TEAR OSMOLARITY VARIATION IN THE DRY EYE*

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INTRODUCTION

THE MEASUREMENT OF TEAR OSMOLARITY HAS PROVEN HIGHLY RELIABLE IN CON-firming the clinical diagnosis of keratoconjunctivitis sicca (KCS) or a "dry eye."[1] The accurate performance of this measurement requires careful technique and the simultaneous use of reliable standard solutions of sodium chloride.[2] Clinical use of the test for the diagnosis and management of dry eye requies a knowledge of expected variations of the test in normal individuals as well as those who have dry eyes. Tear osmolarity measurements in patients with a clinical diagnosis of dry eye have been found to vary more widely than they do in normal patients because of differences in the tear film associated with decreased tear production or increased evaporation.[3,4]

Altered concentrations of lysozyme and lactoferrin have been detected in the tears of KCS patients.[5] Higher concentrations of serum albumin have also been found in tears collected by a technique that stimulates the conjunctival surface more than in tears collected by a no-touch technique.[6] The concentrations of proteins in basal and reflex tears appear to vary depending on whether they are released primarily from the lacrimal gland or in combination with proteins released in association with the stimulated conjunctiva.

In this study we undertook determination of the variations of tear osmolarity in KCS patients and in age- and sex-matched normal volunteers. The associated concentrations of lysozyme, lactoferrin, and serum

albumin in basal and reflex tears were also measured and correlated with the degree of severity of KCS as indicated by the measurements of tear osmolarity.

<center>METHODS</center>

Three groups of patients were studied. The initial experimental group consisted of 67 consecutive patients who had been diagnosed as having KCS on the basis of symptoms and at least one of the following clinical signs: decreased size of the inferior marginal tear strip, excessive debris in the tear film, or a viscous-appearing tear film. The control group consisted of 67 volunteers consecutively selected from a larger group of normal volunteers to match the age within 5 years and sex of individuals in the experimental group. These normal volunteers were patients coming for routine eye examinations or the first fitting of contact lenses. After stating willingness to participate in the study, each patient or volunteer signed an informed consent form prepared according to guidelines approved by the Institutional Review Board.

For 3 weeks, repeated measurements were made of tears collected from a closely controlled group of 13 normal subjects, 6 females and 7 males, all between the ages of 20 and 40 years and all nonsmokers. The subjects had no illnesses, were taking no medications, and reported that they had had at least 4 hours of sleep the night before all tests. None of the subjects wore heavy makeup and all of them were asked not to read continuously for at least 2 hours prior to testing. Nine measurements of each subject's tear osmolarity were made, three during morning visits, three during afternoon visits on the same days, and three during afternoon visits that were not preceded by morning measurements. The entire study of this group of normal subjects took place over an 8-week period.

Tear osmolarity was determined on all subjects by means of a freezing point depression technique that uses a commercially available Biological Cryostat Nanoliter Osmometer (Clifton Technical Physics, Harford, New York). Tear measurements were dne at the end of 12 hours during which no eye drops had been used. The use of any ophthalmic ointments was avoided for a minimum of 5 days prior to the tear tests. In order to minimize the risk of producing reflex tearing, the tear osmolarity test was done prior to any eye examination, including visual acuity testing, and was the initial tear test done in a series of tests that included Periotron® measurement of basal tear volume and measurement of reflex tear flow rate by means of a Schirmer filter paper test without anesthetic.

Using a referent value of 312 mOsm/l, 5 keratoconjunctivitis patients

and matching normal subjects were excluded from the initial group of 67 pairs of patients and controls because of low tear osmolarity values, false-negatives; an additional 12 normal volunteers and their matching keratoconjunctivitis patients were excluded because of high tear osmolarity values, false-positives; 1 matched pair had a false-negative and a false-positive results resulting in exclusion of 16 pairs from the original 27 pairs of consecutively gathered patients and volunteers; this left a sample of 51 patients who had clinical and laboratory evidence of KCS and 51 age- and sex-matched normal volunteers whose clinical and laboratory values confirmed the absence of KCS. The 12 male subjects in this group of 51 keratoconjunctivitis patients were age-matched consecutively with 12 females with KCS to determine any significant differences between the osmolarity of their tears. Statistical comparisons of tear osmolarity and protein data were made for only the right eyes.

In order to compare tear concentrations of lysozyme, lactoferrin, and serum albumin in normals and a more severe dry eye than indicated by a tear osmolarity of 312 mOsm/l or greater, tear osmolarity was used as an index and subgroups of patients were compiled by consecutively excluding patients with tear osmolarities values below 317, 322, 327, 332, and 337 mOsm/l.

Basal tears were collected on 2 × 6 mm filter paper strips (Periopaper®). The volume of the basal tears was measured by means of an electronic-capacitance measuring device (Periotron, Harco Electronics, Winnepeg, Canada). The volume of reflex tears was determined by comparing the wet portion of the Schirmer strip and a normogram constructed by means of a Hamilton syringe and a solution of 1% lysozyme. No more than two-thirds of the paper strip was allowed to become wet and the time necessary for this wetting was used to calculate the reflex tear flow rate. The detail of these methods has been described.[7]

The unwet portion of each Schirmer strip and the blue lacquered line, and the handling portion of the Periopaper® were cut away and discarded. The wet portion of each paper was placed in a plastic vial with a sealed top and stored at − 70°C. At a later time the tears were eluted from the filter paper strips with TIMED acetic acid buffer. The concentrations of lysozyme, lactoferrin, and serum albumin in the eluate were determined, followed by calculations to determine their concentration in the original tear sample. The immunochemical methods employing rocket electrophoresis have been described.[5] In some cases the amount of eluate was not sufficient to determine all three proteins, in which case first preference was given to lactoferrin and then serum albumin, the more stable of the three proteins. A laboratory accident resulted in loss of a

number of protein samples due to thawing.

The average and standard deviation was determined for each of the measurements. Group means were compared by means of the Student's *t*-test for significant differences. The measure of association between variables and their significance were determined by the Spearman rank correlation coefficient.

RESULTS

The original 67 consecutively recruited KCS patients diagnosed by clinical symptoms and signs had a mean tear osmolarity of 324 ± 11 mOsm/l compared to a mean tear osmolarity of 305 ± 10 mOsm/l in the consecutively selected age- and sex-matched normal volunteer controls. The Student's *t*-test demonstrated a significant difference between the two group means at the 99.9% level of confidence.

Five of the original 67 consecutively recruited KCS patients had false-negative tear osmolarity tests, which were recorded as 310, 304, 304, 303, and 307 mOsm/l, respectively. The incidence of false-negatives was 7%. Twelve false-positives were detected in consecutively selected age- and sex-matched control subjects. Values of the tear osmolarity measurement in these normal subjects were 319, 312, 319, 317, 313, 319, 338, 338, 319, 319, 319, and 317 mOsm/l, respectively. The incidence of false-positive values was 18%. Considering the total number of false-negative and false-postive results, the efficiency of the test was 88%. The incidence of false-positives in the closely controlled group of 13 normal volunteers was 14% in the morning measurements and 24% in the afternoon measurements, giving a total incidence of 19% false-positive readings in tear osmolarity tests done during the day.

There were 12 males and 39 females in the experimental group of 51 patients with KCS, diagnosed on the basis of clinical examination and laboratory testing. The individuals in this group ranged in age from 29 to 81 years and had an average age of 59 years. The median age was 62 years. The average age of subjects in the normal control group was 60 years, with an age range from 27 to 83 years. Their median age was 63 years (Table I).

Fig 1 shows the frequency of tear osmolarity values in the 51 pairs of experimental and control subjects. The false-negative and false-positive results have not been included. A bicurve distribution is suggested and indicates two distinct populations. Tear osmolarity measured 324 ± 11 mOsm/l in the KCS group compared to 302 ± 5 mOsm/l in the group of normal volunteers (Table I). There was a significant difference between

TABLE I: TEAR ANALYSIS IN KCS PATIENTS AND IN AGE- AND SEX-MATCHED NORMAL SUBJECTS USING 312 mOsm/l AS THE REFERENT VALUE

	AGE	Tosm	PLz	PLf	SLz	SLf	PAlb	SAlb
KCS								
Mean	59	324	78	171	112	228	58	176
SD	13	1	41	119	71	221	54	166
Range	29	312	11	13	8	25	0	8
	81	361	157	375	300	806	180	577
Median	62	321	65	142	94	227	49	144
n	51	51	29	24	35	36	18	27
Normals								
Mean	60	302	878	216	149	350	98	151
SD	14	5	55	150	109	189	105	136
Range	27	284	12	25	21	13	1	1
	83	311	248	586	538	772	420	455
Median	63	301	66	172	127	386	54	117
n	51	51	30	26	35	34	21	31
Student's t-test								
P	NS*	< 0.001	NS	NS	NS	NS	NS	NS

Tosm = tear osmolarity, P = basal, S = reflex, Lz = lysozyme, Lf = lactoferrin, Alb = serum albumin, SD = standard deviation, NS = not significant at 95% level of confidence.
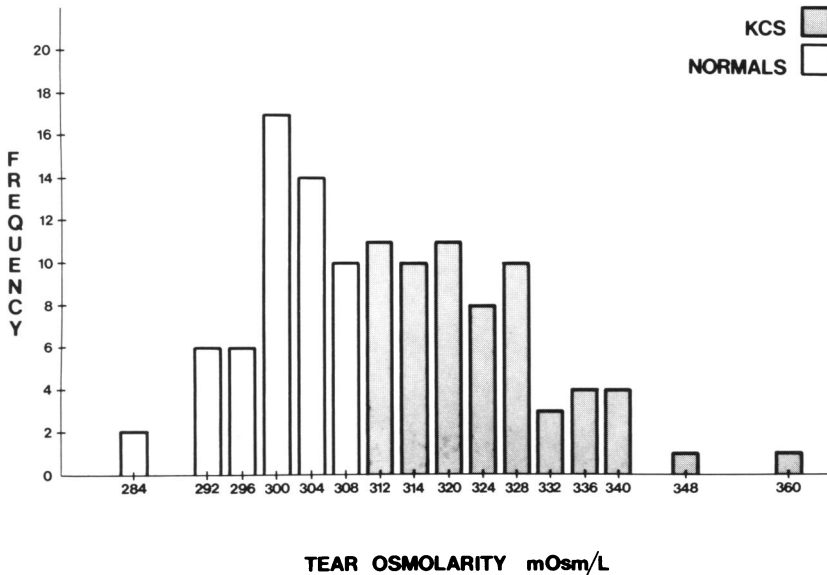
TEAR OSMOLARITY mOsm/L

FIGURE 1

Frequency of tear osmolarity values in keratoconjunctivitis patients and age- and sex-matched normal subjects, false-negative and false-positive measurements excluded.

the results obtained from the two groups at the 99.9% level of confidence.

A comparison of tear osmolarity measurements from age-matched males and females with KCS and the normal control subjects is shown in Fig 2. The mean tear osmolarity of male patients was 328 ± 13 mOsm/l; that of female patients was 323 ± 10 mOsm/l. The mean tear osmolarity of the male control subjects was 306 ± 4 mOsm/l; that of female control subjects was 301 ± 4 mOsm/l. There was a significant difference at the 98% level of confidence between the group means of the normal male and female subjects but there was not a significant difference between the group of means of the male and female KCS patients.

In the group of 13 normal volunteers, the mean osmolarity of tears from right eyes was 307 ± 6 mOsm/l; the mean of left eye measurements was 308 ± 6 mOsm/l. The mean tear osmolarity of tears from the right eyes of the originally selected 67 KCS patients was 324 ± 11 mOsm/l, and the tears from their left eyes was 326 ± 11 mOsm/l. The corresponding values for the age- and sex-matched normal were for right eyes 305 ± 10 mOsm/l and 307 ± 9 mOsm/l for the left eyes. The difference between the mean osmolarity of tears from right and left eyes was not statistically significant in the KCS patients, in their matched normal control subjects or in the 13 normal volunteers.
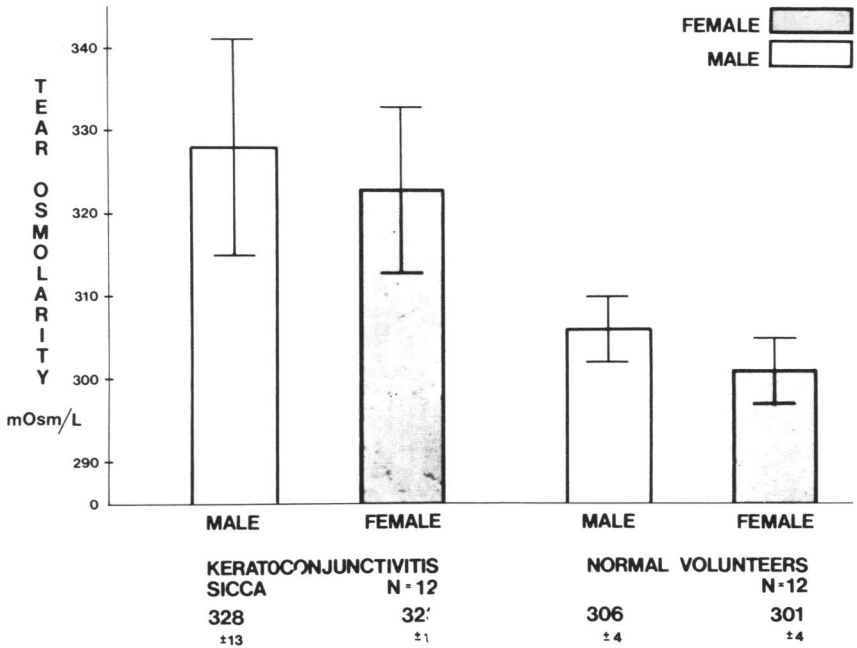
FIGURE 2

Comparison of tear osmolarity measurements in male and female keratoconjunctivitis patients and in age- and sex-matched normal volunteers.

Measurments of tear osmolarity in the group of closely controlled normal subjects revealed a 2 to 3 mOsm/l increase in the mean of afternoon measurements as compared to the morning measurements (Table II). The right and left eye values had to be combined in order to obtain a significant difference between the group means of morning and afternoon measurements. When only the first morning visits of these 13 subjects were compared, there was only a 2 mOsm/l difference in the mean tear osmolarities. This difference was not significant at the 95% level of confidence. When the three morning measurements were combined and compared to three combined afternoon measurements, there was a 3 mOsm/l difference, which was significant at the 99% level of confidence. There was no significant difference between the mean of the three afternoon measurements for each subject that were not preceded by a morning measurement and the mean of three afternoon measurements that followed morning determinations.

The group means and variations in tear osmolarity of the experimental and control groups are shown in Table I. The group means and variations

in the tear concentrations of lysozyme, lactoferrin, and serum albumin are shown in Fig 3. There was no significant difference between the experimental and control groups with respect to the mean concentrations of lysozyme, lactoferrin, and serum albumin in either their basal or reflex tears. However, comparison of these mean values reveal differences that indicate trends of change. The mean basal tear concentration of lactoferrin in the KCS patients, 171 mg/dl, appears to be depressed as compared

TABLE II: A COMPARISON OF MORNING AND AFTERNOON MEASUREMENTS OF TEAR OSMOLARITY IN 13 NORMAL VOLUNTEERS

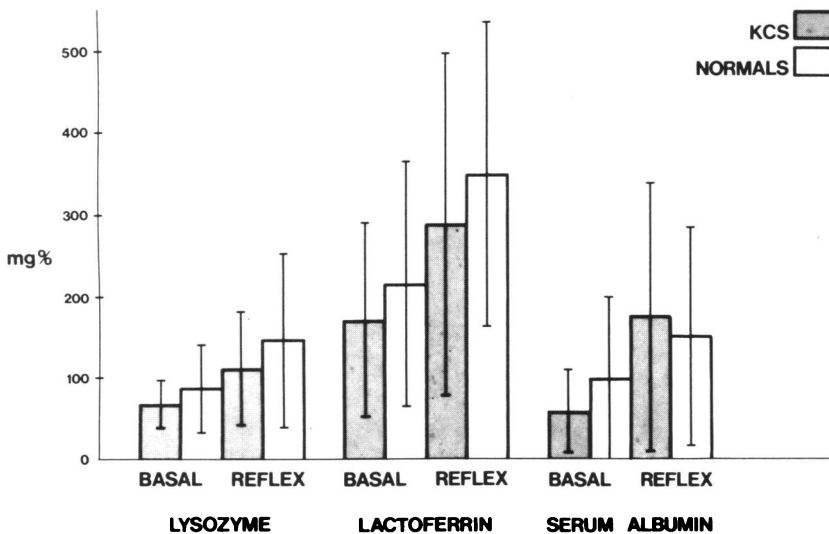|	FIRST MORNING MEASUREMENT (mg/dl) RIGHT EYES (n = 13)		THREE MORNING AND AFTERNOON MEASUREMENTS (mg/dl) RIGHT AND LEFT EYES (n = 78)	
	AM	PM	AM	PM
Mean	306	308	305	308
Standard error	7	6	6	6
Minimum	295	297	295	297
Maximum	316	320	322	324
Range	21	23	27	23
Median	307	309	305	318
t-test	NS*		$P < 0.01$	

*Not significant.

FIGURE 3
Tear protein concentrations in KCS patients and in age- and sex-matched normal volunteers.

to the mean of 216 mg/dl in the basal tears of the normal subjects. The concentration of lactoferrin in the reflex tears of the KCS patients, 288 mg/dl was less than the mean of 350 mg/dl in the reflex tears of the control subjects. However, the Student's *t*-test did not demonstrate statistically significant differences. There were considerable variations in the concentrations of the tear proteins measured in both groups. For example, in the control subjects, the lysozyme concentration in basal tears ranged from 12 to 248 mg/dl, and the serum albumin concentrations in reflex tears varied from the minimally detectable concentration of 1 to 455 mg/dl. In the KCS patient group, the corresponding values ranged from 11 to 157 mg/dl and 8 to 577 mg/dl, respectively.

The mean concentration of lysozyme, lactoferrin, and serum albumin in basal tears compared to reflex tears was significantly different only in the case of lactoferrin in KCS patients ($P < 0.01$). No significant difference between the group mean concentration in basal and reflex tears was detected in comparisons made of lysozyme or serum albumin tear concentrations in normal and KCS patients or in a comparison of lactoferrin concentration in basal and reflex tears in normals.

The change in tear protein concentration in reflex tears compared to basal tears in KCS patients and age- and sex-matched normal volunteers on an individual basis rather than group means is shown in Table III. No statistically significant differences were present in a comparison of KCS patients and age- and sex-matched normals although inspection of the group means indicate a trend towards a greater increase of lysozyme and lactoferrin concentration with reflex tearing in normals, 113 times and 45 times, compared to KCS patients which were only 2 times with lysozyme and 3 times with lactoferrin. The greater differebce between the two patient groups was the increase in lysozyme concentration from basal to reflex tears, a 111 times rise with reflex tearing compared to only a 15 times rise with lactoferrin concentration from basal to reflex tears. The group means of serum albumin concentrations in tears shift in an opposite direction when comparing the change of group means between basal and reflex tears in KCS patients and normals, 9.4 times basal tear concentration *vs* 0.12 times, respectively. The range of response in individual patients includes decrease as well as increase in tear protein concentrations but an increase predominated in lysozyme and lactoferrin concentration comparing reflex and basal tears. An inadequate number of measurements were available to indicate a trend in the case of serum albumin concentrations.

When the referent or cutoff value for tear osmolarity was increased from 312 mOsm/l to 317, 322, and 327 mOsm/l and the mean concentra-

TABLE III: CHANGE IN TEAR PROTEIN CONCENTRATIONS
ASSOCIATED WITH REFLEX TEARING IN KCS PATIENTS AND
AGE- AND SEX-MATCHED NORMAL VOLUNTEERS

	KCS	NORMAL VOL- UNTEERS
Lysozyme		
Mean	× 1.91	× 112.86
Range	×(−) 0.86–16.91	×(−) 511–423
in/dcr/nc	17/4/3	14/7/1
n	24	22
Lactoferrin		
Mean	× 3.13	× 45.27
Range	×(−) 0.60–28.60	×(−) 153–356
in/dcr/nc	16/0/5	6/4/1
n	21	11
Serum albumin		
Mean	× 9.40	× 0.12
Range	×(−) 0.80–53	×(−) 8.92–0.65
in/dcr/nc	3/0/6	6/0/5
n	9	11

× = times basal tear concentration; (−) = decrease;
concentration change: in = increase, dcr = decrease,
nc = no change; n = number of matching basal and
reflex tear determinations.

tions of tear lysozyme, lactoferrin, and serum albumin in the resulting
groups of keratoconjunctivitis patients and control subjects were com-
pared, there were statistically significant differences between the group
means of basal and reflex lactoferrin. Specifically the mean lactoferrin
concentration in the basal tears of the KCS patients was significantly
lower than the mean in basal tears of normal volunteers at the 95% level
of confidence when the tear osmolarity referent value for the diagnosis of
KCS 317 mOsm/l, and at the 99% level of confidence when the tear
osmolarity referent value was 322 and 327 mOsm/l. Significant differences
in the group means of reflex tear lactoferrin was present when the tear
osmolarity referent value was 317, 322, 327, 332, and 337 mOsm/l. In-
creasing the referent value of tear osmolarity in the diagnosis of KCS did
not produce significant differences in a comparison of mean tear protein
concentrations of lysozyme or serum albumin in basal or reflex tears in
KCS patients and normals.

Fig 4 compares the scatter plots of tear osmolarity and the concentra-
tions of lysozyme, lactoferrin, and serum albumin in basal and reflex tears
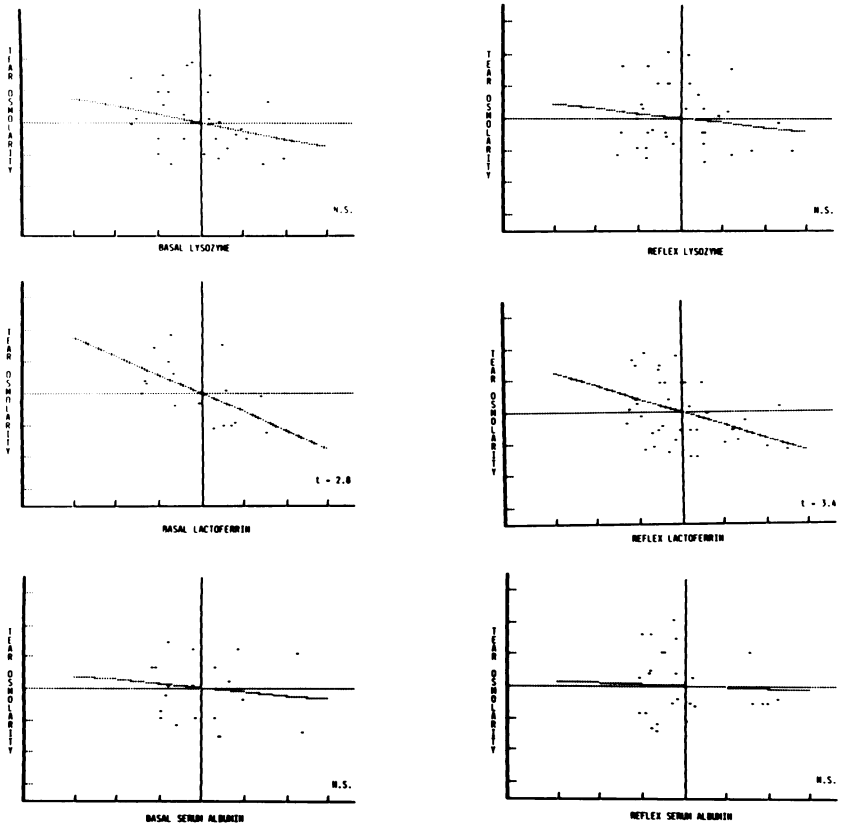
FIGURE 4

Correlation of tear osmolarity and tear protein concentrations in KCS patients. Abbreviations are as in Table I.

of KCS patients. A significant correlation was found in a comparison of tear osmolarity and basal tear lactoferrin. An increase in tear osmolarity was associated with a decrease in lactoferrin concentration. No correlation was found between tear osmolarity and lysozyme or serum albumin concentrations in basal or reflex tears.

DISCUSSION

There is a predominance of females in the general population of KCS patients. This fact as well as the previous finding of higher tear osmolarity in normal females over the age of 40 years than in those under the age of

41 years[7] indicated the need for the use of age- and sex-matched control subjects in a study of tear osmolarity variation. There was no significant difference between the mean osmolarity of tears from the males and females who had KCS. However, the mean tear osmolarity of the normal male subjects was significantly higher than that of the females (Fig 2). These findings are in contrast to those of a previous studies that demonstrated no significant difference between the group mean tear osmolarity values of normal males and females who were not age matched.[7] The advantage of age adjustment in this study was offset by the fact that only a group of 12 normal subjects was included, as compared to 21 males and 85 females in the previous study. In our carefully controlled group of seven male and six female normal volunteers, no significant gender-related difference was found between the group mean tear osmolarity values. Therefore, the weight of evidence appears to support the conclusion that there is no significant difference between the tear osmolarity of normal males and females.

Terry and Hill[8] found no significant difference between tear osmotic pressure of normal men and women in either the open-eye or closed-eye condition. They collected "nonreflex" tear samples from six subjects every hour throughout waking hours. A 5-μl sample was required for the micro-osmometer technique used to determine the osmolarity of the samples, collected in capillary tubes. The mean tear osmolarity during waking was 310 mOsm/kg with a range of 299 to 323 mOsm/kg. Holly and Lamberts[9] measured the tear osmolarity of six normal subjects in a study of nonisotonic solutions. The method of determining tear osmolality required 5 μl samples collected from the inferior fornix over 3 to 20 seconds. Average tear osmolality for the group was 294 ± 8 mOsm/l.

These studies differ from the present study primarily in that relatively large tear samples were collected over longer times than in our study, giving the opportunity for greater contamination of samples by reflex tearing. In the present study, only 100 to 150 nl samples were collected from the inferior marginal tear strip. The collections were done within 2 to 3 seconds, using a hand-drawn microcapillary tube, under the magnification of a slit lamp using only overhead illumination. Since reflex tearing lowers tear tonicity, higher readings than those obtained in the present study are difficult to explain except on the basis of differences in tear-sample collection and handling which may have allowed sample evaporation or solute contamination. Tear samples from the lower fornix have been found to be less hypertonic than tears collected from the interpalpebral fissure.[10]

A more recent study by Benjamin and Hill[11] used a freezing point

method that required only 200 nl of tears. However, 324 samples were collected at 10-minute intervals throughout 8.5 hours during the day. This raises concern about the stimulation of reflex tearing by repeated samplinig. A broad range of tear osmotic pressures were detected, with a mean of 318 mOsm/kg and a median of 315 mOsm/kg. The means of individual normal subjects ranged from 310 to 334 mOsm/kg, with an increase in hypertonicity of 1.43 mOsm/kg as the day progressed. The collection of five to six samples each hour for 8.5 hours seems to produce more reflex tearing than occurs when the subject is at rest.

The time of day that a tear osmolarity test is done also appears to influence the tear osmolarity measurements. One would expect afternoon measurements to be 2 to 3 mOsm/l higher than morning measurements even in normal individuals with normal tear values due to greater opportunity for evaporation from the tear film with an increasing number of waking hours. A lower incidence of false-positive results was evident in the morning measurements of tear osmolarity in the carefully controlled normal volunteers. This significant difference between morning and afternoon tear osmolarity measurements seen in normals is least likely to occur in a keratoconjunctivities population because of the wider variation of measurements. However, KCS patients would be expected to have an even greater difference between their morning and afternoon measurements of tear osmolarity because of their decrease tear volume.[10] The standard error of the mean in normals was 6 to 7 mOsm/l whereas in keratoconjunctivitis patient it was 11 mOsm/l.

Gilbard and Kenyon[12] used the tear osmolarity determination method used in this study to evaluate tear diluents in the treatment of KCS. They found that 90.2% of 41 tear osmolarity measurements from 20 KCS patients were greater than 311 mOsm/l. A 150 mOsm/l solution concentration was found to be superior to more hypertonic and more hypotonic solutions for the relief of symptoms.

Nelson and Wright[13] used the nanoliter micro-osmometer used in this study and found a mean osmolarity of 329 ± 30 mOsm/l in eyes with KCS as compared to 301 ± 7 mOsm/l in normal eyes. They pointed out that the avoidance of reflex tearing is the most critical factor and reported that samples may be stored under oil for as long as 7 days with less than a 0.6% increase in osmolarity.

Previous studies have shown impressive differences in the tear osmolarity measurements of the right and left eyes of KCS patients.[3] In this study no significant differences were observed in the group means of tear osmolarity in the right and left eyes of either the clinically diagnosed KCS patients or the control subjects. Individual patients and normal volun-

teers did sometimes have larger differences between the right and left eye, but the large number of patients and the range of measurements in the group did not permit demonstration of statistical significance between the group means.

Elevated lactoferrin concentrations in basal and reflex tears correlated significantly with the severity of KCS as determined by increased tear osmolarity (Fig 4). As the eye becomes more dry, the concentration of this antibacterial protein decreases. Previous studies of lactoferrin in basal and reflex tears of KCS patients and normal subjects showed in group comparisons no significant increase in lactoferrin in the reflex tears of patients with keratoconjunctivitis, although there was such an increase in the reflex tears of normal subjects.[5] These findings were confirmed in this study, which demonstrated only a significant increase in the group mean lactoferrin concentration in normals when reflex tears were compared to basal tears. No difference in the group mean lactoferrin concentration in basal and reflex tears of KCS patients was observed and no significant difference in the group mean of basal and reflex tears in keratoconjunctivitis patients or normals were noted with lysozyme or serum albumin.

An additional feature of this study was the examination of individual changes in tear protein concentration with reflex tearing (Table III). Most impressive was the range of changes in protein concentrations that occurred with reflex tearing. For example, the changes in concentrations of lactoferrin with reflex tearing ranged from a decrease of 0.6 times to an increase of 28.6 times in different patients with KCS. The lysozyme concentration in normal subjects varied dramatically from a decrease of 511 times to an increase of 423 times. Although the technical limitations of diluting 1 μl samples 50 to 75 times can account for much of this variation, the range of individual variations in the response of tear protein concentrations to reflex tearing is noteworthy, and indicates a potential method of sorting patients with regard to their responses to eye diseases, the environment, and contact lens wear. The mean concentrations of lysozyme and lactoferrin, increased the most in reflex tears, 113 and 45 times, respectively; the mean serum albumin increased only 0.12 times.

Decreased concentrations of proteins in reflex tears occurred in fewer subjects. A majority of subjects had increased concentrations of the anti-bacterial proteins lysozyme and lactoferrin, as has also been shown in previous studies.[5] The number of subjects was too small too allow a conclusion regarding the serum albumin in tears but, more subjects with keratoconjunctivitis had increased concentrations of serum albumin in the reflex tears than did normal subjects, approximately equal numbers of whom had decreased and increased concentrations of serum albumin in

tears with reflex tearing.

In addition, a trend was evident in comparisons of the group means, which, although not statistically significant, indicated that lysozyme, lactoferrin, and serum albumin are present in lower concentrations in the basal tears of keratoconjunctivitis patients than in the basal tears of age- and sex-matched normal subjects (Fig 3). This difference is more evident in the reflex tear concentrations of lysozyme and lactoferrin, but develops in the opposite direction with reflex tear serum albumin. A dilutional effect appears to take place in the serum albumin concentration during reflex tearing.

A final comment is indicated regarding the use of osmolarity and osmolality in describing the measurement of tear tonicity. Colleagues have explained good reasons for using a weight-related unit of measurement, mOsm/kg and the word "osmolality" which reflects osmotic concentration rather than "osmolarity" which denotes solute weight in aqueous solution. Dr Saichi Mishima introduced the use of the word osmolarity to me during our first research on tear film tonicity. Since then, I and many of my colleagues have persisted in the use of the word. I have discussed changing to the word osmolality, but in the dilute solutions with nearly complete dissociation commonly encountered by physiologists, there appears to be no qualitative difference between the two words. As long as we know what we are describing, perhaps the efforts to make an issue of nomenclature in such a simple matter is not warranted.

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DISCUSSION

DR RICHARD O. SCHULTZ. In a previous publication on diagnostic tests for kerato-conjunctivitis sicca, Doctor Farris states that the Schirmer's test without anesthetic and Rose-Bengal staining were the most specific tests (100%), but suffered from low sensitivity (10% and 58%, respectively). The most sensitive tests were believed to be an increase in lactoferrin concentration from basal to reflex tears or percent increase in lactoferrin (96%) and tear osmolarity (76%).

The authors have used the following definitions of specificity and sensitivity. Specificity is the incidence of true negative results in patients without disease and sensitivity is the incidence of true positive results in patients with a disease. It should follow from this information that a significant decreased percentage increase in lactoferrin can be demonstrated in 96% of patients with KCS; and 76% of such patients should show an increase in tear osmolarity.

The current paper stresses that clinical use of tear osmolarity in the diagnosis and management of dry eye patients requires a knowledge of expected variations of the test in normal individuals as well as patients with dry eyes—and, indeed, the title of this paper is "Tear Osmolarity Variation in the Dry Eye."

What have the authors shown us and what have they concluded form their data. First, there are, indeed, variations in tear osmolarity: The salt and protein concentration in tears is higher in normal males compared to normal females, however, there is no difference between age-matched males and females in the KCS group. There is no difference in tear osmolarity between right and left eyes in KCS patients if one looks at averages of 67 patients, ie, group means; however, individual patients were observed to have large differences between right and left eyes. In normal patients, there was no difference between right and left eyes, either in individuals or group means. There are diurnal variations or differences in the osmolarity of tears in normal patients comparing the morning with afternoon and evening.

These are important baseline data and the authors are to be complimented for providing this information. Another point of significance in this paper is that the authors are not stating that ocular surface disease is due to increased osmolarity of tears, but are attempting to correlate tear osmolarity and lactoferrin content with other signs and symptoms of the dry eye syndrome.

One has to look critically, however, at some of the stated correlations. First, there is an assumption based on previous work that tear osmolarity greater than 312 mOsm/l correlates wih KCS. Using 312 mOsm/l as a baseline, 5 KCS patients and 5 normal patients were excluded as false-negatives and 12 normals and 12

KCS patients were excluded because of high values and termed false-positives.

If these data were included, ie, in Table I, the mean values for tear osmolarity would not be significantly different comparing KCS patients to age-matched normals. I think the validity of excluding these data has to be questioned. If one looks at the previous publication from which the 312 figure is derived, it appears that the cutoff point of 312 mOsm/l was obtained by discarding data beyond 2 standard deviations. I do not believe this is a valid procedure when there is bimodal distribution of data such as illustrated in Fig 1. In other words, one cannot discard data beyond 2 standard deviations of the other curve. Further-more, if one looks at Fig 1 of the 1983 publication in CLAO, the significance of 312 mOsm/l is apparently based on the observation that KCS patients have values over that number, but most normals have values less than 312. This ignores the fact, however, that many of the tear osmolarity values in KCS patients which are shown by the black circles fall within the same range that is used to define the normal pupulation. In addition, if the variation on either side of the norm is so great that interpretation of values for any given patient has no meaning, then it is not clear how this test can be used for clinical purposes.

The current paper also presents data on various proteins found in tears empha-sizing differences in concentration of the antibacterial protein lactoferrin. I found some difficulty in trying to relate lactoferrin to the presence of keratitis sicca. In essence, no significant differences were found between KCS patients and nor-mals, and the range of values was enormous. I think the basic reason for this wide range of values relates to methodology. A 50 to 75 times dilution of these tiny samples of tears allows too much room for error. Furthermore, storing the strip at − 70°C can cause dehydration or "freezer burn" thus decreasing the volume of water in the strip. Similarly, there may be artificial absorption of water as the strip warms to room temperature after being removed.

In any event, the implied conclusion regarding the value of lactoferrin assay is not warranted based on data presented. The authors state in the text of this paper that basal tear concentration of lactoferrin appears depressed in sicca patients and that reflex lactoferrin also appears less, but the differences are not statistically significant. They did, however, find a significant correlation in comparison of tear osmolarity with tear lactoferrin and concluded, therefore, that concentrations of lysozyme, lactoferrin, and serum albumin in basal and reflex tears correlate with varying severity of KCS as indicated by measurements of tear osmolarity.

This suggests circular reasoning and perhaps Doctor Farris could comment further on this point. In my opinion, the data presented do not establish either a clinical or diagnostic relationship between the disease keratoconjunctivitis sicca and the concentration of various proteins in tears.

In conclusion, the authors have provided us with some interesting baseline data regarding variations in the salt and protein content of tears. They have also made us aware of the wide range of test results in both the normal and KCS patient populations. Considering the wide range of values, the technical complexity of these assays and the fact that most of these data show trends rather than signifi-cance, I believe it is premature to recommend these tests for the diagnosis and

management of patients with a dry eye.

DR DAVID G. COGAN. I would like to point out the difficulty in determining the osmolality of that portion of the tear film overlying the cornea. Doctor Farris properly pointed out the significant effect of evaporation. We all know how important this is with prolonged occlusion of an eye that has incipient epithelial edema. I had occasion to estimate the expected evaporation of an artificial tear film on an excised cornea by weight analysis and found that about half the volume was lost in a time period between average blinks. In other words, the osmolality doubled progressively in an interblink period. Doctor Kinsey and I thought that it would be impossible to obtain a meaningful measurement with the techniques then available and we gave up further testing.

DR R. LINSY FARRIS. Thank you very much for these good questions. If time permitted, I believe that we could have a seminar devoted solely to the develop-ment and use of diagnostic tests. It appears worthwhile to emphasize from the beginning of my closing remarks that we are attempting to analyze an extremely kinetic tearing system. Each approach in an effort to analyze the system produces change, in a way similar to salivation when one talks about lemons. Perhaps the greatest importance of our study is the measurement of tear osmolarity which permits obtaining a resting state sample of tears very quickly thereby decreasing the opportunity for contamination by reflex tears. We are also measuring tear proteins but thus far have detected no significant differences between the group means of normal and age- and sex-matched KCS patients in this study.

To answer specifically the questions which have been raised, I would reply to Doctor Cogan that since the procurement of a tear sample from the surface of the cornea has not been possible, one may obtain some indirect evidence by viewing the surface epithelium with specular microscopy. Such studies combined with tear osmolarity determinations of the inferior marginal tear strip would possibly develop correlations which would permit indirect measurement of tear film hy-pertonicity on the central cornea. In reply to your comment regarding the short-term effect of hypotonic artificial tear therapy, we have determined that the advantage produced by a one-half isotonic saline compared to isotonic saline is dissipated in 20 minutes. But we have no way of determining what is going on inside the cell. It would seem that the intracellular effect of a hypotonic artificial tear solution would persist longer than this extracellular effect.

In reply to Doctor Shultz' comment about the distribution of the data, I would repeat the plea from one of my references, *Beyond Normality* by Galen and Gambino, in which we are reminded that Gauss' law of errors from which the concept of a bell-shaped curve distribution comes, refers to repeated measure-ments on the same object. There is no reason to believe that a group of normal subjects or different subjects with a disease should have such a distribution of values. In addition, a normal range which encompasses 95% of the test values derived from a normal group of subjects tells us nothing about morbidity or mortality in a group of patients with a disease. The selection of a referent or cutoff

value is done according to the test values obtained in normal subjects and patients with different diseases, and according to the nature of the disease and the clinicians' inclinations. I do not have difficulty diagnosing the severe dry eye with the slit lamp but I do have difficulty with the diagnosis of dry eye in patients with complaints that may represent a mild dry eye producing minimal and variable signs on slit lamp examination. The tear osmolarity test has provided a means to obtain additional information about the tear film. The cutoff or referent value has been set at a level which gives greatest efficiency and favors sensitivity only slightly more than specificity. I have used 312 mOsm/l because I am particularly interested in detecting mild cases even at the risk of overdiagnosis. The penalty of overdiagnosis is not great since artificial tear therapy does not carry great risks. The simultaneous study of tear osmolarity and tear proteins reported in this study indicate 317 mOsm/l would relate the test more closely to lactoferrin, an antibacterial protein in tears which is more stable than lysozyme and exerts its antibacterial effect by binding the iron that bacteria require for their metabolism.

Finally, I would like to tell you how much I have appreciated the opportunity provided by Doctor Schultz to review his commens before the presentation of my paper. This provided an opportunity for me to gather and report some overlooked mean values as well as emphasize some important concerns of the research. This is my first AOS meeting and I am certainly looking forward to such helpful attitudes in future meetings. I want to thank my discussants and the program committee for the opportunity to present this paper.