Assessment of Tear Film Dynamics: Quantification Approach

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ABSTRACT **The dynamics of the tear film are reviewed with specific reference to the biophysical aspects: distribution, turnover and elimination through evaporation, drainage, and absorption. The review concentrates on quantitative assessments and is confined to aspects of the dynamics that can be fully and directly measured. The techniques of fluorophotometry, fluorescein clearance, lacrimal scintigraphy, evaporimetry and osmometry are described. Reports in the literature for values of tear turnover (flow), evaporation and osmolarity for normal and dry eyes are collated. Indices of tear film dynamics based on these measurements, including tear function index, total tear flow, and osmolarity, are discussed in relation to their potential in the differential diagnosis of dry eye and new referent values for the disease suggested. The limitations of derivation and application of these indices are discussed.**

KEYWORDS **absorption, distribution, evaporation, osmolarity, tear dynamics, tear indices, tear turnover**

I. INTRODUCTION

A complete tear film is essential for the health and function of the eye. Normal tear film dynamics require adequate production of tears, retention on the ocular surface, and balanced elimination. Disruption of any of these components can lead to the condition of dry eye. This review will focus on the biophysical aspects of tears. The approach will be essentially quantitative and will concentrate on the aspects of tear film dynamics that can be most fully and directly measured; therefore, it will consider the distribution, turnover (and drainage), evaporation, and absorption of tears (Figure 1). It will address laboratory techniques for the assessment of the tear film rather than the more conventional clinical approaches found in much of the literature. In addition, the dynamic balance of these elements will be considered through a single parameter—that of tear film osmolarity. Consideration will be given to this and other indices of tear film dynamics. The indices will be used in an attempt to define dynamics in the normal healthy eye and differentiate them from those in the dry eye.

II. TEAR DISTRIBUTION

Tears are produced principally by the lacrimal gland under the influence of the parasympathetic and sympathetic nerves.[1] Traditional methods of measuring tear production are based on absorption of tears[2] by Schirmer strips[3] or cotton threads.[4] Both tests are poor quantifiers of tear production; the Schirmer test is marred by low specificity and sensitivity,[2,5] and the exact parameter measured with the cotton thread test has been questioned.[6] Although such tests have clinical utility, particularly in the diagnosis of aqueous deficienct dry eye from the normal,[7] they offer only an indirect measure of tear production[8] and are of limited use in the quantification of tear dynamics.

The distribution of tear fluid on the ocular surface is dependent on the lid blink.[9] Lid closure on blinking proceeds from the temporal to the nasal side of the eye, spreading tears across the ocular surface and facilitating drainage through the lacrimal puncta.[10] The interblink time in normal individuals averages 4 ± 2 secs and is significantly decreased in patients with dry eye (1.5 ± 0.9 secs),[9] the blink rate being increased in dry eye patients to maximize the tear supply to the ocular surface.[11] In concentrated, close reading tasks, the blink rate drops under relaxed conditions by about one half (from 22.4 ± 8.9/mins to 10.5 ± 6.5/mins).[12]

The distribution of tear film can be observed dynamically with use of thin film interferometry. This technique, originated by Doane,[13,14] allows observation of the in vivo tear film through the application of the principle of thin film interferometry. Interference fringes are produced by light reflected at the air-lipid and at the lipid-aqueous

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Abbreviations are printed in **boldface** where they first appear with their definitions.

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OUTLINE

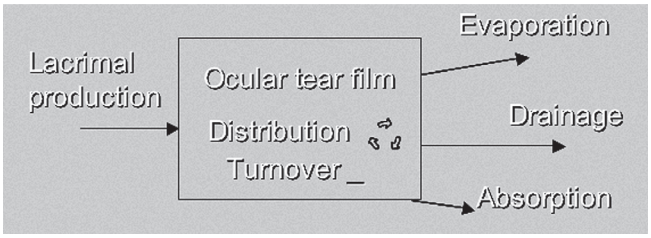

Figure 1. Diagrammatic representation of the input and output components of the tear system.

boundaries of the tear film, due to the changes in refractive index. Specular reflection from the lipid layer precludes a clear view of the aqueous layer of the precorneal tear film, although where the lipid layer is very thin or absent, fringes can be observed from the aqueous phase.[15]

Lipid layer interferometry was developed through the work of McDonald,[16] Hamano et al,[17] Norn,[18] and Guillon.[19] A dynamic interferometry system was first described by Doane.[13] A number of clinical instruments have been developed based on this optical principle, including the Tearscope described by Guillon and Guillon,[20] and instruments developed by Doane[13] and by Goto and Tseng.[21,22] A number of qualitative grading systems for the tear film have been proposed for these instruments.[20,23,24] These are useful for looking at structure of the tear film, and they offer some insight into its stability.[24] Significant differences in appearance (and grade) have been observed in dry eye conditions, with the partial or complete absence of the lipid layer being a feature (Figures 2 and 3).[25]

The development of a quantitative approach to the analysis of interferometric images from the tear film of normal and dry eye patients owes much to the work of Goto and Tseng.[22] Goto and Tseng, with the use a kinetic analysis of sequential interference images, were able to record the lipid spread time of tears in normal and dry eye patients. This spread time, defined by the interval necessary for the lipid film to reach a stable interference image, was 2.17 ± 1.09 secs in the aqueous tear deficiency state, significantly slower than that recorded for normal eyes (0.36 ± 0.22 secs [Figures 2 and 3]).[21,22] Because of this slower spread time, the resultant lipid film was found to be thicker on the inferior cornea than on the superior cornea,[22] the thickness being measured from a "look-up" simulated color chart obtained from the reflectance of thin film interference generated by a white light source.[26] Tear film particle movement, as an indicator of the time necessary to obtain stability of the tear film after the blink, had previously been used by Owens and Philips.[27] Almost 90% of Goto and Tseng's patients with aqueous tear deficiency showed vertical streaking, rather than a normal horizontal propagation of the interferometric pattern on the superior cornea.[22] Owens and Philips measured the displacement of tear film particles just after a blink and found the time necessary to reach zero velocity (tear stabilization time) to be 1.05 ± 0.3 secs.[27] The observed particles were thought to be accumulations of newly secreted lipid from the meibomian glands.

A commercial thin film interferometer (DR-1, Kowa Co Ltd, Japan) was developed by Yokoi and Komuro.[28] In this apparatus, the specular reflection from the tear surface is imaged with a video camera, observed on a TV monitor, and recorded digitally. Yokoi et al have developed a classification system for grading interference pat-

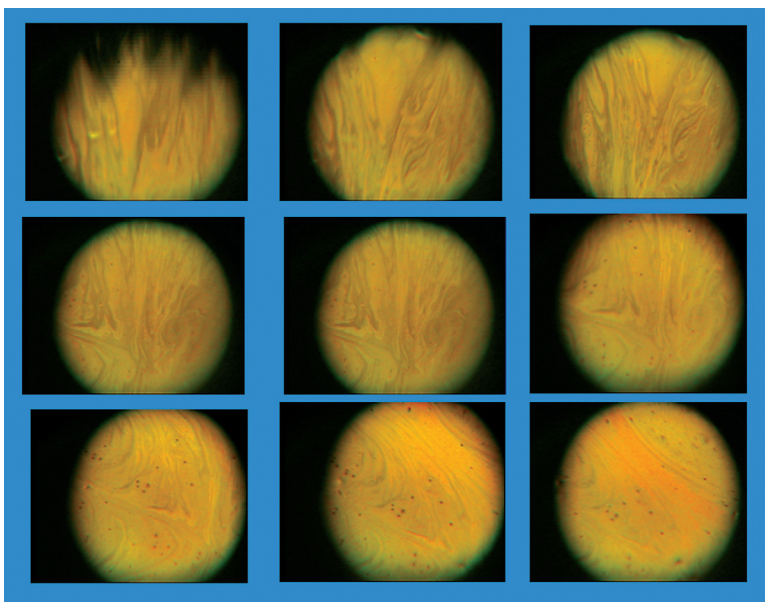

Figure 2. Series of thin film interferometry images obtained by the dynamic technique of Doane[13] from a normal asymptomatic subject. The images are obtained at 1 sec intervals, following a blink. The lipid layer of the normal tear film reaches a relatively stable pattern within the first second after the blink (consistent with the lipid spread time measurements of Goto and Tseng[22]). This pattern is then stable for about 6 secs.

terns observed from the tear film[23]; their research has shown that thicker lipid layers are associated with greater tear film stability.[28] Grading systems have also been developed by Guillon and Guillon,[20] by Mathers et al,[29] by Forst,[30] and by Thai et al.[24] Most grading systems assess the uniformity of the interference fringe pattern; the change in color and loss of uniformity in distribution indicate tear film instability. Such patterns are found more commonly in dry eyes,[28,30] which show reduced break-up times, a thinner lipid layer, and reduced stability.

Assessment of the reflected images from the cornea and tear film have been used to assess tear film quality and stabilization following the blink. Nemeth et al employed a high-speed videotopographic technique to assess the regularity indices (surface regularity index [**SRI**]), and (surface asymmetry index [**SAI**]) in the time interval following a blink.[31] They observed that the SRI and SAI decreased in the first few seconds after a blink, which implied that, after eyelid opening, it took the tear film some time to build up and reach its highest regularity and optical quality. In healthy eyes, tear film build-up time averaged 5-7 secs after the blink. A similar measurement was observed in dry eye patients.

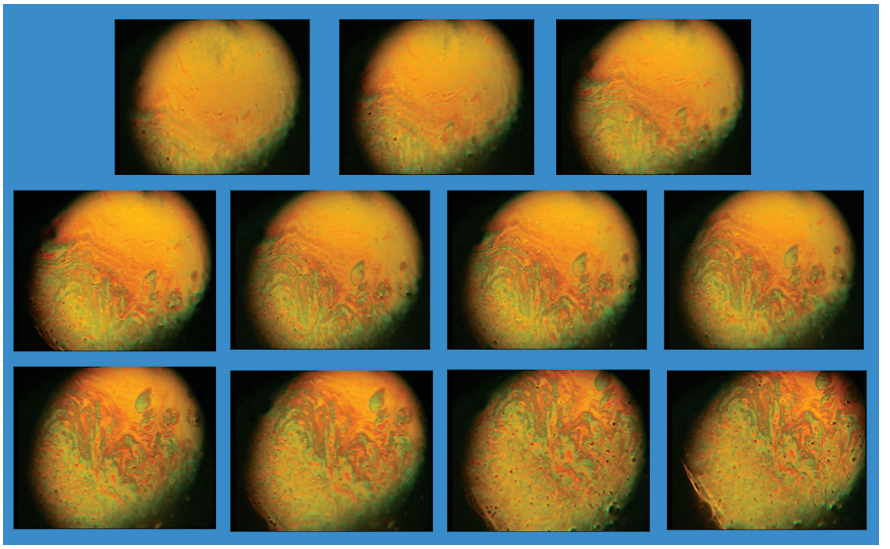

Figure 3. Series of thin film interferometry images obtained from a patient with severe dry eye by the technique of Doane.[13] The patient had primary Sjogren syndrome with a tear turnover rate of 4%/min, evaporation of 25.5 x 10^{-7}g/cm²/sec, volume of 3.9 μl and osmolarity of 337.6mOsm/ml. The images are obtained at 1 sec intervals following a blink. The lipid layer of the film is incomplete and variable in thickness, exhibiting color fringe patterns.[25,81] A stable pattern is reached in 2-3 secs after the blink,[21,22] but this pattern begins to be disrupted within the next 3 secs.

Surface regularity indices have been used by others[32] to assess the severity of keratoconjunctivitis sicca, with the Tomey computerized videokeratoscopy instrument. The SRI, SAI and the irregular astigmatism index (**IAI**) were found to be significantly correlated with the results of standard diagnostic tests (i.e., symptoms, tear break-up time, Schirmer I test, fluorescein staining score, and best corrected visual acuity).[32] These studies suggest that videotopographic techniques offer the possibility of quantitative measurement of tear film dynamics that may have clinical value in the management of ocular surface disorders.[31,32]

III. TEAR TURNOVER AND DRAINAGE

A. Fluorophotometry

A number of tests have been devised to measure the rate of disappearance of a dye marker placed in the tear film with the production of new tears and through tear elimination from the eye. In most studies, the disappearance of sodium fluorescein dye placed in the tear film has been used to record tear turnover by the technique of fluorophotometry.[3-39] Early studies used modified slit lamp fluorophotometers,[34,36,40] but the development of a commercial instrument helped standardize the procedure,[35] i.e., the Fluorotron Master (Coherent Radiation Inc, Figure 4).

The development of software for processing the data from tear turnover measurements ("ANT-SEGMENT tear") by the Leiden Centre for the European Concerted Action on Ocular Fluorometry[39] allowed greater consistency of data analysis in multi-center studies. The decay of fluorescein concentration in the tear film is measured by these techniques over a period of up to 30 minutes following instillation of 1μl of 2% fluorescein sodium into the lower fornix with a measuring pipette; scans are performed every 2 minutes for up to 30 minutes. The change in rate of decay of fluorescence of the tear film is then calculated for the total measurement period. A biphasic decay in fluorescence is observed.[35] The measurements for the first 5 minutes show a rapid decay, thought to be due to the initial reflex tearing produced by the instillation of the fluorescein drop. The later part of the curve (from 5 mins outward) represents the measurement of tear turnover under basal conditions of secretion (Figure 4). It is this part of the curve that is fitted using appropriate software,[39] and the decay in fluorescence is calculated from the log of the

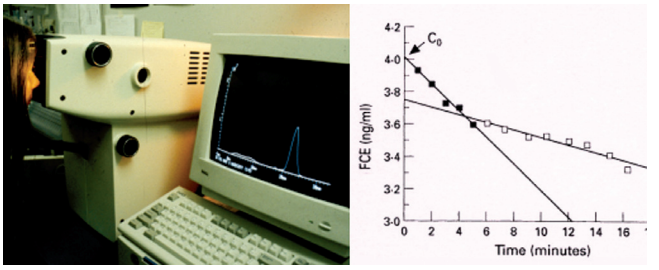

Figure 4. The commercial fluorophotometer (Fluorotron Master, Coherent Radiation Inc, CA, USA) is shown with a typical trace of the decay of ocular surface fluorescence following instillation of fluorescein sodium into the eye. A biphasic curve of fluorescence is seen with initial rapid decay (due to reflex tearing) followed by a more gradual decay (due to basal tear turnover).

curve obtained from the formula below to obtain the basal tear turnover rate;

$$T_0(t_0) = \frac{100\ [C_t(t_0) - C_t(t_0+1)]}{C_t(t_0)} \quad (\%/\text{min})$$

Where $C_t(t)$ = fluorescein concentration in tear film at time t(min).

Assuming a monophasic decay of fluorescence from 5 mins post-instillation with a decay time constant β (min^{-1})

$$C_t(t) = C_t\ (0).e^{\beta t} \quad (ng/ml)$$

the following is obtained:

$$T_t(t_0) = 100\ (1 - e^{\beta t}) \quad (\%/\text{min})$$

This gives a measurement of the tear turnover recorded in % per minute. In order to express the turnover value in terms of the µl/min (sometimes called "flow"), it is necessary to either assume a value for the tear volume (typically 7µl[40]) or to measure the volume from the initial dilution of the instilled sodium fluorescein in the tears. Initial dilution is calculated by back extrapolation to time zero of the initial fluorescence decay. In this technique, it is the monophasic decay of fluorescence in the first 5 minutes after instillation of the fluorescein that is determined.[39,41] Tear volume is derived from the formula[40]:

$$Vt = (Cd.Cm\text{-}1.k\text{-}1\text{-}1)\ Vd$$

Where Cd = Fluorescein concentration in the drop
Cm = Initial fluorescein concentration calculated by back extrapolation with the Fluorotron in ng/ml.
k = Correction factor (k = 250) for the limited spatial resolution of the Fluorotron
Vd = Drop volume in ml.

The turnover in µl/min is then calculated from the product of tear turnover in %/min and tear volume. The values reported for tear turnover (%/min) and tear flow (µl/min) in a number of studies using the commercial fluorophotometer are collated in Table 1.[38,40,42-47] Values in this Table are for normal eyes and those with different categories of dry eye. The data reported for normals in the majority of studies range from 10-20 %/min, which equates to a tear flow rate of just over 1µl/min.[38-40,42,44,45] In contrast, Mathers et al[46] found normal tear turnover in the order of 7%/min or 0.19µl/min, values not dissimilar to those found for dry eyes. The difference in values for normals still remains to be reconciled,[47] but later reports by this group suggest a difference in their calculations producing higher values in the range of 0.34-0.49 µl/min[47,48] The values reported for tear turnover rate in dry eye (aqueous tear deficiency with or without meibomian gland dysfunction) are in the order of a half to a fifth of those for normals found by other researchers[37,38] and in our laboratory (Khanal S, Tomlinson A, unpublished data). Therefore, tear turnover is an ideal measurement for the differential diagnosis of dry eye, particularly in those cases with some element of aqueous deficiency in the etiology.

B. Fluorescein clearance tests

Because fluorophotometry with the commercial instrument is costly and time-consuming,[49] simpler clinical tests have been developed which use the same principle of dye disappearance.[50-52] The technique developed by Xu and Tsubota[52] is a modification of the Schirmer test with anesthesia. The tear clearance rate is obtained after a 10µl drop of 0.5% fluorescein and 0.4% oxybuprocaine has been instilled into the conjunctival sac and the subjects have

Table 1. Tear Turnover Rate. Shows values of tear turnover rate for normal and dry eyes reported in the literature (1992–2004), as measured by the technique of fluorophotometry. Patients classified as dry eye generally have some degree of aqueous tear deficiency and those with MGD (meibomian gland dysfunction) an element of evaporative dry eye.

Report	Type/No subjects	Tear Flow (ml/min)	TTR (%/min)
Kok et al[42]	Normals (n=25)	1.06 ± 0.34*	15.2 ± 4.9
Van Best et al[39]	Normals (n=48)	1.15 ± 0.31	16.4 ± 4.4
Goebbels et al[38]	Normals (n=20)	1.20 ± 0.5	22.2 ± 0.9
Sahlin, Chen[43]	Normals (n=43)	0.76 ± 0.22*	10.9 ± 3.1
Tomlinson et al[6]	Normals (n=20)	1.47 ± 0.77*	21.4 ± 11.1
Tomlinson et al[44]	Normals (n=9)	1.16 ± 0.47*	16.6 ± 6.7
Keijser et al[45]	Normals (n=16)	1.00 ± 0.46*	14.3 ± 6.5
Mathers et al[46]	Normals (n=72)	0.19 ± 0.19	7 ± 4
Goebbels et al[38]	Dry eye (n=20)	0.20 ± 0.2	6 ± 6
Mathers et al[46]	Dry eye (n=37 MGD (n=109)	0.12 ± 0.1 0.14 ± 0.1	5 ± 3 7 ± 6
Khanal,Tomlinson (Current report)	Dry eye (n=8) MGD (n=6)	0.55 ± 0.1 0.72 ± 0.4	8 ± 3 11 ± 6

TTR= tear turnover rate
*Tear flow values estimated from turnover on the basis of a tear volume = 7ml

been instructed to open their eyes for 5 minutes. A standard Schirmer strip is inserted into both eyes, which are then closed for another 5 minutes. The length of the wet portion is measured, and the intensity of the staining is compared with the standard color plate for the Schirmer test with anesthesia or the tear clearance rate. The tear clearance rate is determined by the rate at which the color of the 0.5% fluorescein faded and was graded as 1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, or 1/256.[52] A significant correlation was found between the tear clearance and the basal tear turnover rate and tear flow obtained with fluorophotometry.[52] Each grade of tear clearance showed a 12.5% increase in basal tear turnover rate (i.e. 3.6%/min) and a tear flow change of 0.38μl/min.

Afonso et al described an alternative fluorescein clearance test in which a CytoFluor II fluorophotometer measured fluorescein concentration in tear samples collected from the inferior tear meniscus 15 minutes after the instillation of a 5μl drop of 2% sodium fluorescein.[7] This fluorescein clearance test showed a greater predictive value for symptomatic dry eye (ocular irritation) than the Schirmer I test.[53] A fluorescein concentration of 274 units/μl eliminated 80% of normal subjects (specificity) and identified 85% of the abnormal subjects (sensitivity). But the CytoFluron technique still requires an elaborate system of tear collection for analysis with the fluorophotometer and is not readily available in a clinical situation.

Macri et al described an alternative clinical assessment of fluorescein clearance rate that eliminated the need for the fluorophotometer.[50] In this procedure, the color of the tear meniscus in the lateral third of the lower lid was compared visually with one of the colors of a standardized visual scale. A score of 3 on the 6-point scale corresponded to a fluorophotometric value of 274 fluorescein units/μl, which had been shown previously to be the threshold between normality and abnormality[52]; values above 4 indicated delayed tear fluorescein clearance and dry eye. The fluorescein clearance test with the standardized visual scale showed a strong correlation with irritation symptoms, corneal fluorescein, and Schirmer I test scores and meibomian gland and eyelid pathology.[50] The sensitivity of the fluorescein clearance test was found to be 67% in diagnosing meibomian gland dysfunction and 95% in diagnosing aqueous tear deficiency; the specificity was 97%. The diagnostic precision of the fluorescein clearance test was 78% for meibomian gland dysfunction and 94% for aqueous tear deficiency.[50]

The tear turnover measured by any of the dye disappearance tests, while partially dependent on the rate of tear production, is not a direct or independent measure of that production facility.[8] In fact, tear turnover is more a direct measurement of tear elimination from the eye by drainage through the lacrimal drainage system.[43] Tear turnover is not limited by the drainage ability of the lacrimal system, which has a mean capacity of 50μl/min,[43] well above the 1μl/min of recorded turnover in normal eyes under basal conditions (Table 1).

C. Lacrimal Scintigraphy

Another technique for measurement of tear turnover and direct observation of tear drainage uses a radioactive dye placed in the tear film. The technique of lacrimal scintillography (or scintigraphy) was first described by Rossomondo et al in 1972.[53] In this technique, a radioactive tracer, such as technetium 99 (99M Tc), is introduced into the lower marginal tear strip (0.013 mls[54]). The distribution of the tracer is imaged serially by a gamma camera (Ohio Nuclear Series 100) as it passes down the lacrimal drainage system (Figure 5A and 5B). Images are typically taken every 10 seconds for 1 minute and then at less frequent intervals until all of the tracer has drained into the nasal cavity. The system has been used to quantify tear turnover from the eye and drainage through the lacrimal system.[54-56] Drainage through the system is not linear, as a significant number of normal nasolacrimal ducts offer physiological obstruction to tear flow (Figure 5C).[55]

A number of models have been used to analyze flow or drainage from the system.[56] Hilditch et al have observed that in the asymptomatic lacrimal system, variable tear flow is a normal feature of the drainage facility.[56] Therefore, such systems cannot be analyzed by a linear compartmental model. Hilditch et al have proposed a compartmental model for the analysis of lacrimal scintigraphy data, incorporating separate components for the conjunctival sac, lacrimal sac, the nasal lacrimal duct, and the nasal cavity.[56] Although most quantitative lacrimal scintigraphy measurements describe the transit time of the radioactive tracer through the system, this compartmental model has been used to estimate tear flow.[56] On the assumption that the slow component of clearance from the conjunctival sac represents the net fractional turnover of that compartment, and taking a compartmental volume of 7μl, a mean tear flow of 0.45μl/min is estimated.

An alternative calculation based on the four compartmental model estimates tear flow in the order of 1 to 8μl/min.[56] Using a single compartment model for decay of the radioactive tracer on the conjunctival surface, Craig and Tomlinson recorded mean values of reflex and basal turnover of 3.33 ± 1.95μl/min and 0.56 ± 0.32μl /min by gamma scintigraphy.[57] Lacrimal scintigraphy, despite its advantage in providing visual evidence of the drainage through the lacrimal drainage system, is extremely expensive, with its dependence on the availability of a gamma camera, and Institutional Review Board approval is required for the use of radioactive substances in normal eyes. As a result, this technique has not been widely used since the introduction of the commercial fluorophotometer systems.

The mechanism of lacrimal drainage and the influence of blinking on the mechanics of the system have been observed by high-speed photography[58] and by intracanalicular pressure measurements.[59,60] An anatomical approach has been adopted by Paulsen et al, who studied 31 lacrimal systems obtained from the heads of adults during surgical procedures on cadavers.[61] They observed that the surrounding vascular plexus of the lacrimal sac and the nasal lacrimal duct is com-

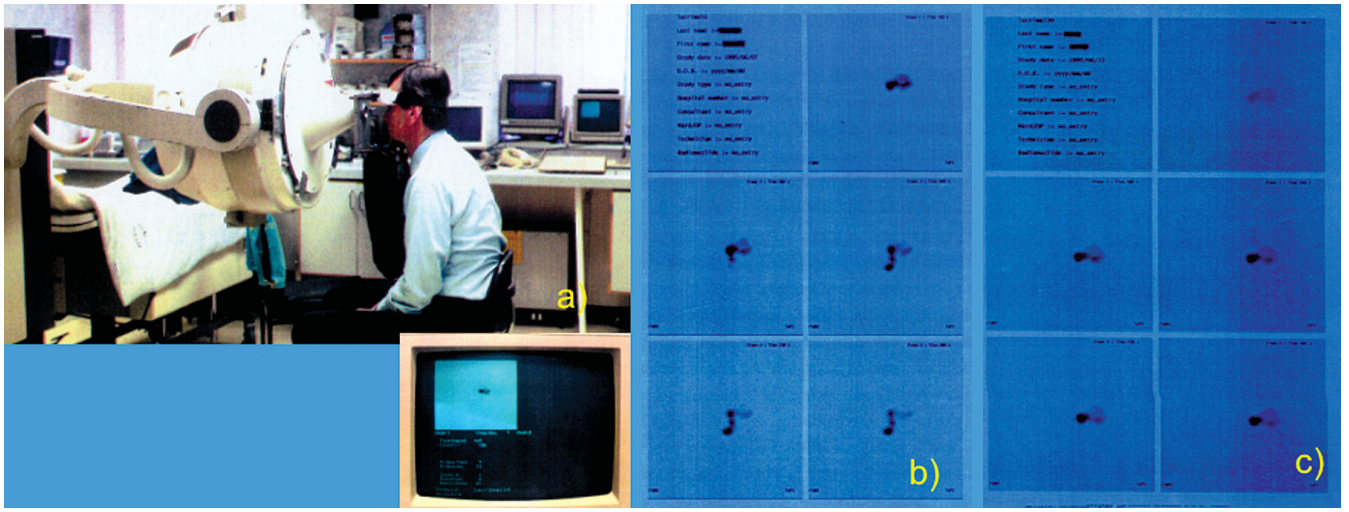

Figure 5. Shows the gamma camera (A) used in the recording of intensity of a radioactive dye at various stages as it passes through the lacrimal system (B). In many cases of normal systems, the tracer does not proceed beyond the lacrimal sac (C).[55,57]

parable to a "cavernous body." While regulating the blood flow, the specialized blood vessels of this body permit opening and closing of the lumen of the lacrimal passage, which is effected by the bulging and subsiding of the cavernous body. Thus, it regulates tear outflow from the eye.[61] Attempts have been made to quantify the regulation of tear outflow by measurement of the transit time of a fluorescein drop from the conjunctival sac into the inferior meatus of the nose.[62] Application of a decongestant drug or placement of a foreign body on the ocular surface both prolonged the dye transit time significantly, indicating a restriction in drainage through the lacrimal system in these conditions. Paulsen et al concluded that the cavernous body of the lacrimal sac and nasal lacrimal duct[63] plays an important role in the physiology of tear outflow regulation; it is subject to autonomic control and is integrated into a complex neural reflex feedback mechanism between the blood vessels, the cavernous body, and the ocular surface.[62]

IV. ABSORPTION OF TEARS INTO THE OCULAR TISSUE

Another method by which tears can be eliminated from the eye is by absorption into the tissues of the ocular surface and the drainage system. The possibility has been suggested that tear fluid is absorbed by the epithelial lining of the drainage system before it reaches the nose.[64]It has been shown in an animal model that lipophilic substances are absorbed from the tear fluid by the epithelium of the nasolacrimal duct[63] and that the cavernous body surrounding this duct may play a role in drainage of absorbed fluid.[65] No quantification of fluid volume eliminated by this route has been reported. However, tears absorbed in the blood vessels of the cavernous body may, because these vessels connect to the blood vessels of the outer eye, act in a biofeedback mechanism for tear production.[65,66]

Observations of the absorption of tear film onto the anterior ocular surface have been made in relation to studies of corneal permeability.[67,68] The proportion absorbed in the absence of compromised corneal function appears to be small at 0.24μ ± 0.13% of the dye instilled in the eye (Paugh JR: personal communication). Reports of absorption of tear fluid into the ocular surface during measurements of tear turnover by fluorophotometry are contradictory. Measurement of corneal or conjunctival absorption is dependent on the observations of the absorption of the dye tracer used in fluorophotometry. The assumption is made that the rate of absorption of tear fluid is the same as the rate of absorption of the fluorescein dye. This is

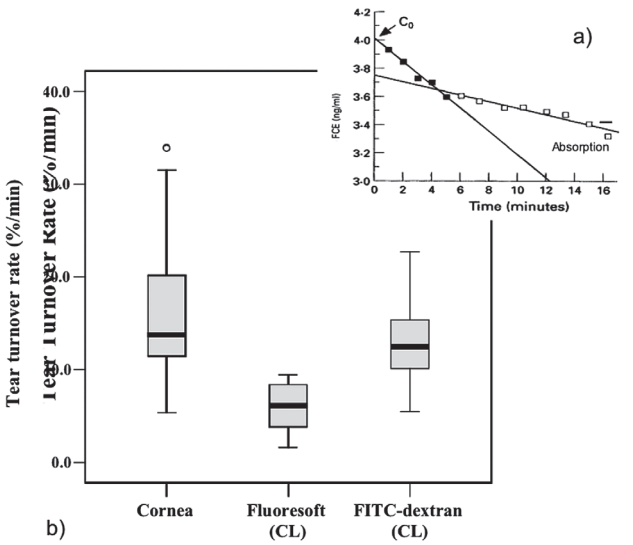

Figure 6. The effect of absorption of the fluorescein into the surface observed with the fluorophotometer is to decrease the apparent decay rate of fluorescence in the latter stages of measurement (A). The effect is demonstrated by the turnover rates obtained in a series of eyes in which measurements were taken from the naked cornea (with Fluorosoft, a lower molecular weight fluorescein), from corneas wearing hydrogel contact lenses, which absorbed the Fluorosoft dye, and from corneas with hydrogel lenses, which did not absorb a high molecular weight FITC- Dextran dye (B). Absorption of the Fluorosoft dye into the hydrogel lens reduced the apparent rate of tear turnover in the center plot.

unlikely, given the different molecular size of the two solutions. Surface absorption of fluorescein during fluorophotometry can have marked effects on the apparent rate of decay of fluorescence from the tear film. Absorption and retention of fluorescein dye on the ocular surface will artificially raise the fluorescence values obtained over the later period of measurement (beyond 15 minutes post instillation), thus decreasing the slope of the curve and apparent rate of tear turnover (Figure 6). Miller has observed that this retention of fluorescein could increase the time necessary to remove 95% of the initial fluorescein concentration on the eye (T95) by a factor of 2.[69] The tear elimination times for sodium fluorescein is about 20 minutes, whereas values for the FITC-Dextran (with a molecular size that cannot be absorbed by the ocular surface) are in the order of 11 minutes.[69]

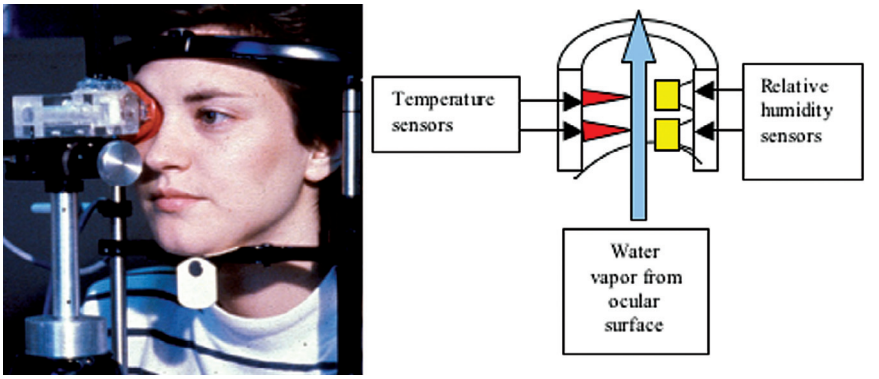

Figure 7. Tear film evaporation rate measured by a modified ServoMed EP-3 (Kinna, Sweden) evaporimeter. This technique involves the measurement of vapor pressure gradient from recordings of relative humidity and temperature at two points above the ocular surface.[80]

The effect of absorption of the dye marker in fluorophotometric measurement of tear turnover has been illustrated by recent experiments with absorption of dye into a contact lens on the eye.[70] In an experiment in which lower molecular weight fluorescein (Fluorosoft; MW 630Da) is absorbed into a soft contact lens, the apparent tear turnover rate was reduced by almost half compared to values for the naked cornea or for those obtained with high molecular weight FITC-Dextran (MW 70,000Da [Figure 6]). The FITC-Dextran is not absorbed into the contact lens. These findings are at odds with a previous report of MacDonald and Maurice[71] that disappearance of fluorescein into the ocular tissue increased the apparent rate of fluorescein decay, compared to that measured with a high molecular rhodamine dextran tracer which was not absorbed into the ocular surface.

V. EVAPORATION OF THE TEAR FILM

Evaporation from the ocular surface is very effectively reduced by the lipid barrier composed of polar lipids.[72] In conditions of 30% humidity at temperatures of 34ºC, the evaporation of water from an open bath is 160 x 10^{-7}g/cm^2/sec.[73] The polar lipids of the ocular tear film reduces this by about 80-90% in the normal eye. Evaporation of fluid from the ocular tear film have been measured by numerous investigators since the first report in 1980 by Hamano et al.[74] Their original device was disruptive to the tear film, as it required the measurement chamber to be placed close to the ocular surface, thus preventing normal blinking and tear redistribution. In 1982, Tomlinson and Cedarstaff employed the technique of resistance hygrometry to measure tear film evaporation within a goggle, a technique that allowed the normal blink mechanism to apply.[75] This technique was based on the principle that the increase in electrical resistance of air passed over the eye was a direct measure of the increase in fluid content (humidity) of that air, due to evaporation from the surfaces within the goggle.

The technique of measuring the humidity of air inside a sealed goggle has also been employed by Rolando and Refojo (1983),[76] Tsubota and Yamada (1990),[77] and Mathers et al (1993).[78] Recently the technique was further refined by Goto et al, who used microbalance technology to allow "continuous readings" of tear film evaporation.[79] The measurement of the vapor pressure gradient calculated from the relative humidity and temperature at two points above an evaporating ocular tear film is a technique developed later by Trees and Tomlinson in 1990[80] and subsequently used in our laboratory (Figure 7).[44,80,81]

Tear film evaporation rate has been reported in different units by various researchers[80-83]; most use units of x10^{-7}g/cm^2/sec, but others report values in g/m^2/hr. This difference may be resolved and all values rendered to the same units (x10^{-7}g/cm^2/sec) by dividing values in g/m^2/hr by a factor of 3.6. The values of evaporation rates recorded for normal and dry eyes are collated in Table 2. Evaporation rate is also reported in units of μl/min by some researchers.[79,82] The evaporation rate in μl/min is numerically equal to 1/100 of the value of the evaporation rate stated in units of 10^{-7}g/cm^2/sec when the area of the evaporating ocular surface is 167mm^2. The use of different techniques for measurement of tear film evaporation makes it difficult to compare findings in normal and dry eyes among different studies because the absolute values recorded are technique-dependent. However, there is a pattern to the observations reported in the literature, with significant increases from normal tear film evaporation seen in patients with both aqueous tear deficiency dry eye and meibomian gland dysfunction (Table 2).[46,47,76-78,82,84] Strictly, these comparative differences are of diagnostic significance only where values in normals and dry eye are recorded by the same technique in the same laboratory.

Mishima and Maurice in 1961 were the first to establish that the lipid layer retarded evaporation in an animal model of the rabbit eye.[85] Iwata et al developed another in vitro rabbit model with a cornea covered with a chamber through which dry air was passed; from the weight of water collected,

they determined the evaporative rate as 10.1 x 10^{-7}g/cm^2/sec.[86] They found that a four-fold increase in evaporation occurred with the removal of the rabbit's tear film lipid layer.

A similar increase in human tear film evaporation was measured by Craig and Tomlinson in patients with incomplete or absent lipid layers.[81] Evaporation measurement is important in the differential diagnosis of dry eye. A number of studies, as well as findings in our laboratory, report measurement of tear film evaporation in aqueous deficient and meibomian gland dysfunction patients.[46,47,76-78,82,84] In most cases, the evaporation rate is greater in the dry eye than in the normal eye, as the increased water loss from the tear film contributes to the dry eye condition. In one study, almost 90% of the dry eye patients showed lower readings of tear film evaporation than normals.[77] This was explained by considering the relative contribution of tear evaporation to tear dynamics in the dry eye condition; the proportional loss through evaporation in the dry eye was greater than in normals, although the actual water loss (in absolute terms) was decreased compared to normal values. Mathers in a comprehensive review of evaporation from the ocular surface has discussed the contribution of evaporation to the total tear flow in the eye.[82] In the normal eye (Table 2), the actual loss of fluid from the eye by evaporation is approximately 10-15% of the tear flow or turnover of the system, i.e., averaging 0.13µl/min for a tear flow in the order of 1µl/min (Table 1). Dependent on the value accepted for tear turnover rate in normals, the proportion lost through evaporation increases, particularly in the reports of Mathers et al,[47,48] where the turnover rate is measure at 0.2 to 0.49 µl/min. (This issue is further addressed in Section VIII. Indices of tear film dynamics.)

Table 2. Tear Evaporation Rate. Shows values of tear evaporation rate for normal and dry eyes reported in the literature (1992 – 2004). Measurements are by various techniques. Patients classified as dry eye (DE) generally have some degree of aqueous tear deficiency and those with MGD (meibomian gland dysfunction) an element of evaporative dry eye.

Report	Type/ Number subjects	Evaporation x 10^{-7}g/cm^{-2}/s	Evaporation (ml/min)
Tsubota,Yamada[77]	Normals (n=40)	15.6 ± 3.8	0.16 ± 0.04
Mathers et al[46]	Normals (n=72)	15.1 ± 8.6	0.15 ± 0.09
Mathers [83]	Normals (n=20)	14.8 ± 6	0.15 ± 0.07
Shimazaki et al[84]	Normals (n=24)	13.1 ± 6	0.13 ± 0.05
Mathers, Daley[47]	Normals (n=34)	13.0 ± 6	0.13 ± 0.05
Tomlinson et al[43]	Normals (n=9)	7.2 ± 3	0.07 ± 0.03
Goto et al[79]	Normals (n=22)	4.1 ± 1.4	0.04 ± 0.01
Mathers et al[46]	DE (n=37) MGD (n=109)	23.9 ± 17.5 22.8 ± 16.3	0.24 ± 0.18 0.23 ± 0.16
Mathers[83]	DE/MGD (n=20) MGD (n=24)	59.1 ± 28 49.9 ± 21	0.58 ± 0.23 0.49 ± 0.29
Shimazaki et al[84]	MGD (n=37)	14.8 ± 1.5	0.15 ± 0.15
Mathers, Daley[47]	DE (n=22)	25.0 ± 35	0.25 ± 0.04
Goto et al[79]	MGD (n=21)	7.6 ± 2.8	0.08 ± 0.03
Tsubota, Yamada[77]	DE (n=72)	9.5 ± 5.6	0.10 ± 0.06
Khanal, Tomlinson (Current report)	DE (n=8) MGD (n=6)	14.6 ± 11.3 20.6 ± 7.9	0.15 ± 0.11 0.21 ± 0.02

VI. TEAR FILM OSMOLARITY

Attempts to quantify tear film dynamics are frustrated by the lack of direct and independent measurements of lacrimal production, limiting this review to consideration of tear distribution and turnover, evaporation, and drainage. Knowledge of the elimination that occurs (loss due to drainage and evaporation) gives an estimate of the tear production, although we lack knowledge of the amount of tear fluid absorbed into the ocular tissues. Tear osmolarity provides a single measurement that may reflect the balance of input and output from tear dynamics. Osmolarity is the end product of variations in tear dynamics. Normal homeostasis requires regulated tear flow, the primary driver of which is osmolarity.[87] Tear hyperosmolarity is the primary cause of discomfort, ocular surface damage, and inflammation in dry eye.[88-92] Therefore, tear film osmolarity is attractive as a single parameter of tear film dynamics.

Measurements of osmolarity are obtained from small (0.2 µl) samples of tears taken from the lower meniscus and placed in a freezing point depression osmometer (Clifton Technical Physics, Hartford, NY, [Figure 8]).[81,87,88] This osmometer has been accepted as the "gold standard" in the diagnosis of dry eye [90,93]; a clinical cut-off of 312mOsm is suggested as the diagnostic level for aqueous deficient and evaporative dry eye.[90] Hyperosmolarity causes surface damage in animal models of aqueous deficient and evaporative dry eye[92,94]; corneal epithelial cells are damaged by hyperosmolarity in vitro[92,95] and in vivo.[96] The measurement of tear film osmolarity from a sample taken from the inferior tear meniscus has been questioned by Bron et al, who suggest that the osmolarity of the precorneal tear film may be higher than that of the meniscus because the

preocular tear film may be "compartmentalized" and susceptible to greater evaporative fluid loss.[97] However, Benjamin and Hill found that the tear fluid from the conjunctival sac had significantly higher osmolarity than that from the inferior tear prism.[98]

The sampling of tear film osmolarity with an instrument such as the Clifton freezing point pressure technique is technically difficult and requires considerable expertise to achieve consistency in measurement.[99-101] Alternative techniques have been used to obtain osmolarity values for human tear fluid.[101-103] The widespread clinical use of osmometry will require a simpler, quicker and less expertise-dependent technique for measurement.[104]

A number of workers have reported values for tear film osmolarity in normals and in various dry eye states (Table 3). Although there is some overlap in the values between normal and dry eye, those for dry eye are higher and osmolarity offers a means for the general diagnosis of dry eye.[46,88,90,105] This will be discussed again later.

VII. TEAR FILM DYNAMICS AND AGE

Changes with age in the aspects of tear film dynamics considered in this review have been addressed by a number of researchers. It is attractive in hypotheses of dry eye to have age-related changes, even in asymptomatic individuals, which may contribute to the increased prevalence of dry eye with age. Mathers et al have shown significant correlations with age for tear evaporation, flow, volume and osmolarity in an extensive series of normal and dry eye patients.[106] But for normal (asymptomatic of dry eye) populations, age does not appear to be significantly related to changes in evaporation, osmolarity, lipid structure, break-up time, or tear production rates.[57] In other studies, age-related changes have not been found for tear evaporation,[76,107] turnover,[43] osmolality,[105] or lipid layer.[18] At present, it is difficult to resolve these conflicting reports. It is possible that work that shows age-related effects for tear parameters measured on consecutive patients in a corneal service of a hospital[46] may be susceptible to the participation bias observed by Schein et al[108] in dry eye studies of clinic-based popoulations. However, this could be true of other studies as well.[43,76] However this issue is resolved, and despite the fact that the prevalence of dry eye conditions increases in older age groups, it is important to account for the overwhelming majority of older individuals who do not develop dry eye.

VIII. INDICES OF TEAR FILM DYNAMICS

It would be useful in the study of dry eye to be able to describe and quantify tear film dynamics in a single index that determines the balance of input and output of the system (Figure 1). Such an index could define the imbalance that leads to the condition of dry eye. The majority of the output, and by implication the input, of the lacrimal system can be determined through measurement of tear turnover and water loss by evaporation. The degree of elimination due to absorption is more difficult to assess. Also the measurement of tear production is difficult to measure independently and directly.[8] Tear film distribution on the surface of the eye at this time can only be assessed qualitatively. Despite these difficulties, attempts have been made to derive indices to describe the tear film dynamics.

A. Tear function index for tear film dynamics

One of the earliest indices for tear film dynamics was the Tear Function Index (**TFI**) devised by Xu et al.[8] This index combined values obtained for tear secretion (from the Schirmer test with anesthesia) with measurements for drainage (turnover as measured by the fluorescein clearance test[52]) in the following formula:

$$\text{TFI} = \frac{\text{Schirmer value with anesthesia}}{\text{Tear clearance rate}}$$ [8,9]

This index considered two of the three main factors determining tear dynamics,[8] namely, secretion and drainage; it has been argued that tear secretion is the most important determinant of tear dynamics.[8] Moreover, as tear secretion could not be measured independently and directly, the Schirmer test result had to represent the production component of dynamics.[8]

The ability of the TFI to discriminate between normal and dry eye patients, with and without Sjogren syndrome, was found to be considerably better than the Schirmer test or the tear clearance rate values alone.[1] A value of the log to the base 2 of the TFI below 96 gave a sensitivity and specificity in the diagnosis of dry eye of 67.4% and 60%, respectively. For a value of the log of the TFI below 34, sensitivity and specificity for Sjogren syndrome dry eye were 78.9% and 91.8%, respectively.[8] The major deficiency of the TFI as an index of tear dynamics is that it fails to take into account the elimination of tear fluid from the eye through evaporation. Evaporation is a key variable in differentiating some groups of dry eye.[48]

B. Total tear flow as an index of tear dynamics

Recently, Mathers has suggested an index that captures the principal sources of elimination of tear fluid from the eye.[87] It takes values for drainage, as measured by tear turnover rate and surface fluid loss by evaporation. Mathers' index represents "total tear flow" expressed in μl/min, which is obtained by combining rates for evaporation and tear turnover. As the drainage facility is not necessarily affected in dry eye states,[43] the tear flow is determined from tear turnover rate (effectively a measurement of drainage).[43] The combination of tear turnover and evaporation rates gives an estimation of the tear production facility of the eye.[87] Therefore, dry eye may result when tear flow (turnover) is reduced due to tear production deficiency (aqueous deficient dry eye) or a high level of evaporation (evaporative dry eye) as a result of meibomian gland dysfunction or blepharitis. In the assessment of the balance of production and outflow from the eye, the proportion of elimination due to evaporation as a part of the total tear flow is an essential consideration.

In Table 4, the values for tear turnover and evaporation rates and the total tear flow have been collated from all the literature between 1992 and 2004, and also for Mathers publications (1993 to 2004). The numbers shown in Table 4 are weighted averages of the published values. This approach has been taken because the values recorded for tear turnover in normals (Table 1) by Mathers and co-workers in some reports[46,82] are considerably below those recorded by others. This affects any attempt to define the differences between normals and dry eye patients in terms of this Index.

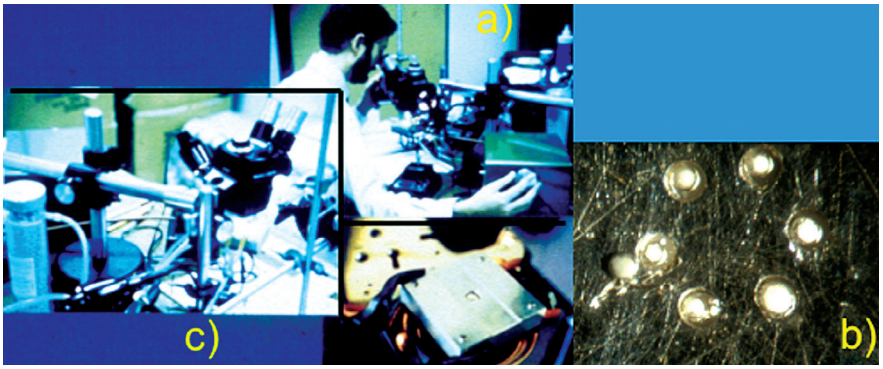

Figure 8. Freezing point depression osmometry with the Clifton Technical Physics device. (A) Nanoliter samples of tears from the interior tear prism are placed below heavy oil in the apertures of the 'freezing plate' by a micromanipulator (B). Cooling of the plate by the instrument allows direct reading of the osmolality from the instrument at the point at which the samples freeze (C).

Mathers has reported that approximately one-third of the resting tear flow evaporates in the normal eye.[82,87] This increases to 75% of the total tear flow in the dry eye,[87] although the total tear flow in normals and dry eye patients recorded by Mathers is similar at around 0.5 µl/min. In a later paper, Mathers reported a percentage loss of tear fluid from the eye through evaporation to be around 55% in dry eyes.[82] Despite the difference in measurement of tear flow (turnover) rates between Mathers' group and other workers, the total tear flow combined with evaporation as a proportional value still provides a useful index in differentiating dry eye. In Table 4, if all the literature is considered, the proportion of evaporation to total tear flow is 14% for normals and more than 60% for dry eye—regardless of whether the dry eye is due to aqueous deficiency or meibomian gland dysfunction. Mathers' data (Table 4) suggests that normals have a proportional value of evaporation to total tear flow of around 44% compared to over 60% for the aqueous deficient dry eye and meibomian gland dysfunction groups. Therefore, irrespective of whether dry eye is due to aqueous tear deficiency or meibomian gland dysfunction/blepharitis (evaporative dry eye), a dry eye syndrome may be indicated if the total tear flow is less than 0.5 µl/min, or the proportion of loss due to evaporation, compared to the total tear flow, is greater than 50%.

The importance of tear evaporation measurement in the definition of dry eye is illustrated by a recent re-analysis by Mathers and Choi[48] of the data from the Iowa study, which formed the basis for earlier papers.[82,87] In this later report, they applied cluster analysis with hierarchial clustering (Ward minimum variance method[109]) to determine groupings that corresponded to clinically relevant and identifiable diagnoses. Diagnoses of physiologic dry eye and seborrheic or obstructive meibomian gland dysfunction were based on physiological parameters. Using this approach, the subjects in the study were divided into nine categories on the basis of only 5 of the 13 physiological variables. Interestingly, evaporation was found to be a key variable in differentiating some clusters, suggesting that it is a key process and plays an important role in ocular surface disease.[48,78,108] There appears to be no real substitute for evaporation in the ultimate classifications that Mathers has reported.[48]

C. Tear osmolarity as an index of tear film dynamics

The use of a single value for tear osmolarity as an index is attractive, as it represents the end product of changes in tear dynamics.[87] However, the use of this value is complicated by the overlap in values obtained between normal subjects and those with keratoconjunctivitis sicca; significant overlap occurs in values of tear osmolarity between 293 and 320mOsm/L.[88] This complicates the choice of a referent value for the diagnosis for dry eye. Farris and Gilbard deliberately chose

Table 3. Tear Osmolarity. Shows values for osmolarity for normal and keratoconjunctivitis sicca patients measured by freezing point depression nanoliter osmometry. KCS patients classified as dry eye (DE) generally have some degree of aqueous tear deficiency and in, extreme cases, primary Sjogren syndrome. Those with meibomian gland dysfunction (MGD) have an element of evaporative dry eye.

	Tear Film Osmolarity (mOsm/L)	
Report	**Normal**	**KCS (DE, MGD, SS)**
Gilbard et al[90]	302 ± 6 (n=31)	343 ± 32 (DE=38)
Farris et al[88]	304 ± 8 (n=180)	326 ± 20 (DE=111)
Mathers et al[45]	303 ± 10 (n=72)	313 ± 9 (DE=37) 314 ± 10 (MGD=109)
Craig et al[105]	305 ± 7 (n=25)	323 ± 15 (DE=17) 331 ± 21 (SS=30)
Khanal and Tomlinson (Current report)		323 ± 17 (DE=8) 321 ± 12 (MGD=6)

Table 4. Total Tear Flow: An Index of Tear Dynamics. The table shows the weighted, average values for rates of tear turnover, evaporation and total tear flow in normal and dry eyes reported in all the literature from 1992–2004 and in papers published by Mathers and co-workers (1993–2004). The proportional values of evaporation to total tear flow for each subject group and data set are calculated. Patients classified as dry eye (DE) generally have some degree of aqueous tear deficiency and those with meibomian gland dysfunction (MGD) an element of evaporative dry eye.

Source of data	Tear Turnover/ Drainage (μl/min)	Evaporation (μl/min)	Total Tear Flow* (Drainage + Evaporation) (μl/min)	Evaporation/ Total Tear Flow (%)
Literature 1992-2000 [6,39,42-47,77,79,83,84]				
Normals	0.82	0.13	0.95	14
DE	0.15	0.22	0.37	60
MGD	0.14	0.23	0.37	62
Mathers, 1993-2004 [46,47,83]				
Normals	0.19	0.15	0.34	44
DE	0.20	0.24	0.34	67
MGD	0.14	0.23	0.37	62

* Mathers[87]

312mOsm/L to provide maximum sensitivity in diagnosis,[88] preferring some possible overdiagnosis to the underdiagnosis that might result from using maximum specificity. Others have suggested other referent values for dry eye. Craig has suggested values over 320mOsm/L.[25] Mathers and Choi suggested values = 318mOsm/L[48] as the definition for physiologic dry eye by a criterion of one standard deviation from the mean in their cluster analysis of patients with the condition.[108] If the 95% confidence intervals are applied to the data shown in Table 3, it will be seen that the overlap between normal patients and those with some form of dry eye (Figure 9) is not great. However, it is more difficult to differentiate the dry eye subgroups using osmolarity, i.e.,to differentiate between aqueous deficient dry eye and meibomian gland dysfunction,[46,] and between aqueous deficient dry eye with and without primary Sjogren syndrome.[105] It is perhaps not surprising that there is clearer differentiation, by osmolarity, between normal and dry eye than between categories of dry eye, given that osmolarity is the "average" end product of the disease process and that this end point may be similar irrespective of the process by which it is achieved.

D. Other indices of tear film dynamics

Other reported indices for tear physiology incorporate different parameters than those considered in this review and use techniques of derivation that involve more sophisticated statistical analyses. In a study of tear physiology in patients with rheumatoid arthritis (with and without primary Sjogren syndrome, Craig[25,105] employed discriminant function analysis[110,111] in an attempt to predict the disease status on the basis of tear parameters. The initial analysis incorporated tear osmolarity, evaporation, grade of lipid layer structure, break-up time, symptoms, Schirmer test, and rose bengal staining. The development of a discriminant function on the basis of these parameters allowed the correct grouping of subjects into normals and rheumatoid arthritis (with and without primary Sjogren syndrome) in over 93% of patients. However, this analysis did incorporate two tests, the Schirmer test and

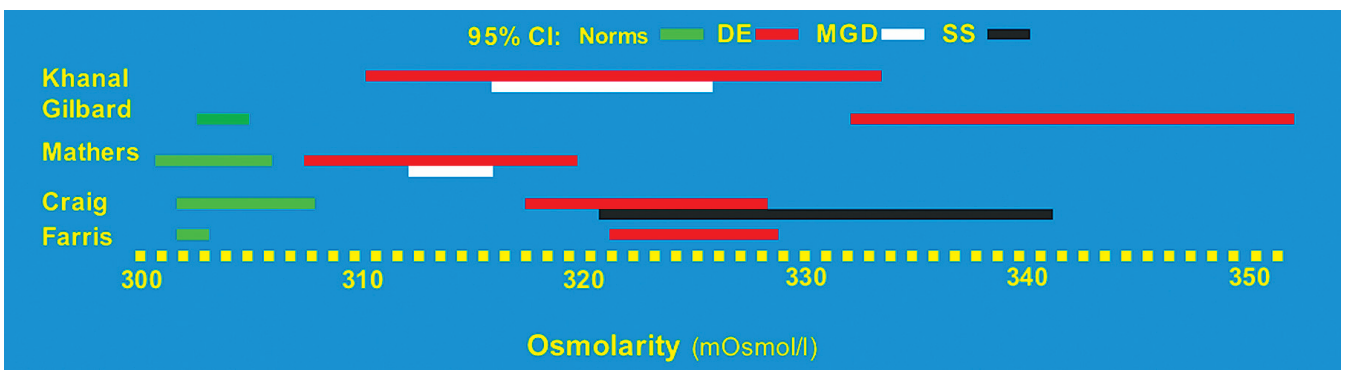

Figure 9. Osmolarity values for normal and dry eye subjects reported in the literature (values shown are for the 95% confidence intervals) for normals. DE = aqueous tear deficiency, MGD = meibomian gland dysfunction, and SS = Sjogren syndrome. (Khanal and Tomlinson, unpublished data, and references[45,88,90,105]).

rose bengal staining, commonly employed by clinicians in the independent clinical classification of the groups. A discriminant analysis with a step-wise variable selection was performed without these clinical tests or symptoms included to determine the most influential objective tear physiology parameters for predicting group assignment.[25] Two functions were derived; Function 1 had the best combination of parameters, which were osmolarity, evaporation rate, and grade of lipid layer structure. The discriminant function equation is:

$$\text{Function 1} = 0.06 \text{ osmolarity} + 0.23 \text{ evaporation rate} + 0.13 \text{ lipid grade} - 20.61$$

The overall accuracy in classifying 72 subjects with this discriminant function was 68% when the following cut-off values were used: <0.4 for controls =0.4 to 0.5 for rheumatoid arthritis alone; and >0.5 for rheumatoid arthritis with primary Sjogren syndrome. Osmolarity was the single best predictor, with an overall accuracy of 54%. In distinguishing between normal subjects and those with rheumatoid arthritis alone, the sensitivity of the discriminant function was 96% and the specificity 87%. For correctly identifying subjects with rheumatoid arthritis alone, the sensitivity of the function was 60% and the specificity was 81%. For identifying Sjogren syndrome subjects alone, the sensitivity was 41% and the specificity, 84%. The sensitivity of the function was poorer for diagnosing the rheumatic conditions because of the spread of data and the greater degree of overlap between the tear characteristics of the two rheumatic groups. The tear characteristics of these two patient groups were more similar to each other than they were to normals.

Clearly, in order to obtain true values for sensitivity and specificity of any discriminant function, it is necessary to test the function on independent samples of dry eye and normal patients. It would be instructive to apply discriminant function analysis to the data obtained for the aspects of tear film dynamics discussed in this review, namely tear turnover rate, tear evaporation rate, and tear osmolarity. Discriminant function analysis is the most appropriate technique for discriminating between patients on the basis of measurement where the clinical classification of the patients is already known.[112] In order to do this, a significant patient sample is required with a full data set for all of the parameters. Such data is not currently available to the authors.

Mathers et al[46] have applied another form of multivariate analysis, step-wise linear regression,[111] to define a multiple linear regression model for aspects of tear physiology measured during their seminal study in Iowa of normal and dry eye patients. They used regression analysis to predict osmolarity and tear evaporation. A regression model for tear osmolarity based on the results on the Schirmer test, evaporation rate, gland drop-out, lipid volume, and tear volume (from fluorophotometry), and age of subject accounted for 47% of the variance in tear osmolarity.[46]

The other form of statistical analysis, cluster analysis, applied to this same data by Mathers and Choi[48] employed hierarchial clustering of the physiological variables that had been measured. Potential age effects in the physiological variables had been removed by local polynomial regression. This analysis created new classifications that partially corresponded to clinically relevant and identifiable diagnoses. The 513 subjects in the study were divided into nine categories on the basis of only 5 of the total of 13 variables that had been measured; these variables were gland drop-out, lipid viscosity, evaporation rate, Schirmer test value, and lipid volume. This cluster analysis suggested that tear data can be used to classify subjects with similar characteristics that have clinical relevance and statistical validity.[48] Mathers and Choi argue that the approach places ocular surface disease classification on a stronger scientific footing, although the use of cluster analysis is unusual in a situation where a pre-existing classification of patients is available. The range of measurements considered by them goes beyond the basic parameters of tear film dynamics discussed in this review, but it would be of interest to apply their analysis to data sets of tear turnover, evaporation rate, and osmolarity.

IX. CONCLUSION

The diagnosis of dry eye by the assessment of tear film dynamics has a number of problems associated with it; these include the difficulties of clinical measurements, validity of any index in the differential diagnosis of dry eye, and the inability to capture other aspects of dry eye etiology that are not directly associated with the mechanisms of tear dynamics.

The preferred "quantification approach" to tear film dynamics in this paper involves measurements that are technically difficult to achieve, expensive, and time-consuming, and do not lend themselves readily to simple clinical assessment. The techniques for measurement of tear turnover rate (by fluorophotometry), tear film evaporation (by evaporimetry) and tear osmolarity by freezing point depression) give the most direct and quantifiable measures of tear film dynamics available to date, but all suffer from the limitations noted above. Alternative clinical tests are available for two of these measures. Tear distribution has been measured and observed using a simple clinical device of the Tearscope[56] (Keeler, Windsor, UK). Tear turnover can be measured more rapidly and cost-effectively by the tear clearance tests[50,52] with a standardized visual scale for evaluation of tear fluorescein clearance.[50] These tests are simple and easy to use and have high correlations with fluorophotometry results.[50,52]

Tear evaporation is more difficult to measure with a surrogate clinical test. The patency of the meibomian glands of the lid, which produce the lipid secretions and which inhibit tear evaporation, may be assessed by a slit lamp examination of the gland orifices,[113] by an estimate of gland drop-out with transillumination of the lower lid,[114] or by meibometry, a technique for assessment of the lipid obtained from the lower lid.[115] However, these clinical tests are not quantifiable as direct measures of fluid loss in the same way as tear evaporation rate. A measure of tear film osmolarity by a rapid and

less expertise-dependent technique must await development of new clinically applicable instruments. Such instruments are in development,[102,104] but are not currently available to supersede the Clifton nanometer freezing point depression osmometer. It is true that surface damage in vivo can be produced by hyperosmolarity,[96] and correlations between hyperosmolarity and ocular surface damage have been found.[116-118] However, methods of grading surface damage through staining per se are not sufficiently exact to substitute for measurement of osmolarity.

The validity of any index for tear film dynamics needs to be validated in the differential diagnosis of dry eye. The validity of the TFI devised by Xu et al,[8] demonstrated relatively high sensitivities and specificities for diagnoses compared to tear clearance and the Schirmer test values alone. However, their assessment of specificity and sensitivity appears to have been carried out on the sample for which the cut-off values were originally determined. To assess the validity of this or any other index, such as total tear flow or osmolarity, assessment must be carried out on an independent sample of normal and dry eye patients.

Any index of tear film dynamics is necessarily one that attempts to quantify measures of tear production and elimination and is a somewhat mechanistic index of tear physiology. As a result, it does not capture the other aspects of the etiologies for dry eye, such as dysfunctional neurology,[1] hormonal influences,[119] or the inflammatory nature of the condition.[120] However, it has the utility that it addresses the essential feature of the disease, that of the imbalance of tear physiology and the resultant "dryness" of the eye.

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