie to some degree certain inconsistencies in the fitting and performances of hydrophilic contact lenses, particularly where local hydration levels may be critical to the orientation and movements of those devices on the eye.

Acknowledgement. This study was supported in part by a grant from The National Institutes of Health, EYO2383, to Richard M. Hill.

References

Benjamin WJ, Hill RM (1983) Human tears: osmotic characteristics. Invest Ophthalmol Vis Sci 24:1624–1626

Gilbard JP, Farris RL (1979) Tear osmolality and ocular surface disease in keratoconjunctivits sicca. Arch Ophthalmol 97:1642–1646

Hamano H, Kawabe H, Mitsunaga S (1982) Even field biodifferential interference microscope. Folia Ophthalmol Jpn 33:383–388

Iwata S, Lemp MA, Holly FJ, Dohlman CH (1969) Evaporation of water from the precorneal tear film and cornea on rabbit. Invest Ophthalmol Vis Sci 8:613–619

Lemp MA, Holly FJ, Iwata S, Dohlman CH (1970) The precorneal tear film: 1. Factors in spreading and maintaining a continuous tear film over the corneal surface. Arch Ophthalmol 83:89–94

McDonald JE (1969) Surface phenomena of the tear film. Am J Ophthalmol 67:56–64

McDonald JE, Brubaker S (1971) Meniscus induced thinning of tear films. Am J Ophthalmol 72:139–146

Mishima S, Maurice DM (1961) The oily layers of the tear film and evaporation from the corneal surface. Exp Eye Res 1:39–45

Terry JE, Hill RM (1978) Human tear osmotic pressure. Arch Ophthamol 96:84–96

Received January 9, 1986 / Accepted June 20, 1986