SIMULTANEOUS EVALUATION OF TEAR TURNOVER AND CORNEAL EPITHELIAL PERMEABILITY BY FLUOROPHOTOMETRY IN NORMAL SUBJECTS AND PATIENTS WITH KERATOCONJUNCTIVITIS SICCA (KCS)

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ABSTRACT

Purpose: To simultaneously determine the tear turn over rate (TT) and corneal epithelial permeability (P_{dc}) in normal subjects and patients with KCS by a single-drop fluorophotometric technique using disodium fluorescein (DSF) or carboxyfluorescein (CF).

Methods: DSF was instilled in one eye chosen at random and CF in the fellow eye of 13 normal subjects and in 13 patients with KCS. TT and P_{dc} were determined using a single-drop technique on a Fluorotron Master®.

Results: In normals and KCS subjects, TT was found to be independent of age and sex and essentially identical for DSF and CF. TT was approximately 42% lower in KCS subjects than normals (Table). TT was independent of Schirmer's I and P_{dc}.

	TT - DSF (%)	TT - CF (%)	P_{dc} - DSF (nm/sec)	P_{dc} - CF (nm/sec)
Normal (±SD)	19.25 ±7.70	18.83 ±7.76	0.1868 ±0.0855	0.1710 ±0.2509
KCS (±SD)	11.23 ±5.38	10.79 ±7.69	0.7385 ±0.5429	0.4995 ±0.7590

P_{dc} values were similar for DSF and CF in normals and increased with age. In KCS subjects, P_{dc} was 3-4 times higher compared to normal subjects ($p<0.01$) and was directly correlated with the severity of corneal punctate staining. P_{dc} was independent of TT.

Conclusion: Patients with KCS are more susceptible to the therapeutic and toxic effects of topical medications and preservatives due to increased corneal epithelial permeability. With the decreased TT in KCS patients, there is also slower elimination of substances from the tear film. This, combined with the increase in epithelial permeability, puts the KCS eye doubly at risk for the toxic effects of topically applied substances.

INTRODUCTION

The integrity of the tear film is extremely important for maintenance of the cornea and conjunctiva. Clinical tests are available to assess tear secretion, tear evaporation, tear drainage, and the ocular surface, but few actually measure the interaction of these components. It is not surprising that isolated tests often fail to correlate with patient symptoms or with other clinical or laboratory tests.

Fluorophotometric determination of tear turnover allows physiologic assessment of the combined effects of tear secretion, tear evaporation, tear drainage and the ocular surface on tear turnover. In addition, determination of corneal epithelial permeability by fluorophotometry gives a sensitive measure of epithelial integrity. For most fluorophotometric methods, determination of tear turnover and corneal epithelial permeability requires two separate measurements. In addition, most methods for determining corneal permeability require knowledge of the tear turnover rate.

In this thesis, a new fluorophotometric method that allows simultaneous determination of tear turnover rate and corneal epithelial permeability is evaluated in normal subjects and patients with keratoconjunctivitis sicca (KCS). The purpose of this thesis is to demonstrate that fluorophotometry can be a useful tool in studying tear film dynamics and corneal epithelial permeability changes due to KCS.

DEFINITIONS

A 7 to 8 μm aqueous-dominated, trilaminar precorneal tear film[1] is dogma at present. Although recent evidence suggests that the tear film may be much thicker (40 μm) and composed mostly of mucin,[2] the classic description of the tear film will be used in this thesis. Dry eye is a general term that refers to abnormalities of any layer of the tear film, lid resurfacing abnormalities, or ocular surface disease. KCS refers to an eye with insufficient aqueous secretion to maintain a normal ocular surface, which is the combined conjunctival and corneal epithelial surfaces. For this thesis a normal ocular surface is defined by the absence of rose bengal and fluorescein staining and squamous metaplasia.

NORMAL TEAR FILM STRUCTURE

General

The classic description of the tear film is a three-layered structure composed of a lipid layer, an aqueous layer, and a goblet cell secreted mucin layer. The epithelial secreted glycocalyx should be thought of as an additional layer. The tear film is 7 μl total volume and approximately 8 μm in total thickness. The upper and lower tear menisci hold 75% to 90% of this

volume.[3,4] The remainder is in the tear film and in the inferior and superior conjunctival fornices. Normal tear secretion is 1.2 μl/min and can double during reflex tearing.[3,5]

Lipid Layer

The meibomian glands and, to a lesser extent, the glands of Zeis and Moll secrete nonpolar wax and cholesterol esters that float on the aqueous layer. This 0.1-μm layer of lipid accounts for less than 1% of the total thickness of the precorneal tear film.[6] The lipid layer increases to 100 μm when the lids are closed.[4,6] Opening the eyes causes the polar lipid to spread rapidly to form a monomolecular film. Less polar lipids then flow over this monolayer to ultimately form a thicker bilayer of lipid. The rapid spreading of lipid ahead of tear macromolecules aids in establishing a surface tension gradient. This gradient causes the aqueous tears to flow in the direction of the advancing lipid front, which results in thickening of the tear film.[4,7,8]

The lipid layer affects evaporation of the aqueous tears. An absence or deficiency of the lipid layer results in an increase in evaporation.[9] It also forms a protective hydrophobic layer, which stabilizes the tear film by decreasing surface tension and preventing contamination and disruption of the tear film by environmental pollutants and skin lipids.[8,10] Therefore, the lipid layer can be responsible for an increase in the tear film thickness after a normal blink or may cause a decrease in tear film thickness when there is a deficiency or absence of lipid.

Aqueous Layer

Fluid secreted by the main and accessory lacrimal glands (the glands of Krause and Wolfring) form the aqueous layer of the tear film. Classically, tear secretion has been thought to consist of basal and reflex tears.[11] The main lacrimal gland was thought to contribute reflex tears and the accessory glands basal tears. It is likely, however, that all tearing is reflex or irritant in nature and that nonreflex or basal tear secretion does not exist.[12] This is supported by the fact that the main lacrimal and accessory lacrimal gland tissues are histologically identical.[13] Minor stimuli such as blinking, air currents, and cold air are capable of increasing tear secretion.[12] The exception is emotional tearing, which is thought to be different from reflex tearing.[14] Increased tear secretion will increase tear turnover as well as the thickness of the tear film.

The 7-μm-thick aqueous layer forms 99% of the tear film.[15] It contains over 60 different proteins, which result from a combination of lacrimal secretion and transudation of serum proteins from the conjunctival vessels.[16,17]

The main function of the aqueous layer (and the tear film) is to provide a smooth and uniform optical refractive surface for vision. It also serves as the major source of oxygen for the ocular surface and anterior segment of the eye. Immunoglobulins, lysozyme, lactoferrin, and tear-specific albumin

present in the aqueous layer provide antimicrobial protection. The aqueous layer also decreases friction and lubricates the lids as they move across the ocular surface. Increased reflex tearing washes away debris, noxious substances, and foreign bodies from the cornea.[5]

Mucin Layer

There are two sources of ocular surface mucin- conjunctival goblet cells and ocular surface epithelial cells. Conjunctival goblet cells contribute most of the mucin to this layer.[18] Goblet cells are spread over the entire conjunctival surface and are more concentrated inferonasally on the bulbar conjunctiva and on the plica semilunaris and caruncle.[19] Blinking spreads the mucin over the ocular surface.

A nongoblet cell source of mucin has been identified.[20,21] Interepithelial vesicles located near the apical cell margin, containing glycoproteins histochemically different from goblet cells, fuse with the apical cell membrane, evert, and form the glycocalyx.[20]

For many years the mucin layer was thought to be 0.02 to 0.05 μm in thickness.[22] It is probable that earlier work measured the thickness of the glycocalyx. The thickness is now thought to be at least 0.4 to 1.0 μm over the cornea and 2 to 7 μm over the conjunctiva.[23] In a recent report, the thickness of the tear film was measured at 40 μm, and after application of acetyl cysteine to remove the mucin layer, the remaining precorneal tear film measured 7 μm in thickness.[2] Therefore, contrary to common belief, the tear film could be more mucin-based than aqueous-based.

The function of the mucin layer is open to question. Holly and Lemp[24] suggested that the main function of the mucin layer was similar to a wetting agent and rendered the hydrophobic corneal surface hydrophilic. However, the corneal epithelium is intrinsically wettable by aqueous solutions,[25,26] and mucin probably functions more as a lubricant than a wetting agent.[5] Mucin may also aid in hydrating[23] and protecting the underlying ocular surface epithelia.

Ocular Surface Epithelium and Glycocalyx

The corneal epithelium is composed of 5 to 6 orderly layers of nonkeratinized, stratified squamous cells. The bulbar conjunctival epithelium is composed of 6 to 9 less orderly layers of nonkeratinized columnar cells. The corneal and conjunctival barrier functions are formed and maintained by tight, intercellular desmosome attachments between the adjacent superficial cells.[27,28] The superficial epithelial cells have a large number of microvilli and microplicae on the apical surface. These structures increase the surface area of the ocular surface epithelia, facilitating absorption of substances from the tear film. The microvilli and microplicae are covered by the glycocalyx, which anchors the goblet cell secreted mucin to the underlying ocular surface epithelium.

CLASSIFICATION OF DRY EYE

Dry eye conditions can be classified into aqueous-adequate and aqueous inadequate forms (Fig 1). Aqueous-adequate forms of dry eye are those conditions in which symptoms of dry eye are present and aqueous tear production is normal. Aqueous-inadequate disorders are those due to Sjögren's syndrome and those due to non-Sjögren's etiologies, such as acquired immunodeficiency syndrome (AIDS),[29] graft-versus-host disease,[30] and aging. KCS tends to be much more severe in Sjögren's syndrome patients than in non-Sjögren's syndrome patients. The actual tear film structure in patients with KCS is not known. It is assumed that decreased aqueous tear secretion causes tear film insufficiency, which results in ocular surface disease. If this assumption is correct, changes in tear secretion should be correlated with ocular surface changes. Unfortunately, they are not. Commonly used clinical tests for KCS measure tear secretion and the status of the ocular surface.

MEASUREMENT OF TEAR SECRETION AND EPITHELIAL INTEGRITY

Schirmer's Tests

In 1903, Schirmer[31] introduced the tests that bear his name. His original Schirmer I, II, and III tests were used to quantify reflex tearing due to conjunctival, nasal, and light stimulation, respectively. Wetting of the Schirmer strip is biphasic with an initial rapid wetting phase over the first 1 to 2 minutes followed by a slower phase.[32, 33] The initial rapid rate can be attributed to the absorption of fluid from the inferior tear meniscus and conjunctiva sac and to an initally higher reflex tearing rate. Jones[11] introduced the basal tear secretion test, which is the Schirmer I test performed with anesthesia. A kinetic Schirmer test can be performed by measuring the amount of wetting every minute and plotting the amount of wetting versus time to determine initial and final wetting rates and an exponential decay constant.[4, 32]

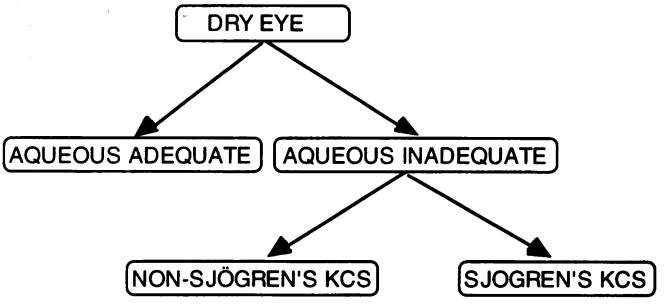

FIGURE 1

Classification of dry eye.

In clinical practice, the Jones basal tear secretion test and Schirmer's I test are usually performed. In patients with KCS due to Sjögren's syndrome, Schirmer's II test may be useful, as there is an increased loss of reflex tear secretion compared with non-Sjögren's syndrome KCS.[34]

Tear secretion measured by Schirmer's test does not differ between the sexes, but is reduced in women after the sixth decade, perhaps owing to hormonal changes related to menopause. Reflex tear production decreases with increasing age,[31, 35-40] but basal tear secretion does not.[41]

The main criticisms of Schirmer's test are its poor reproducibility, intrasubject variability, and low sensitivity and specificity. Schirmer's I test values are not correlated with tear meniscus height,[41] tear evaporation,[42] fluorescein tear breakup times,[42, 43] or tear lactoferrin levels.[37] In patients with less than 15 mm of wetting in 5 minutes, there is correlation of Schirmer's I test values and tear lysozyme levels.[44] In normal subjects, kinetic Schirmer test parameters were not correlated with symptoms, tear meniscus height, non-invasive tear break-up time, or tear protein levels.[45]

Cotton Thread Test

The first cotton thread tear test was introduced in 1976.[46] A cotton thread is placed in the inferior tear meniscus, and the amount of wetting is recorded over 15 seconds. This test probably does not measure basal secretion rates and may actually be a measure of tear volume.[27, 47, 48] No correlation between Schirmer's test and cotton thread values has been found.[49]

Tear Film Dilution Tests

In KCS, tear volume is decreased owing to the lack of tear secretion.[3] Semiquantitative evaluation of tear production can be done by placing a drop of 2% fluorescein[50] or 10 μl of a 1% fluorescein/rose bengal solution[51] into the eye. The diluted appearance of a tear sample can then be matched against standards. Decreasing tear production with age has been shown by this method, but no differences between sexes has been found.[51]

Fluorescein Staining

Fluorescein is a water-soluble dye that can penetrate between damaged corneal epithelial cells and accumulate in the intercellular spaces.[52] Recent studies suggest that fluorescein also acts as a vital stain, adhering to devitalized cells.[53-55] In KCS, fluorescein staining is usually confined to the interpalpebral conjunctiva and cornea. Staining in KCS may be due to pooling of fluorescein in areas of epithelial cell loss,[52] penetration of fluorescein between cells resulting from compromise of epithelial tight junctions,[56] or staining of devitalized cells.[53-55] Fluorescein staining of the cornea is not highly specific for KCS, as it does occur in normal eyes on occasion.

Rose Bengal Staining

Rose bengal has been used widely to diagnose KCS. Although it has been thought to be a vital dye staining dead or devitalized cells,[57] it may actually stain areas where the mucin layer is discontinuous.[54,55] The degree of rose bengal staining does not appear to be correlated with the Schirmer I or tear breakup tests.[39] In clinical trials of artificial tear preparations, symptoms improve and rose bengal staining decreases, but Schirmer test values remain the same.[58,59]

Summary

The mainstay of measuring tear secretion and diagnosing the aqueous-indequate eye is the Schirmer test. It is a rather gross test that is not highly specific for KCS, and it does not appear to correlate with most of the other clinical diagnostic tests for dry eye. Tear film dilution tests may be more useful but have not been widely used. Clinical assessment of corneal epithelial integrity is based on subjective observation of corneal epithelial staining with fuorescein or rose bengal. These tests are, at best, crude measures of the function of the tear film and corneal epithelium.

FLUOROPHOTOMETRY

Fluorophotometry allows in vivo assessment of the tear film dynamics of tear turnover, tear volume, and tear secretion. It can also be used to measure corneal permeability, which reflects the integrity and physiology of the corneal epithelium.

THE FLUOROPHOTOMETER

In 1954, Langham and Wybar[60] were the first to use fluorophotometry when they studied aqueous humor dynamics. In 1963, Maurice[61] modified their fluorophotometer by introducing a blue filter in the excitation beam pathway. In 1975, Cunha-Vaz and associates[62] introduced scanning fluorophotometry which led to commercial development of the Fluorotron Master (Coherent Radiation, Inc, Palo Alto, Calif). Brubaker modified the Fluorotron Master by introducing an XY scanning fluorophotometer.

The basic fluorophotometer uses a beam of light passing though an excitation filter (430 to 490 nm) to excite the transparent layers in the eye. The emitted light from ocular tissues passes back through a fluorescence emission filter (510 to 630nm). Both the excitation (blue light) and the fluorescent light pass through the same lens system before being separated by their respective filters (Fig 2). The area of measurement is called the focal diamond. The amount of fluorescence in the focal diamond of the excitation and emission beam is measured with a photomultiplier and registered by a computer. For anterior segment fluorophotometry an anterior segment attachment is used. With this attachment the focal diamond is

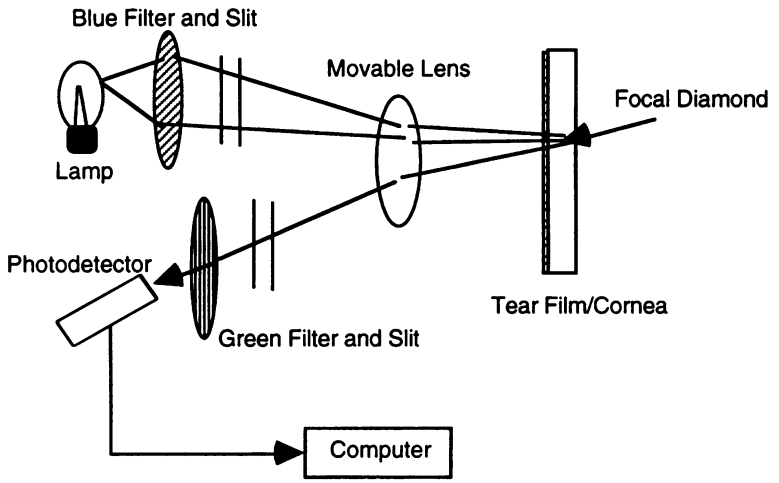

FIGURE 2
Diagrammatic representation of fluorophotometer.

moved forward along the desired axis (usually the optical axis of the eye being measured) by moving the lens system with a stepped motor. Over approximately 8 seconds, 12 points/mm are scanned. The focal diamond is 50 μm wide, 1.9 mm high, and approximately 0.50 mm deep.[63]

Because the depth of the focal diamond approximates corneal and tear film thickness, it has insufficient resolution to distinguish fluorescence between the two (termed limited spatial resolution). This limited spatial resolution poses a significant problem in determining corneal permeability, as tear film fluorescence must be eliminated before corneal fluorescence can be measured.

THE FLUOROPHORES

Disodium Fluorescein (DSF)

DSF is the most frequently used fluorophore in assessing tear turnover and corneal permeability. It is a hydrophilic molecule with a molecular weight of 376.27. Its excitation and emission wavelengths are 475-490 nm and 510-520 nm respectively with a peak fluorescence at pH 7.4. It has a relatively low toxicity topically and intravenously.[64]

Carboxyfluorescein (CF)

Carboxy-fluorescein (bis-(N,N-bis-(carboxymethyl)-aminoethyl)-fluorescein tetrasodium) has a molecular weight of 710.47. It is, however, more hydrophilic than DSF.[65] Its activity and excitation and emission spectrums are similar to those of DSF. The CF molecule is larger than DSF and has been shown to penetrate soft plastics much more slowly than DSF.[66]

FLUOROPHOTOMETRIC DETERMINATION OF TEAR TURNOVER RATES

Because of induced reflex tearing, measurement of dynamic changes in tear turnover is difficult. However, with fluorophotometry small amounts of fluorescein can be used to avoid this problem. Once a drop of fluorescein has been instilled, measurement of tear turnover can be delayed until reflex tearing has subsided. Several studies have determined tear turnover by fluorophotometry.

Historical Survey

In 1966, Mishima and associates[3] studied the dynamics of tear flow in humans using a slit-lamp fluorophotometer. They found that the disappearance or decay of fluorescein in the tear film occurs in a single exponential manner. However, the decay was biphasic, being more rapid in the first 5 minutes, which they attributed to reflex lacrimation. After 5 minutes they believed the decay to be physiologic. They suggested that including measurements obtained in the first 5 minutes after drop instillation may lead to an artificially elevated tear turnover rate. Therefore, tear turnover rates should be determined after the effects of reflex tearing have subsided. Their initial tear turnover rates showed individual variation and were slower in older subjects. There was no difference in physiologic tear turnover rates between the two age-groups (Table I). This may suggest that reflex, but not physiologic tearing, decreases with age.

Puffer and associates[67] in 1980, measured tear turnover in 52 normal subjects using 1 μl of 10% DSF. Fluorophotometric measurements were made at 5 minute intervals over 45 to 60 minutes. Fluorescence was plotted against time, and the resultant slope represented the tear turnover rate. On the basis of decay curves, they separated their subjects into three groups. The first group eliminated DSF from at least one eye in 30 minutes. In this group tear turnover was 29 ±18 %/min in right eyes and 25 ±13 %/min in left eyes. In the second group, measureable DSF was present after 30 minutes in one eye with the exponential loss occuring between 15 to 30 minutes. In this group tear turnover was 11.1 ±4.7 %/min in right eyes and 11.0

TABLE I: INITIAL AND PHYSIOLOGIC TEAR TURNOVER RATES IN YOUNG AND OLD SUBJECTS

AGE (YRS)	INITIAL TT (%/MIN)	RANGE (%/MIN)	PHYSIOLOGIC TT (%/MIN)	RANGE (%/MIN)
20-45	52	8-200	16	9-25
50-89	38	12-72	18	3-40

TT, Tear turnover

From Mishima et al.[3]

±4.6%/min in left eyes. In the third group there was one eye that did not lose any DSF after 30 minutes. The overall mean tear turnover for the three groups was 15.4 ±11.9%/min. The investigators suggested that the rate of fluorescein loss by diffusion into the cornea and conjunctiva was slow and probably relatively slower than loss of water by evaporation. They attributed most of the loss of DSF to drainage into the nasal lacrimal system.

In 1987 Webber and Jones[68] studied 16 subjects using a continuous reading fluorophotometer after application of 1 μl of 2% DSF. They obtained a mean tear turnover rate of 14.9 ±5.6%/min (range: 5.2 to 27.2%/min) with no significant differences between the right and left eyes. They also added two important findings. First, they found that tear turnover rates were significantly higher in the morning than the afternoon, suggesting a circadian rhythm to tear turnover. Previous attempts to demonstrate a circadian rhythm to tear secretion using Schirmer's test were not successful.[35] Second, when they used 10% DSF, they found that tear turnover was significantly underestimated. This underestimation was due to quenching (absorption of excitation light without re-emission) and self-absorption effects of fluorescein in the tear film. These effects were more marked near the time of dye instillation causing the fluorescence and its rate of decay to be underestimated.[68] The near zero turnover rates seen in some eyes by Puffer and associates[67] may have been due to this phenomena. Therefore, careful attention must be paid to the amount and concentration of fluorescein used in order to prevent dye quenching and self-absorption which cause anomalous tear turnover rates.

Occhipinti and colleagues[69] placed one drop from a saline moistened fluorescein-impregnated strip into the eyes of 12 normal subjects, 21 to 28 years of age. After 2 to 5 minutes, serial measurements were made. They obtained tear turnover rates of 27 ±14%/min (range 7 to 51%/min) for right eyes and 32 ±18%/min (range 11 to 60%/min) for left eyes. They found no correlation with Schirmer's I test values (when less than 30-mm wetting per 5 minutes).

Kuppens and associates[70] determined the tear turnover rates in normal subjects (15.7 ±5.3%/min), chronic open-angle glaucoma patients using timolol (10.1 ±3.2%/min) and chronic open angle glaucoma patients not using timolol (12.3 ±4.1%/min). Tear turnover was significantly lower for both groups of glaucoma patients. Tear secretion was decreased 22% when compared with normals. Tear turnover rates were not correlated with intraocular pressure, age, or Schirmer's test values.

Kok and associates[71] measured tear turnover in 25 rigid contact lens wearers, ages 20 to 42 yrs. With use of 1 μl of 2% DSF and the Fluorotron Master fluorophotometer, the eyes were scanned every 2 minutes for 30 minutes. Tear turnover rates were 15.2 ±4.9%/min in contact lens wearers and 17.5 ±6.8%/min in noncontact lens wearers. Therefore, the presence or absence of rigid contact lenses had no bearing on tear turnover rates.

Table II summarizes the tear turnover rates for normal subjects obtained by various investigators.

Tear Volume and Flow Determinations

Fluorophotometry can be used to determine tear volume, tear flow, and tear turnover. After instilling a known volume and concentration of fluorescein, the decay in tear film fluorescence can be measured over time. The slope of the exponential regression of the decay curve gives the decay constant or tear turnover rate. Tear volume (V) can be calculated by knowledge of the initial volume (V_i) and concentration (C_i) of fluorescein and determination of the fluorescein concentration (C_f) of a microsample volume (V_f) of tears taken after fluorescein instillation using the following equation

$$V=V_f\,(C_f/C_f) - V_i$$

Multiplying the turnover rate (%/minute) by the tear volume, the tear flow rate (μL/min) can be obtained.[69] Some investigators report no influence of age on tear flow rates,[3] while others have shown reduced tear flow in older subjects.[12] No gender differences in tear flow rate or tear volume have been found.[72, 73] Tear turnover is affected by conjunctival permeability.[74]

What Does Tear Turnover Measure?

Strictly speaking, tear turnover (TT) measures much more than tear secretion. It is proportional to the sum of the effects of secretion by the lacrimal gland (T_s), transudation of fluid through the conjunctiva(C_t), tear drainage through the nasal lacrimal duct system (T_d), tear evaporation (T_e), conjunctival permeability (P_c) and corneal permeability (P_{dc}), or

$$TT \subset (T_s + C_t) - (T_d + T_e + P_c + P_{dc})$$

TABLE II: SUMMARY OF TEAR TURNOVER RATES

INVESTIGATORS	YEAR	NO. EYES	AGE (YR)	MEAN (%/MIN)	RANGE OR SD
Mishima et al[3]	1966	18	20-45	16	9-25
Puffer et al[67]	1980	30	19-50	15.4	± 11.9
Jordan and Baum[12]	1980	18	25-45	12	± 3
Webber and Jones[68]	1986	20	21-39	14.9	± 5.6
MacDonald and Maurice[74]	1991	12	25-66	24	1-42
Kok et al[71]	1992	25	20-42	17.5	± 6.8
Kuppens et al[70]	1992	27	40-80	15.7	± 5.3

Tear turnover time can be thought of as a physiologic measure that takes into account everything that affects the tear film. For example, normally: $T_d > T_e > P_c > P_k$. After punctal occlusion, where $T_d = 0$, tear film evaporation and conjunctival and corneal permeability become the major influences on tear turnover. Tear turnover may more accurately reflect tear film dynamics than measurements of the individual components.

Potential Sources of Error

There are several sources of error that can occur in the measurement of tear turnover rates. Due to reflex lacrimation, there is a biphasic decay of fluorescein from the tear film, with the first 5 minutes having a faster rate of decay. Therefore, physiologic turnover rates should be calculated using fluorophotometric scans obtained after the first 5 minutes. Because of the limited spatial resolution of the fluorophotometer, fluorescence depends not only on the concentration of fluorescein in the tear film but also on the tear film thickness Blinking is important in spreading the tear film over the ocular surface. It has been shown that forced blinking can substantially increase the thickness of the tear film, while a weak blink decreases it.[7] Therefore, fluorophotometric measurements must be made at a consistent and fixed amount of time after a blink. Also, after initial instillation of fluorescein, tear film thickness increases so that measurements obtained in the first 5 minutes are not comparable to those obtained afterward.

Because of the limited spatial resolution of the fluorophotometer, fluorescence in the cornea is also measured with tear film fluorescence. After application of fluorescein, it will diffuse into the corneal stroma over time. As long as the tear fluorescein levels are much higher than corneal stromal values, they do not contribute significantly to the fluorescence level. These effects can be minimized by performing the scans within the first 20 to 30 minutes.

Finally, the cornea possesses inherent autofluorescence. Because of the limited spatial resolution of the fluorophotometer, corneal and tear film fluorescence levels must be corrected by subtracting corneal autofluorescence levels.

FLUOROPHOTOMETRIC DETERMINATION OF CORNEAL PERMEABILITY

Methods of Measurement

Four different methods have been used to determine corneal permeability. The first determines the amount of corneal fluorescence at a given time point after topical administration of DSF (drop-method).[56, 75-77] As will be discussed, corneal permeability determined by this method is dependent on individual tear turnover rates. In the second method (bath method), subjects immerse their eye(s) in a bath containing DSF.[78, 79] The eye is rinsed and measurements are taken at specified time points over the next 60 min-

utes. This method takes tear turnover into account. In the third method (modified-drop method), DSF is applied as a drop and the initial tear film concentration measured by direct sampling.[80] Fluorophotometric scans are done every 90 seconds for 15 minutes and then 45 minutes after dye instillation. This method also takes tear turnover into account. The fourth method is a novel method for simultaneously determining corneal permeability and tear turnover.[81] It is this method that serves as the basis for the following experiments.

Historical Survey

Drop Method: Whaltman and Patrowicz,[75] in 1970, compared the penetration of 2% DSF in various vehicles by measuring corneal stromal DSF concentrations. At 1 hour, peak corneal DSF concentrations were 3.18 ±0.41 x 10^{-4}mg/mL (SE, standard error) for aqueous solutions and 2.60 ±0.24 x 10^{-4} and 4.59±0.47 x 10^{-4} mg/mL for polyvinyl alcohol-based and hydroxypropylmethyl cellulose-based vehicles, respectively. They found significant interindividual and intraindividual variation in ocular corneal penetration of DSF (twofold to fivefold). This variability is likely due to the fact that tear turnover was not taken into account. Single-versus multiple-drop applications of DSF had no effect on corneal stromal concentrations. Doubling the concentration of DSF doubled the peak concentration. These data suggest that the vehicle and concentration used affect penetration into the corneal stroma.

In 1971, Marsh and Maurice,[76] studied the effects of nonionic detergents and other surface-active agents on corneal permeability. A single drop of detergent increased corneal permeability five times over that of controls. Therefore, preservatives that are present in topical medications and lubricants or in the fluorescein solution can affect corneal permeability.

In 1981, Berkowitz and associates,[82] measured the corneal penetrance of DSF following penetrating keratoplasty. At 1 week, corneal permeability was 67 times that of controls. Corneal stromal fluorescence was measured 45 minutes after instillation of one drop of 2% DSF. Control values were 0.25 ±0.013 μg/mL and healed keratoplasty values were 0.835 ±0.093 μg/mL. Increased stromal fluorescence was correlated with the amount of punctate keratopathy present and the size of the epithelial defect. This study emphasized that epithelial integrity is important in maintaining the corneal epithelial barrier function and that punctate epithelial defects increased corneal permeability to DSF.

In 1989, Göbbels and Spitznas[56] compared the corneal permeability of normal subjects with that of patients with dry eyes. Using 40 μLof 2% DSF, they measured mean corneal stromal DSF concentrations after 45 minutes. Corneal stromal fluorescence was 222.14 ±110.45 ng/mL in normal subjects and 631.50 ±252.32 ng/mL in dry eye patients (~threefold increase). They also showed that benzalkonium chloride increased corneal permeability.

In addition, these investigators showed that treatment of dry eye patients with artificial lubricants decreased corneal permeability.[83] Stromal fluorescence values were 201.7 ±104.2 ng/mL in normal control subjects and 635.2 ±176.7 ng/mL in dry eye subjects. After treatment with topical nonpreserved artificial tears, fluorescence decreased from 699.7±186.3 to 369.8 ±156.3 ng/mL. In dry eye patients using artificial lubricants preserved with benzalkonium chloride, stromal fluorescence did not change significantly (584.9 ±198.6 ng/mL). This emphasizes that use of benzalkonium chloride-preserved artificial tears does not restore the corneal barrier function.

In 1993, Chang and Hu[77] using the drop-technique with 20 μL of 2% DSF, determined corneal permeability in normal subjects. They obtained a mean value for stromal fluorescence of 373.2 ±551.2 ng/mL. They also found that corneal autofluorescence increased with age.

All of these studies failed to take tear turnover into account. Failure to do so did result in variable values of corneal permeability between subjects. For example, for a tear turnover rate of 3%/min over 45 minutes, the amount of fluorescein in the tear film that can diffuse through the corneal epithelium is 12 times larger than that for a tear turnover rate of 40%/min.[84] Therefore, any method used to determine corneal permeability must also take into account the tear turnover rate for each individual subject. To do so requires knowledge of the initial (time zero) concentration of DSF in the tear film.

Bath Method : One approach to obtaining the initial concentration of DSF in the tear film involves the subject placing his or hers eye in an eye bath containing a known concentration of DSF.[78] After 3 minutes, the eye is rinsed, and measurements are taken four times over the next hour. The initial tear film concentration of DSF is assumed to be equal to that of the eye bath. Permeability was calculated using the following relationship:

$$\text{Corneal permeability } (P_{dc}) = d_c \cdot C_c(t_b)/C_b \cdot t_b \cdot 10^6/60$$

In this formula, d_c is the average thickness of corneal stroma along the optical axis. $C_c(t_b)$ is the inital tear film concentration of corneal fluorescein directly after rinsing the eye and is determined by exponential regression of the fluorophotometric data points to time zero. C_b was the concentration of DSF in the eye bath, and t_b was the bathing time (min^{-1}). The factor $10^6/60$ is to convert mm/min into nm/sec. A correction factor of 1.56 (for a corneal thickness of 0.52 mm) was used to correct for the limited spatial resolution of the fluorophotometer. The bath method assumes that diffusion of DSF from the central cornea to the limbus can be neglected and that fluorescein loss from the cornea is negligible. In addition, it is assumed that the concentration of DSF in the tear film is equal to that of the eye bath.

de Kruijf and associates[78] obtained corneal permeability values of 0.008 to 0.090 nm/sec with a mean of 0.038 ±0.017 nm/sec in a group of normal

subjects. Corneal permeabilities were not correlated with age. They found similar values in both eyes, which were reproducible within 10% over a 3-month period. When phosphate buffer was used instead of balanced tear bath solution, corneal permeability values increased five to six times.

Khalil and colleagues[79] determined corneal permeability of patients with Graves' disease (0.053 ±0.043 nm/sec) to be increased over that of controls (0.038 ±0.017 nm/sec). This increase was found to correlate with the degree of exophthalmos.

Modified-Drop Method : Göbbels and Spitznas[80] described a modification of the drop method, which takes tear turnover into account. After placing 1 µl of 10% DSF, a tear specimen was taken from the inferior tear meniscus and the initial concentration of DSF (C_0) was measured by fluorophotometry. After 4 minutes, the decrease in the concentration of DSF in the tear film was measured every 90 seconds over a period of 15 minutes (C_t). After 45 minutes, the increase in corneal stromal fluorescence (ΔF) was measured:

$$\Delta F = C_{45} - C_0$$

The degree of corneal permeability was expressed as the corneal epithelial transfer coefficient (K_e) from time 0 to 45 minutes or:

$$K_e = \Delta F / \int C\,(t)$$

In control eyes K_e was 0.41 ±0.23 x 10^{-5} min^{-1}, and in dry eyes K_e was 1.21 ±0.47 x 10^{-5}. When dry eye patients were treated with artificial lubricants, K_e values improved with unpreserved artificial lubricants but not with lubricants containing benzalkonium chloride. Table III summarizes corneal epithelial permeability values from various investigators.

Corneal Permeability of Carboxyfluorescein

In 1987, Araie and Maurice[65] used an isolated rabbit cornea preparation to determine the permeability of corneal epithelium, stroma, and endothelium. They found the epithelial permeability for DSF to be 2.6 ±0.9 x10^{-5} cm/min (0.433 ± 0.15 nm/sec) and CF to be 1.6 ±0.5 x10^{-5} cm/min (0.267

TABLE III: CORNEAL EPITHELIAL PERMEABILITY IN HUMANS TO DSF DETERMINED BY THE BATH METHOD

INVESTIGATORS	YEAR	NO. EYES	AGE (YR)	MEAN (ng/sec)	SD
de Kruijf et al[78]	1987	86	15-67	.038	±.017
Khalil et al[79]	1990	46	not stated	.038	± .017
Mc Namara et al[86]	1994	7	not stated	.076	±.012

DSF, disodium fluorescein.

±0.083 nm/sec). They compared this to an in vivo corneal epithelial permeability of 0.7 ±0.4 x10^{-5} cm/min (0.118 ±0.067 nm/sec) for DSF (Table IV).

McCarey and Reeves[85] used carboxyfluorescein (CF) to determine corneal epithelial permeability in rabbits. The rabbit cornea was bathed in 2.7 x 10^{-3} M CF for 5 minutes and then completely rinsed from the eye. Fluorescence was measured at 3, 60, and 120 minutes and corneal permeability values of 0.0188 to 3.757 nm/sec, with a mean of 0.0614 ±0.0316 nm/sec, were obtained. They observed that larger permeability values were obtained when naturally occurring epithelial defects were present. In the rabbit, corneal permeability was not correlated with the contralateral eye or with subsequent measurements during the day.

Simultaneous Determination of Corneal permeability and Tear Turnover: Paugh and Joshi, in 1993,[81] suggested a novel method of simultaneously determining corneal permeability and tear turnover. After measurement of baseline corneal autofluorescence, a 2 µL drop of 0.75% DSF was applied to the eye. Fluorophotometric scans were obtained immediately and as often as possible for the next 8 minutes and then every 2 minutes for an additional 12 minutes. At the end of 20 minutes, the eye was washed and the corneal fluorescence measured. Corneal permeability was determined by dividing the resultant increase in corneal stromal fluorescence by the integral of the tear film fluorescein concentration calculated over 20 minutes. Tear turnover can also be calculated. McNamara and associates[86] used this method to measure corneal permeability in seven healthy subjects. Using a 2 µL drop of 0.35% DSF, they obtained a mean corneal permeability value of 0.076 ±0.012 nm/sec (SE). They felt that the repeatability was unreliable for monitoring individual patient changes, but with an

TABLE IV: CORNEAL EPITHELIAL PERMEABILITY OF CF IN RABBITS

INVESTIGATORS	METHOD	YEAR	DYE	MEAN (ng/sec))	SD
Araie and Maurice[65]		1971			
In vitro			DSF	.433	±.15
	In vitro		CF	.267	±.083
	In vivo		DSF	.118	±.067
McCarey and Reeves[85]	In vivo	1994	CF	.061	±.032

CF, carboxyfluorescein; DSF, disodium fluorescein.

appropriate sample size the technique might be valuable to compare moderately large differences in treatment effects between groups of subjects.

In this thesis, the simultaneous method was investigated further. Both DSF and CF were used to determine corneal permeability and tear turnover in normal subjects and patients with KCS due to Sjögren's syndrome. Factors that might influence tear turnover and corneal epithelial permeability were investigated.

EXPERIMENTAL STUDIES

MATHEMATICAL DESCRIPTION OF TECHNIQUE

Determination of Fluorescein Mass

The theoretical basis for this technique has been described previously.[81] Figure 3 shows a sheet of infinitesimal thickness dq, which is perpendicular to the axis of the fluorophotometer and an element of area Q corresponding to the area of the focal diamond. The volume of this element is Qdq, and the mass of fluorescein within it is dm_q.

$$dm_q = C_q Q dq$$

C_q is the concentration of fluorescein. When the focal diamond is at position x, the fluorescence recorded, f_x, is proportional to dm_q and a spread function, S_x, which is dependent on the optics of the fluorphotometer. Therefore:

$$f_x = S_x dm_q \qquad (1)$$

As the focal diamond scans through the element, a profile of the fluorescence readings corresponding in shape to the spread function and the area under the profile is da_q and can be expressed by:

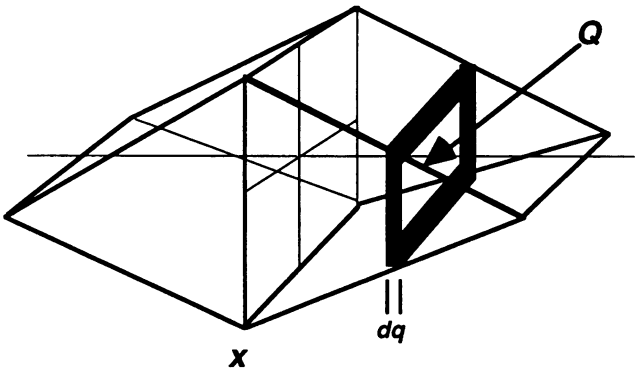

FIGURE 3
Three-dimensional representation of focal diamond.

$$da_q = \int f_x dx \quad (2)$$

Substituting equation (1) into (2):

$$da_q = \int S_x dm_q dx \quad (3)$$

or

$$da_q = dm_q \int S_x dx \quad (4)$$

as $\int S_x dx$ can be represented by a constant, S, then

$$da_q = S dm \quad (5)$$

As the instrument scans through a finite thickness, q , of the tear film and cornea, the area, a, under the corresponding fluorescent profile is given by:

$$a = \int da_q \quad (6)$$

or:

$$a = S \int dm_q \quad (7)$$

Because the total mass, m, of fluorescein in this tissue is equal to $\int dm_q$, the area under the fluorescent profile is given by:

$$a = Sm \quad (8)$$

Therefore, the area under the fluorescence curve is proportional to the mass of fluorescein in the cross section of the focal diamond regardless of its distribution.

Epithelial Permeability

If we assume that the transfer of fluorescein across the corneal epithelium follows simple first-order kinetics, then:

$$dm_c/dt = P_{dc} Q (C_d - C_c) \quad (9)$$

m_c is the mass transferred into the cornea from the tears, P_{dc} is the epithelial permeability, Q is the area of fluorescein transfer, C_d is the fluorescein concentration in the tears, and C_C is the fluorescein concentration in the cornea. As the concentration of fluorescein in the tear film is much greater than that of the cornea, equation (9) becomes:

$$dm_c/dt = P_{dc} Q C_d \quad (10)$$

Integration of equation (10) gives:

$$P_{dc} = \frac{M_c}{(Q \int C_d dt)} \quad (11)$$

The tear film concentration of fluorescein is equal to the mass of fluorescein in the tear volume, or:

$$C_d = \frac{M_d}{q_d Q} \quad (12)$$

Substituting equation (12) into (11):

$$P_{dc} = \frac{q_d m_c}{\int m_d dt} \quad (13)$$

From equation (8), the area under the fluorescence curve equals a constant, *S*, times the mass of fluorescein. Substituting for *md* and *mc* :

$$P_{dc} = \frac{q_d a_c}{\int a_d dt} \quad (14)$$

As the corneal posseses inherent autofluorescence, a^0_c, this value must be subtracted from the final value, a^f_c, when no fluorescein remains in the tear film to obtain the true value penetration of fluorescein into the cornea, a_c, or:

$$a_c = a^f_c - a^0_c \qquad (15)$$

The value used for thickness of the tear film, q_d, is assumed to be constant at 8 μm.

Because of the limited spatial resolution of the Fluorotron, the peak fluorescence curve contains values from both the tear film and cornea. Therefore, fluorescein must be thoroughly irrigated from the eye prior to measuring a^f_c. The denominator of equation (14) is the area under the curve, a_t, obtained by plotting the tear-corneal fluorescence peaks versus time. In addition, the true area under the tear film peak, a_d, will contain a contribution from the cornea, a^f_c. Therefore:

$$a_d = a_t - a^f_c \qquad (16)$$

Tear Turnover Rate

After the first 5 minutes following instillation of fluorescein, dye disappearance follows first-order kinetics. Tear turnover rate can be calculated according to the the following equation:

$$C_d = C^0_d \, e^{-kt} \qquad (17)$$

The concentration of fluorescein in the tear film, C_d, is proportional to the area under the tear-cornea scan, a_d, assuming a constant tear film thickness. The tear turnover rate, k, can be determined by an exponential regression of equation (17) or by the linear regression of the natural logrithm of a_d against time.

MATERIALS AND METHODS

Fluorophotometer

Fluorophotometry was done using a Fluorotron Master (Coherent Radiation, Inc, Palo Alto, Calif) with an anterior-segment attachment using standard excitation and emission filters and scanning software.

Fluorophores

A 0.75% solution was prepared by diluting a 10% stock solution of disodium fluorescein (DSF, Alcon Laboratories, Fort Worth, Tex) with balanced salt solution (BSS, Alcon Laboratories, Fort Worth, Tex). CF was obtained from a commercially available source as a 0.35 % solution. Subsequent dilutions of both DSF and CF were made by diluting with BSS.

Fluorophotometry

Subjects were seated and positioned at the fluorophotometer and baseline scans were obtained to determine corneal autofluorescence. An eye was

chosen at random to first receive a 2-μL drop of DSF. Fluorophotometric scans of the tear film and cornea were done immediately after fluorescein instillation, in rapid succession for 8 minutes, and then every 2 minutes for the next 12 minutes. At the end of 20 minutes, the eye was washed three times with balanced salt solution and three consecutive fluorophotometric scans of the cornea were done. After completing the first series of scans, the fellow eye received 2 μL of CF. Fluorophotometric scans were repeated for the fellow eye. Subjects were asked to blink just before the start of a scan but not during the scan. All scans were done along the visual axis of the cornea.

Data Analysis

The computer printout of the fluorophotometric scan was used to identify the position of peak fluorescence. The area under the fluorescence peak was estimated by measuring the fluorescence values in 16 equal increments on each side of the peak value. A total of 33 fluorescence values were used. The area under each peak represented the combined fluorescence of the tear film and cornea prior to washout and only corneal fluorescence postwash. The peak area was plotted against time and the area under the resultant curve calculated by determining the time integral of tear mass using a commercially available curve-fitting software program (KaleidaGraph, Synergy Software, Reading, Pa). To determine a_d, the area due to tear film fluorescence, the area due to corneal fluorescence, a^f_c was subtracted from a_t. To determine the true value for a_c, the area due to baseline autofluorescence, a^0_c was subtracted from the postwash area values, a^f_c. Tear film thickness was assumed to be 8 μm. Corneal permeability (ng/sec) was calculated from equation (14). The tear turnover rate (%/min) was equal to the slope of the line obtained by linear regression of the natural log of a_d against time for the time periods 0 to 5, 5 to 20 and 0 to 20 minutes.

Subjects

The study protocol and informed consent were reviewed and approved by the local Institutional Review Board. Informed consent was obtained from all study participants. Normal subjects were those who had previously been characterized as having no symptoms of dry eye and no evidence of fluorescein or rose bengal staining. Fluorescein breakup times were normal, and Schirmer's I tests were >10 mm wetting per 5 minutes. KCS patients were those who had previously been diagnosed as having KCS due to Sjögren's syndrome. To be classified as having KCS, patients had to have at least one of the following symptoms at the time of diagnosis: burning, foreign body sensation, or dryness. In addition, results of one or more of the following tests had to be abnormal: Schirmer's I test (≤ 5 mm wetting per 5 minutes), fluorescein or rose bengal staining (scores ≥3 out of 9 for both eyes using van Bijsterveld's grading system),[87] and a fluorescein tear breakup time (<

10 seconds). Sjögren's syndrome was diagnosed when symptoms of KCS and/or xerostomia were present in patients with a systemic immunopathy as determined by elevated titers of one of the following autoantibodies: rheumatoid factor, antinuclear antibody, SSA (anti-Ro) antibody, or SSB (anti-La) antibody.

Study 1 : Establishment of the Technique
To refine the details of this fluorophotometric technique, corneal epithelial permeability values and tear turnover rates were determined in 12 normal subjects (11 female and 1 male) using DSF in one eye chosen at random for each patient. The mean age was 40.8 ±12.6 years (range, 22 to 64). To determine the effects of reflex tearing, turnover times were calculated from 0 to 5 (reflex turnover), 0 to 20 (overall turnover), and 5 to 20 minutes (actual or physiologic turnover). Two subjects underwent subsequent measurements at later dates to determine the repeatability of the values.

Study 2 : Comparision of DSF and CF in Normal Subjects
To compare corneal epithelial permeability values and tear turnover rates using DSF (0.35%) and CF (0.75%),13 normal subjects (8 female and 5 male), ages 23 to 67 years, underwent fluorphotometry. One eye of each patient (chosen at random) received DSF, and the fellow eye received CF. Tear turnover rates were determined from 0 to 5, 0 to 20 and 5 to 20 minutes using both DSF and CF.

Study 3 : Establishment of Fluorescein Concentration in KCS Patients
The maximum concentrations of DSF and CF that could be used in patients with KCS to avoid quenching and self-absorption while ensuring an adequate fluorescein distribution in the tear film were determined. A single patient with moderately severe KCS due to Sjögren's syndrome underwent measurement of corneal epithelial permeability and tear turnover using various concentrations of DSF and CF (Table V). DSF was applied to one eye chosen at random and CF to the fellow eye. Determinations for paired eyes were done on different days over 1 month. Corneal epithelial permeability was plotted against fluorescein concentration to determine the range of concentrations over which corneal permeabilities remained stable.

Study 4 : Comparision of DSF and SF in KCS Patients
To compare corneal epithelial permeability and tear turnover, 13 patients (12 female and 1 male) with KCS underwent fluorophotometry using DSF and CF concentrations determined in study 3. The mean age of the patients was 48.5 ±15.3 years (range 19 to 70). Schirmer I test values and the grade of punctate staining were recorded for each eye. Punctate staining was recorded using a grading system of 0 to 4 . One eye was chosen at random from each patient to receive DSF and the other to receive CF.

TABLE V: CONCENTRATIONS OF DSF AND CF USED TO DETERMINE THE EFFECTS OF QUENCHING AND MIXING IN PATIENT WITH KCS

CF (%)	DSF (%)
.750	.350
.500	.250
.250	.200
.100	.150
.075	.100
.050	.050

CF, carboxyfluorescein; DSF, disodium fluorescein; KCS, keratoconjunctivitis sicca.

Study 5 : Corneal Permeability and Tear Turnover After Punctal Occlusion
One patient with KCS, whose symptoms were not responding to conventional medical therapy, underwent punctal occlusion of the inferior and superior puncta in both eyes. Corneal epithelial permeability values, tear turnover rates, and the degree of punctate staining were determined before punctal occlusion, immediately after punctal occlusion, and 6 and 35 days later using DSF.

STATISTICS

Assuming a two-tailed comparision of means at the 95% confidence level with an *a*-error of 5% and *b*-error of 5%, a sample size of 13 subjects for studies 2 and 4 was calculated. Mean values are reported with their standard deviation unless noted otherwise. Statistical analysis was done using a commercially available software program (Staview, Abacus Concepts, Berkeley, Calif). Correlation between continuous variables was determined by simple linear regression. Fisher's exact test was used to determine significance. Comparisons of tear turnover rates and corneal permeability values between groups were done with an unpaired Student's T test. Comparisons of tear turnover rates and corneal epithelial permeability values between eyes were done with a paired Student's T test. Comparison between continuous and nominal values was done using analysis of variance. Statistical significance was assumed at the 0.5 level.

RESULTS

Study 1 : Establishment to the Technique
Corneal Autofluorescence : For the first nine subjects, a single baseline corneal autofluorescence reading was obtained. For the last three subjects,

three baseline readings were obtained. The mean area under the baseline autofluorescence peaks (a^0_c) was 159.33 ±38.97 area units (range, 104.10 to 212.10 area units). There was no correlation between a^0_c and age. Individual postwashout values of a^f_c were relatively stable after irrigation (Table VI). There was no correlation of a^f_c with age.

There was a significant correlation between a^f_c (area under the postwash corneal fluorecence peak) and tear turnover rate (r^2=.362, P=.038) as shown in Fig 4.

TABLE VI: AREA UNDER CORNEAL FLUORESCENCE PEAKS (a^f_c) AFTER WASHOUT

SUBJECT	READING 1 (AREA UNITS)	READING 2 (AREA UNITS)	READING 3 (AREA UNITS)	MEAN a^f_c (AREA UNITS)	SD (AREA UNITS)
1	406.7	322.6	354.0	361.1	42.5
2	416.9	390.0	420.7	409.2	16.7
3	197.2	199.2	178.4	191.6	11.5
4	141.3	130.9	141.6	137.9	6.1
5	1308.0	1083.5	1183.7	1191.7	112.5
6	331.4	214.9	279.8	275.4	58.4
7	249.1	216.1	213.8	226.3	19.8
8	368.6	275.8	251.2	298.5	61.9
9	379.6	313.3	337.1	343.3	33.6
10	169.8	250.8	271.1	230.6	53.6
11	144.6	175.9	125.8	148.8	25.3
12	218.7	341.0	301.6	287.1	62.4

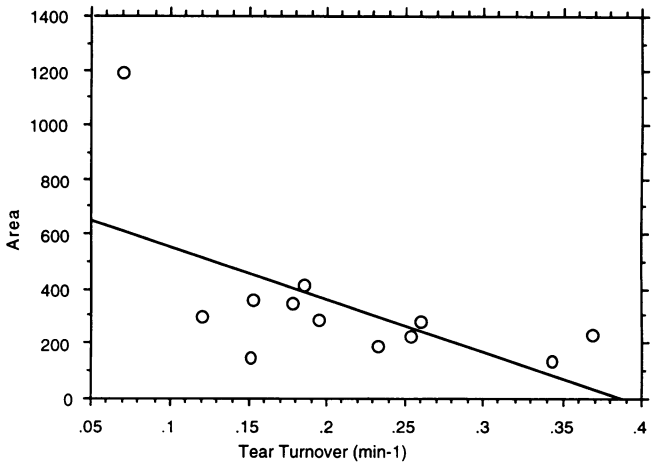

FIGURE 4

Regression plot of area under postwash corneal fluorecence peak (a^f_c) versus tear turnover rate (r^2=.362,P=.038).

Corneal epithelial permeability and tear turnover: Mean corneal epithelial permeability was 0.1239 ±0.0948 nm/sec. Mean tear turnover rate was 20.94 ±8.75%/min (Table VII). There was no significant correlation between corneal permeability and tear turnover rates. However, there was significant correlation between corneal epithelial permeability and age (Fig 5, r^2=.419, P =0023).

Repeatability: Two subjects were tested and epithelial permeability and tear turnover rates determined at two different days about 2 weeks apart (Table VIII).

TABLE VII: CORNEAL EPITHELIAL PERMEABILITY VALUES AND TEAR TURNOVER RATES IN NORMAL SUBJECTS

SUBJECT	PERMEABILITY (nm/sec)	TEAR TURNOVER (% min^{-1})
1	.0673	15.23
2	.2771	18.51
3	.0202	23.31
4	.0336	34.31
5	.3026	7.03
6	.1656	25.95
7	.0387	25.43
8	.1465	12.07
9	.1302	17.89
10	.1531	36.96
11	.0196	15.14
12	.1321	19.44
Mean	**.1239 ±.0948**	**20.94 ±8.75**

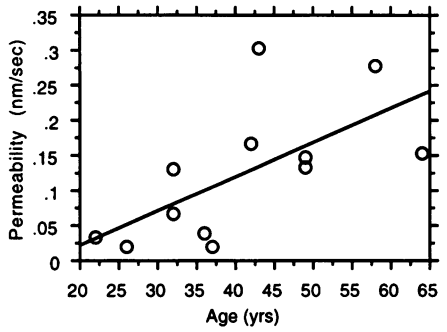

FIGURE 5

Linear regression of corneal epithelial permeability (nm/sec) versus age (r^2=.419, P=.023).

For subject 5, corneal epithelial permeability and tear turnover rates were 55% and 47% higher, respectively, when retested. For subject 6, corneal epithelial permeability decreased approximately 50%, while the tear turnover rate was essentially unchanged.

TABLE VIII: CORNEAL EPITHELIAL PERMEABILITY VALUES AND TEAR TURNOVER RATES IN TWO NORMAL SUBJECTS ON DIFFERENT DAYS

SUBJECT	DAY	PERMEABILITY (nm/sec)	Tear Turnover (min^{-1})
5	0	.3026	7.03
5	19	.5526	15.08
6	0	.1656	25.95
6	13	.0833	26.20

Study 2- Comparision of DSF and CF in Normal Subjects
Baseline and postwashout values : Mean baseline values for a^0_c were 166.3 ±166.3 (range, 130.0 to 218.4) area units for the DSF group and 166.0 ±24.8 (range, 111.6 to 215.0) area units for the CF group. There was no correlation between a^0_c and age. Postwashout values for a^f_c were 439.0 ±70.4 (range, 5.73 to 126.0) area units for the DSF group and 197.4 ±27.5 (range, 9.6 to 57.8) area units for the CF group. The lower mean value for the CF is expected because of its higher molecular weight. There was no correlation of a^f_c with age for the DSF group, but there was for the CF group (r^2=.473, P =.009).

Tear Turnover Rates: Tear turnover rates for normal eyes instilled with DSF are shown in Table IX. Mean turnover rates for DSF from 5 to 20 minutes were significantly lower than those from 0 to 5 minutes (P =.035), but not those from 0 to 20 minutes (P =.058). Tear turnover rates for the fellow eyes using CF are shown in Table X. Mean turnover rates for CF from 5-20 minutes were significantly lower than those from 0 to 5 minutes (P =.011) but not those from 0 to 20 minutes (P =.131).

Physiologic tear turnover rates (0 to 5 minutes) obtained by using DSF were not significantly different from those obtained with CF (P =.8923). Tear turnover rates using either DSF or CF were not correlated with age or sex (Fig 6).

Corneal Epithelial Permeability : The mean corneal epithelial permeability values were 0.1868 ±0.0855 nm/sec for the DSF group and 0.1710 ±0.2509 nm/sec for the CF group (Table XI). These values were excluded in calcu-

TABLE IX: TEAR TURNOVER RATES IN NORMALS USING DISODIUM FLUORESCEIN OVER DIFFERENT TIME INTERVALS

SUBJECT	0-5 MIN	0-20 MIN	5-20 MIN
1	21.36	28.86	17.36
2	11.01	39.85	10.05
3	24.67	23.64	25.37
4	32.02	87.17	29.47
5	22.81	30.82	13.47
6	26.40	24.11	27.94
7	24.99	24.96	24.71
8	17.08	--------	16.59
9	18.45	30.07	18.58
10	22.34	24.07	22.21
11	13.93	8.78	6.13
12	15.08	48.95	10.43
13	26.40	24.11	27.94
Mean	**32.95 ±19.64**	**21.27 ±9.15**	**19.25 ±7.70**

TABLE X: TEAR TURNOVER RATES IN NORMALS USING CARBOXYFLUORESCEIN OVER DIFFERENT TIME INTERVALS

SUBJECT	0-5 MIN	0-20 MIN	5-20 MIN
1	25.15	50.75	26.81
2	13.66	21.64	11.28
3	26.56	76.70	15.27
4	12.55	17.12	12.66
5	35.74	83.11	16.87
6	27.90	18.97	30.99
7	33.95	84.57	24.45
8	5.20	6.33	7.09
9	20.49	58.37	19.56
10	21.53	21.08	22.47
11	17.63	38.31	13.90
12	87.91	14.78	12.41
13	27.96	18.97	31.10
Mean	**44.91 ±30.09**	**21.78 ±8.88**	**18.83 ±7.76**

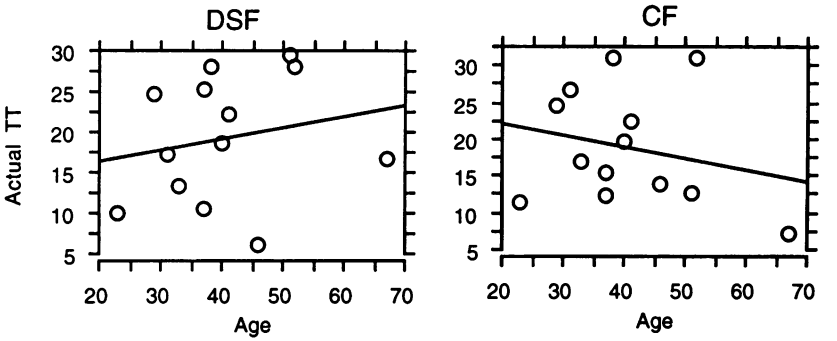

FIGURE 6

Simple regression of tear turnover (TT) rate versus age for disodium fluorescein (DSF) and carboxyfluorescein (CF). There was no correlation with age for either group.

TABLE XI: CORNEAL PERMEABILITY VALUES FOR NORMAL SUBJECTS USING DSF AND CF

SUBJECT	DSF CORNEAL PERMEABILITY (nm/sec)	CF CORNEAL PERMEABILITY (nm/sec)
1	.2231	-.0277
2	.0477	5.4718*
3	.0626	.1916
4	.2234	.1788
5	.8839*	.6453
6	.1733	.0811
7	.1786	-.2595
8	.3254	.5431
9	.3114	.1646
10	.1928	-.0274
11	.1879	.0086
12	.1600	.3379
13	.7880*	.2150
Mean	**.1868 ±.0855**	**.1710 ±.2509**

CF, carboxyfluorescein; DSF, disodium fluorescein.

* These values not used in calculating mean values.

lating the means for reasons discussed below. There was no significant difference in permeability values between the two groups (P =.817). Several of the permeability values for the CF group were negative, with postwashout values of a^f_c less than baseline values of a^0_c . This implies that CF did not penetrate the cornea.

The correlations between tear turnover rates and corneal epithelial permeability were not significant for either the DSF (r^2=.005, *P* =.840) or the CF (r^2=.2634, *P* =.088) groups.

The permeability values from two subjects in the DSF group (subjects 5 and 7) and one from the CF group (subject 2) were not used in calculating the means owing to their extremely high values compared with the rest of the subjects. Baseline values for a^o_c and postwashout values for a^f_c for subject 5 (DCF group) and subject 2 (CSF group) were not that dissimilar from those of other subjects. This suggests that the aberrant permeability values were not due to errors in measuring cornea fluorescence. Careful inspection of the fluorescein concentration against time curve for each subject showed that the initial values were very low. This resulted in low values for $\int a_d dt$ and resultant elevated permeability values. The low fluorescence values may be due to quenching or poor mixing of fluorescein. Because the tear turnover times between 0 to 5 minutes were not unusually elevated for either patient, the low fluorescence values were not due to dilution by reflex tearing (Table XII). The permeability value from a second subject from the DSF group (subject 7) was also excluded from determining the mean permeability for this group. Careful analysis of Table XII shows that the elevated permeability value was due to the high postwashout area values (a^f_c). This suggests that there was insufficient washout of residual DSF.

Two additional subjects (5 and 12) in the CF group had moderately high permeability values. In these subjects, the value for $\int a_d dt$ was quite low owing to quenching, increased reflex tearing, or both. Both subjects had elevated tear turnover rates of 35.74% and 87.91%, respectively, during the first 5 minutes. This suggests that reflex tearing caused a rapid dilution of fluorescein during the first 5 minutes.

TABLE XII: DATA FOR THREE SUBJECTS WITH ABNORMALLY HIGH CORNEAL EPITHELIAL PERMEABILITY

SUBJECT	DYE	BASELINE AREA (a^0)	POST-WASHOUT AREA (a^f_c)	$\int a_d dt$	PERMEABILITY (ng/sec)	TEAR TURNOVER (%/min)
5	DSF	209.5	298.2	13,385	2.4986	13.47
7	DSF	165.2	947.3	132,329	.7880	27.94
2	CF	111.6	129.7	441	5.4718	11.28

CF, carboxyfluorescein, DSF, disodium fluorescein.

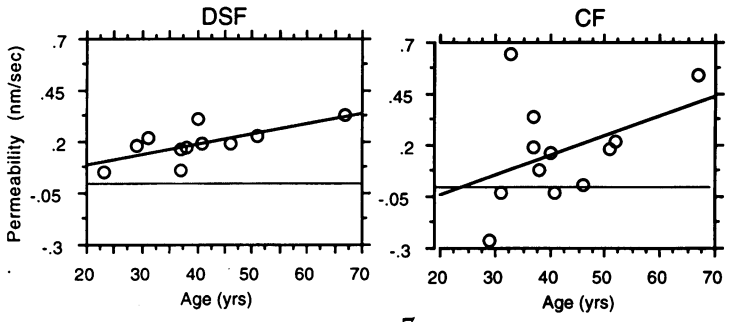

FIGURE 7

Simple regression of corneal epithelial permeability versus age for disodium fluorescein (DSF) and carboxyfluorescein (CF).

There was a statistically significant correlation between corneal epithelial permeability and age for the DSF group (Fig 7; r^2=.447, P =.024) but not for the CF group (r^2=.173, P =.179). If the permeability data for subjects 2, 5, and 12 were eliminated, the CF group values did correlate with age (r^2=.726, P =.002).

Study 3: DSF and CF Concentration in KCS Patients

Corneal epithelial permeability values and tear turnover rates for various DSF and CF solutions are shown in Tables XIII and XIV. The effects of DSF concentration on corneal epithelial permeability are shown graphically in Fig 8. With concentrations higher than 0.5%, permeability values were elevated owing to quenching and/or self-absorption because of the the relative increase in fluorescein concentration due to the decreased tear volume in this patient with KCS. With DSF concentrations less than 0.1%, permeability values were lowered owing to poor mixing in the tear film. Because concentrations in the range of 0.1% and 0.5% gave stable permeability levels, a DSF concentration of 0.2% was chosen to use in patients with KCS.

The effects of CF concentration on epithelial permeability are shown graphically in Fig 9. These data are more variable and more difficult to interpret. In spite of this, a CF concentration of 0.2% was chosen for use in KCS patients on the assumption that quenching at various concentrations of CF should be similar to DSF and, also, to allow comparision between the groups using similar concentrations of DSF and CF.

Study 4: Comparison of DSF and CF in KCS Patients

On the basis of quenching data from Study 3, DSF and CF were both used at concentrations of 0.2%.

Tear turnover: Tear turnover rates for 0 to 5, 0 to 20 and 5 to 20 minutes were calculated for both the DSF and CF groups (Table XV).

TABLE XIII: CORNEAL EPITHELIAL PERMEABILITY VALUES AND TEAR TURNOVER RATES AT DIFFERENT CONCENTRATIONS OF DSF

DAY	DSF CONCENTRATION (%)	PERMEABILITY (nm/sec)	TEAR TURNOVER RATE (%/min)
1	.750	3.3829	1.67
5	.500	.8764	8.31
14	.250	.6388	10.59
19	.100	.7694	3.34
26	.075	.2348	4.23
36	.050	.3372	13.89

DSF, disodium fluorescein.

TABLE XIV: CORNEAL EPITHELIAL PERMEABILITY VALUES AND TEAR TURNOVER RATES AT DIFFERENT CONCENTRATIONS OF CF

DAY	CF CONCENTRATION (%)	PERMEABILITY (nm/sec)	TEAR TURNOVER RATE (%/min)
1	.350	.3242	10.60
5	.250	1.2330	4.54
14	.200	.8638	7.54
19	.150	.4982	7.46
26	.100	.6946	6.14
36	.050	.5469	4.41

CF, carboxyfluorescein.

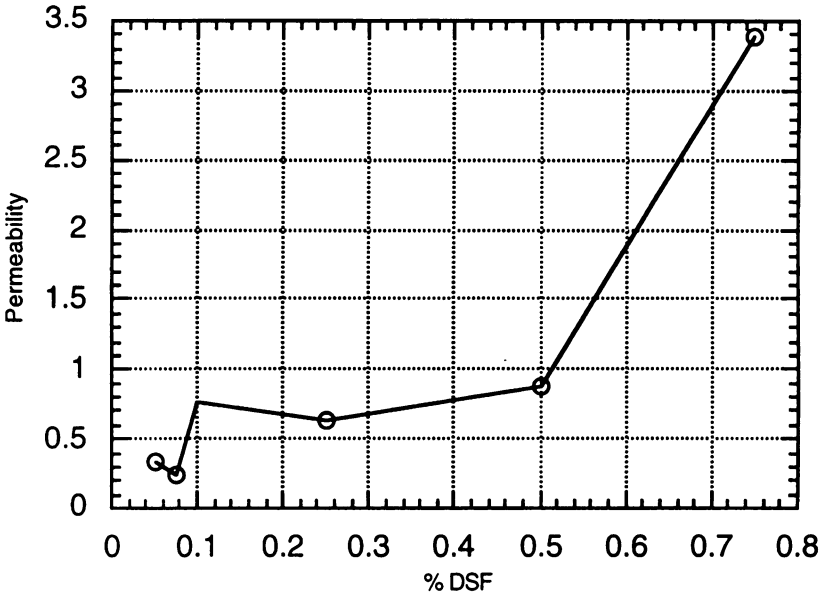

FIGURE 8
Effects of disodium fluorescein (DSF) on corneal epithelial permeability.

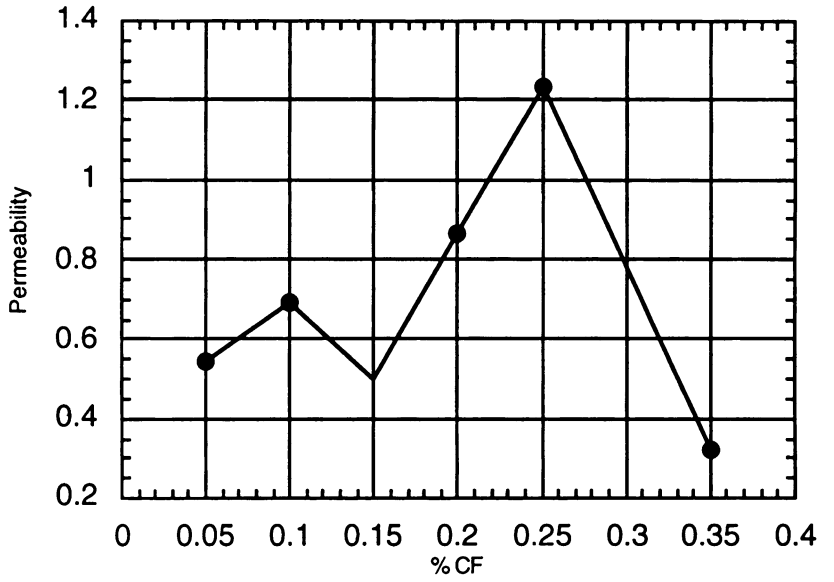

FIGURE 9
Effects of carboxyfluorescein (CF) concentration on corneal permeability.

Mean tear turnover rates from 5 to 20 minutes were significantly lower than

TABLE XV- COMPARISION OF MEAN TEAR TURNOVER RATES FOR THE DSF AND CF GROUPS

	TEAR TURNOVER RATES (%/min)		
GROUP	0-5 MIN	0-20 MIN	5-20 MIN
DSF	28.30 ±26.44	13.15 ± 6.28	11.22 ±5.38
CF	40.71 ±21.20	11.78 ±10.61	10.70 ±7.69

CF, cvarboxyfluorescein; DSF, disodiumfluorescein.

those from 0 to 5 minutes for both the DSF (P =.030) and CF (P <.0001) groups. The mean tear turnover rate from 5 to 20 minutes was also significantly lower than that from 0 to 20 minutes for the DSF group (P =.025) but not the CF group (P =.598). The actual tear or physiologic turnover rates (5 to 20 minutes) were not significantly different between the two groups (P =.867). There was no correlation between tear turnover rates and age.

Mean actual tear turnover rates for the DSF and CF groups were not correlated with Schirmer I test values. However, tear turnover rates between 0 and 5 minutes (reflex tear turnover) were correlated with Schirmer I test values for both the DSF (r^2=.495, P =.004) and CF (r^2=.342, P =.021) groups (Figs 10 and 11).

Corneal Permeability: Data for the DSF and CF groups are shown in Tables XVI and XVII. There were no differences in corneal epithelial permeability values between the DSF and CF groups (P =.365). Permeability values did

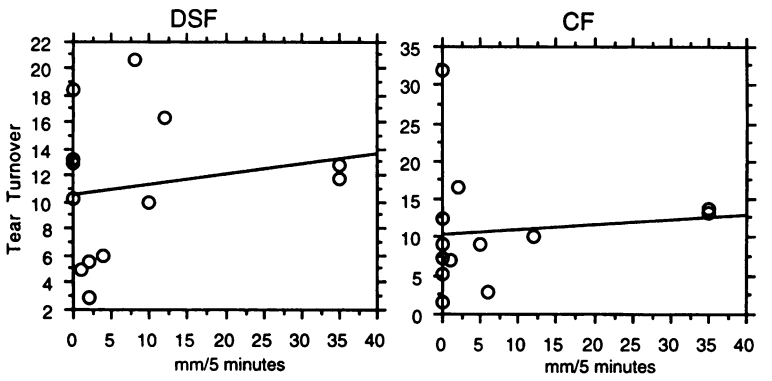

FIGURE 10

Simple regression of actual tear turnover rates in patients with keratoconjunctivitis sicca versus Schirmer I test values for disodium fluorescein (DSF) and carboxyfluorescein (CF) groups. There was no correlation in either group.

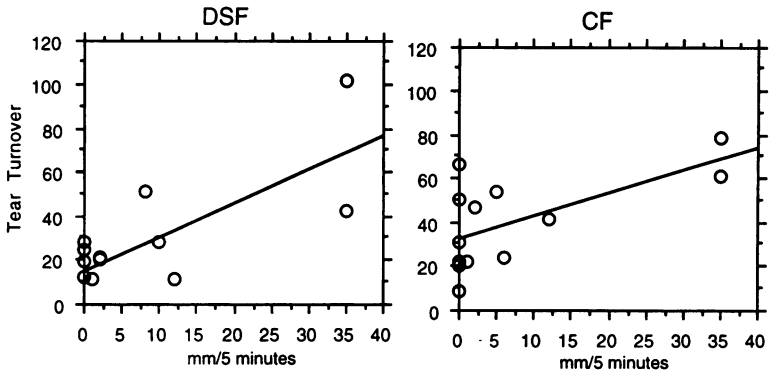

FIGURE 11

Simple regression of reflex tear turnover rates in patients with keratoconjunctivitis sicca versus Schirmer I test values for disodium fluorescein (DSF, r^2=.495,*P*=.004) and carboxyfluorescein (CF, r^2=.342,*P*=.021) groups.

TABLE XVI: DATA FOR EYES OF PATIENTS WITH KCS WHICH RECEIVED DSF

SUBJECT	SCHIRMER'S I TEST	PUNCTATE STAINING	TEAR TURNOVER (%/min)	PERMEABILITY (nm/sec)
1	35	0	12.88	.0598
2	12	0	16.36	.2042
3	2	1	2.86	1.1116
4	1	1	4.96	.9528
5	2	1	5.54	.7420
6	0	3	13.03	2.0822
7	4	2	6.00	.5842
8	10	1	9.98	.6002
9	0	0	10.26	.4450
10	0	2	13.19	.8611
11	8	1	20.62	.2927
12	0	0	18.40	.3632
13	35	1	11.84	1.3011
Mean	**8.4 ±12.5**	**1.0 ±0.9**	**11.23 ±5.38**	**.7385 ±0.5429**

DSF, disodium fluorescein; KCS, keratoconjunctivitis sicca

TABLE XVII: DATA FOR EYES OF PATIENTS WITH KCS THAT RECEIVED CF

SUBJECT	SCHIRMER'S I TEST	PUNCTATE STAINING	TEAR TURNOVER (%/min)	PERMEABILITY (nm/sec)
1	35	0	13.80	-1.1500
2	12	0	10.20	.5309
3	0	1	1.57	.1972
4	1	1	6.99	.4517
5	2	1	16.47	.3860
6	0	3	12.46	2.2915
7	6	2	2.95	.8755
8	5	0	9.08	.3011
9	0	0	5.18	.6208
10	0	2	7.19	.6641
11	0	1	9.10	.6056
12	0	0	31.94	-.1882
13	35	1	13.28	.9080
Mean	**7.4 ±12.5**	**0.9 ±1.0**	**10.79 ±7.69**	**0.4996 ±0.7590**

CF, carboxyfluorescein; KCS, keratoconjunctivitis sicca

not correlate with age (DSF, r^2=.045; CF, r^2=.007), Schirmer I test values (DSF, r^2=.034; CF, r^2=.0523), or tear turnover rates (DSF, r^2=.098; CF, r^2=.061).

Epithelial permeability was higher in eyes with more severe punctate corneal staining (Fig 12). For the DSF group, corneal epithelial permeability was significantly higher in eyes with grade 4 staining compared with grades 1-3 (P =.0036 to .00046) and in eyes with grade 2 compared with grade 1 (P =.016). For the CF group, corneal epithelial permeability was significantly higher for grade 4 compared with grades 1 to 3 (P = .04 to .003) but not between grades 1 and 2, grades 1 and 3, and grades 2 and 3. Tear turnover rates were not significantly different for any of the grades.

Comparison of Normal Subjects and Patients with KCS: Table XIX summarizes the tear turnover rates and corneal epithelial permeability for normal subjects and patients with KCS. Tear turnover rates in KCS patients were significantly lower than in normal subjects for both the DSF group (P =.005) and the CF group (P =.031). There were no signifcant differences between reflex turnover rates (0 to 5 minutes) for normal subjects and patients with KCS. Permeability values were significantly higher for KCS patients compared with normal subjects for the DSF group (P =.0081) but not the CF group (P =.1669).

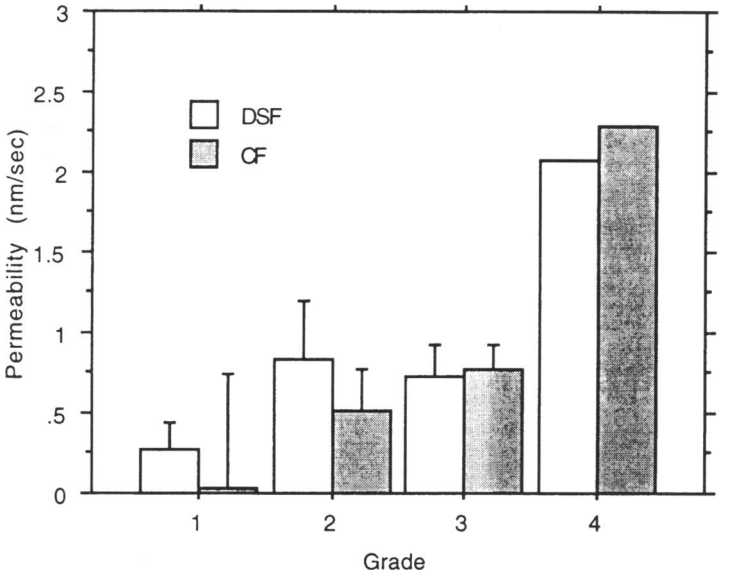

FIGURE 12
Corneal epithelial permeability in disodium fluorescein (DSF) and carboxyfluorescein (CF) groups according to grade of punctate staining.

TABLE XIX: SUMMARY OF TEAR TURNOVER RATES AND CORNEAL EPITHELIAL PERMEABILITY VALUES IN NORMAL SUBJECTS AND PATIENTS WITH KCS

GROUP	DSF		CF	
	TEAR TURNOVER (%/min)	PERMEABILITY (nm/s)	TEAR TURNOVER (%/min)	PERMEABILITY (nm/s)
Normal	19.25 ±7.70	.1868 ±0.0855	18.83 ±7.76	.1710 ±0.2509
KCS	11.23 ±5.38	.7385 ±0.5429	10.79 ±7.69	.4996 ±0.7590

CF, carboxyfluorescein; DSF, disodium fluorescein; KCS, keratoconjunctivitis sicca

Study 5: Corneal Permeability and Tear Turnover after Punctal Occlusion
A patient with severe KCS underwent fluorophotometry after occlusion of all puncta. Tear turnover and corneal epithelial permeability values are shown in Table XX. After 6 days, tear turnover rates were increased by 32% and 45% in the right and left eye, respectively. Permeability values were decreased 63% and 26% in the right and left eye, respectively. These decreases remained stable for up to for 35 days.

TABLE XX: TEAR TURNOVER RATES IN A PATIENT WITH KCS BEFORE AND AFTER PUNCTAL OCCLUSION.

	TEAR TURNOVER RATES (%/min)		PERMEABILITY (%/min)	
	RIGHT EYE	LEFT EYE	RIGHT EYE	LEFT EYE
Preocclusion	4.96	4.96	2.2439	0.9358
Postocclusion:				
Immediately	8.15	3.29	1.1567	0.6012
6 days	6.56	9.69	0.8388	0.4442
35 days	7.29	8.01	0.8366	0.6916

KCS, keratoconjunctivitis sicca

DISCUSSION

FLUOROPHOTOMETRIC TECHNIQUE

The fluorophotometric technique used in this research allowed simultaneous measurement of tear turnover rates and corneal epithelial permeability. This is an important advantage over other methods. Also, the technique is convenient for the patient and the investigator in that it requires only a single application of fluorescein and measurements can be completed within 25 to 30 minutes for one eye. It does not require sampling of the tear film (which is sparse in patients with KCS) or a fluorescein bath. By reducing the concentration of DSF and CF to avoid quenching and self-absorption, the technique can also be used in patients with KCS.

In the first part of this investigation, tear turnover values obtained for normal subjects using either DSF or CF were similar to those obtained by others (Tables II and XIX). Permeability values are similar to those obtained in rabbits.[65, 88] These values, however, were an order of magnitude higher that those obtained by the bath method in humans (Tables III and XIX).

The 10-fold discrepancy between mean corneal epithelial permeability values measured with this technique and the those reported with the bath technique are difficult to explain. In the simultaneous method, permeability is dependent on tear film thickness (q_d), the area under the corneal fluorescence peak after washout (a_c), and the area under the curve generated by plotting the area under the tear film fluorescence curves, (a_t), against time ($\int a_d dt$), or:

$$P_{dc} = \frac{q_d\, a_c}{\int a_d dt}$$

In the simultaneous method, errors in tear film thickness (a_c) would not be significant enough to change the values by a factor of 10. If there were

an error of as much as 50% in determining the integral $\int a_d dt$, permeability values would be reduced by only one half. There are no potential errors of a large enough magnitude to explain the difference.

With the bath method, corneal permeability is dependent on corneal thickness (d_c), the corneal concentration of fluorescein at the start of bathing ($C_c(t_b)$), the concentration of fluorescein in the bath (C_b), and the time the eye is exposed to the bath (t_b), or:

$$P_{dc} = d_c \cdot C_c(t_b)/C_b \cdot t_b \cdot 10^6/60$$

Errors in measurement of corneal thickness and the original DSF concentration after irrigating the eye ($C_c(t_b)$) are unlikely to account for the discrepancy between permeability values. However, the assumption that the concentration of fluorescein in the tear film right after bathing the eye is the same as that of the bath may not be correct. It is possible that there is interaction between DSF and other ions in the bath, which results in a reduction in the solubility of DSF and an overestimation of the bath concentration of the dye.[89]

In any event, the large difference is not readily explainable by errors in measurements and probably represents methodologic differences. The major difference in the techniques is that the simultaneous method depends on tear film thickness, while the bath method depends on corneal thickness. This may be the key difference. Perhaps the classic assumptions about the nature and structure of the tear film or its interaction with the cornea are not correct. Further investigation is needed to explain the difference in permeabilities between the two methods. However, from a relative standpoint, permeability values can be compared in patients as long as the same technique is used to obtain them.

TEAR TURNOVER

Reflex Tear Turnover

Mishima and associates[3] showed that decay of fluorescein in the tear film was biphasic, with a more rapid turnover during the first 5 minutes after drop instillation followed by a single exponential decay. Tear turnover rates for DSF and CF in our studies were also found to be biphasic. In normal subjects, the mean tear turnover rates were found to be 71% and 139% higher during the first 5 minutes compared with the next 15 minutes for the DSF and CF groups respectively. In patients with KCS, the mean tear turnover rates were 150% and 280% higher during the first 5 minutes compared with the next 15 minutes for the DSF and CF groups respectively. The increased tear turnover values obtained for the first 5 minutes were due to reflex tearing. Reflex tear turnover rates were correlated with Schirmer I test values. This is not surprising, because the Schirmer I test is a measure of reflex tearing. Tear turnover rates during the next 15 minutes (physiologic or actual tear turnover) were independent of Schirmer I test

values. This emphasizes that tear turnover rates determined during the first 5 minutes reflect reflex tearing and not the normal physiologic tear turnover rate. Reflex tear turnover rates did not vary with age. Many investigators have shown that Schirmer I test values decrease with age.[31,35-40] This may be due to the increased irritation of the Schirmer test strips compared with a drop of fluorescein.

Using Schirmer II tests (nasal stimulation), Tsubota[34] has reported that patients with KCS due to Sjögren's syndrome have less reflex tearing than non-Sjögren's KCS patients. Our data show that reflex tear turnover rates in patients with KCS and normal subjects were not significantly different. Again, this may be due to increased irriation involved in a Schirmer II test compared with instillation of a drop of fluorescein.

Physiologic (Actual) Tear Turnover

To control potential errors in measuring tear turnover, physiologic tear turnover rates were calculated from measurements made between 5 and 20 minutes to avoid problems due to reflex tearing and significant corneal penetration of fluorescein. Eyelid blinking was controlled to prevent variation in tear film thickness, which occurs between lid blinks.[7] The effects of autofluorescence of the cornea on the overall fluorescence of the tear film/cornea fluorescence curves were taken into account.

Actual tear turnover (5 to 20 minutes) was found to be independent of age (Fig 5). Previously, basal tear secretion (measured by Schirmer's test with anesthesia) was shown to decrease with increasing age.[41] It is interesting to speculate why tear turnover does not decrease with age, while basal tear secretion does. Tear turnover is mostly determined by the interaction of tear secretion, tear film evaporation, and tear drainage. As Jordan and Baum[12] have suggested, tear drainage also may decrease with age and balance out the loss of basal tear secretion, causing tear turnover to remain unchanged.

Tear turnover (TT) reflects not only tear secretion but also transudation of fluid from the conjunctiva (C_t), tear drainage (T_d), tear evaporation (T_e), conjunctival permeability(P_c) and corneal permeability (P_{dc}), or:

$$TT \subset (T_s + C_t) - (T_d + T_e + P_c + P_{dc})$$

This relationship is useful when considering what might affect tear turnover. Therapeutic intervention with punctal occlusion to decrease T_d or environmental efforts to reduce T_e do prove helpful in treating patients with KCS.

TEAR TURNOVER AND EPITHELIAL PERMEABILITY IN NORMAL SUBJECTS

Corneal Autofluorescence

The area under the baseline corneal fluorescence scans (a^0_c), which is directly related to corneal autofluorescence, did not show any significant increase with age. Corneal autofluorescence was previously shown to be in-

creased in individuals with diabetic retinopathy but was independent of age.[90]

Corneal Penetration of Fluorescein

The area under the postwashout corneal fluorescence scans (a^f_c), which is directly related to the amount of fluorescein in the corneal stromal, was found to be dependent on tear turnover rate (Fig 3). With a decreased tear turnover rate, fluorescein has more time to penetrate the cornea resulting in higher corneal stromal concentrations. Unless the tear turnover rate is taken into account, measurement of corneal stromal fluorescein concentrations does not accurately reflect permeability. Studies that use a single-drop technique and fail to take tear turnover into account do not measure the effects of permeability as much as they do tear turnover.

Tear Turnover and Corneal Permeability

Tear turnover was found to be independent of age; this has also been noted by others.[70] Our data also show that turnover rates were independent of the sex of the subject. Rates were essentially identical for DSF and CF (Tables X and XI) and similar to values obtained by others (Table II). This suggests that either DSF or CF may be used for determination of turnover rates.

Mean permeability values for DSF and CF were similar in normal subjects. Because the molecular weight of CF is about twice that of DSF, it would be expected to penetrate the cornea less readily. CF is also more hydrophilic than DSF. Otherwise, they share similar characteristics. Permeability values for the CF group varied substantially between subjects. This variation could reflect normal physiologic variation, the presence of undetectable epithelial defects, or methodologic problems. The unusually high values in several subjects in the CF group could be explained by reflex tearing, quenching, or poor dye mixing. A 0.35% solution of CF is more viscous than DSF and could cause more reflex tearing and mixing problems than DSF. Because of these problems, DSF may be preferable for permeability measurements.

Corneal epithelial permeability was found to increase with age. This was not observed by de Kruijf and associates[78] who measured corneal epithelial permeability using the bath method. A decrease in corneal barrier function in older individuals may make them more susceptible to the therapeutic and toxic effects of topical medications and preservatives.

Tear turnover and corneal epithelial permeability were not correlated in normal subjects. Although a low tear turnover allows more time for fluorescein to penetrate the corneal epithelium, this does not directly influence the permeability of the cornea. In other words, the longer a substance remains in the tear film, the greater the opportunity for it to penetrate the epithelium.

Repeatability: In two subjects, tear turnover and epithelial permeability were determined on different days. In one, turnover rates increased but permeability values remained relatively stable. In the other, turnover rates were similar but corneal permeability decreased. Similar variation over time has been noted by others.[86] It is possible that these variations are normal physiologic variations that are dependent on such factors as the environment, medication use, sleep patterns and health status.

TEAR TURNOVER AND EPITHELIAL PERMEABILITY IN PATIENTS WITH KCS

Self-absorption and Quenching

In patients with decreased tear volume, self-absorption and quenching can occur if the concentration in the tear film is too high. In patients with KCS, use of solutions of DSF and CF between 0.1 to 0.5% avoided these problems.

Tear Turnover and Corneal Permeability

Physiologic tear turnover rates were similar for both the DSF and CF groups (Tables XVI and XVII). These rates were approximately 42% lower when compared with normal subjects (Table XIX). This is not unexpected and is consistent with the finding of decreased Schirmer's test values in patients with KCS.[87] Turnover rates were independent of Schirmer I test values, age, and punctate corneal staining.

Corneal permeability values for the DSF and CF groups were not significantly different, although the mean value for the CF group was lower than that of the DSF group (Tables XVI and XVII). In patients with KCS, permeability values were independent of Schirmer I test values, physiologic tear turnover, and age. Permeability increased with increasing amounts of corneal punctate staining. Since punctate staining indicates loss of tight junctions and epithelial cell integrity, this correlation is not unexpected and has been noted in rabbits.[85]

Compared with normal subjects, permeability values were four times higher for the DSF group and three times higher for the CF group. Göbbelsand Spitznas[80] also found permeability values to be three times higher in KCS patients. As with older subjects, because of the increase in permeability, patients with KCS will be more susceptible to the therapeutic or toxic effects of topical medications and preservatives. With the decreased tear turnover rate in KCS patients, there is also slower elimination of topical solutions from the tear film. This, combined with increased epithelial permeability, puts the KCS eye doubly at risk for the toxic effects of topically applied substances. Göbbels and Spitznas[56, 83] have shown that the benzalkonium chloride increases corneal permeability in patients with KCS. Unpreserved artificial tears have been shown to reduce (or improve) corneal permeability in KCS patients.[56] Frequent use of unpreserved tears has the effect of increasing the tear turnover rate. This causes substances to be

eliminated from or diluted in the tear film. Our data show that corneal epithelial permeability is not dependent on tear turnover. Therefore, simply increasing tear turnover by the addition of topical lubricants may not reduce epithelial permeability. However, one study has shown that permeability values do improve with unpreserved topical lubricants.[80] It is possible that by increasing tear turnover, artificial lubricants wash away or dilute substances, which may adversely affect permeability.

The finding that tear turnover and corneal epithelial permeability are independent of one another also suggests that it is not the quantity or volume of the tear film that is important to maintain epithelial integrity. Rather, it may be the composition of the tear film or its interaction with the epithelium, or both, that is of ultimate importance. This is supported clinically by the lack of correlation between rose bengal staining and Schirmer's tests.[39] Also, in clinical trials, artificial tears can improve symptoms and rose bengal staining without improvement in Schirmer's test values.[58, 59] This emphasizes the need for further research to determine the nature of the tear film in normal subjects and patients with KCS.

Punctal occlusion was found to have a positive effect on tear turnover and corneal epithelial permeability. An increase in tear turnover rates and a decrease in permeability values were maintained for at least 35 days after occlusion. This supports the clinical impression that punctal occlusion is efficacious in treating KCS.[91, 92]

SUMMARY

The simultaneous determination of tear turnover and corneal permeability provides valuable information in the normal as well as the KCS eye. It may prove useful in studying the effects of various medical and surgical treatments for patients with KCS. The finding of decreased tear turnover and increased corneal epithelial permeability in patients with KCS emphasizes that these eyes are more susceptible to the toxic and therapeutic effects of topical preparations. The finding of a 10-fold higher mean permeability value compared with those values reported in the literature with the bath method is perplexing. This certainly deserves futher investigation. Because both methods appear to have sound reasoning behind them, it may be that neither method measures actual corneal permeability. Further knowledge of the tear film and the corneal/tear film interface is required to resolve this problem.

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