Tear Meniscus Changes during Cotton Thread and Schirmer Testing

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Purpose. To elucidate the effect of the cotton thread test (CT-T) and Schirmer test (S-T) on the tear reservoir by evaluating the radius of tear meniscus curvature.

Methods. The radii (R) of the central lower tear menisci were measured by a newly developed video meniscometer in 11 eyes of 11 normal volunteers (6 men, 5 women; mean age, 27.7 ± 3.6 years [SD]) and 9 eyes of 9 patients with tear deficiency and severe dry eye in whom the puncta had been therapeutically occluded (9 women; mean age, 50.6 ± 10.4 years). In this dry eye group, the absence of reflex tearing, coupled with the absence of lacrimal drainage due to punctal occlusion allowed more precise observation of the removal of tears from the meniscus. A 1-minute CT-T was performed, followed after an interval of 10 minutes by a 1-minute S-T. Tear meniscus curvature was documented before (R_0) and during the tests at 30 seconds (R_{30}) and 60 seconds (R_{60}).

Results. In the normal group, respective R values (CT-T; S-T; mean ± SD mm) were R_0 (0.26 ± 0.11; 0.26 ± 0.07), R_{30} (0.27 ± 0.16; 0.20 ± 0.13), and R_{60} (0.29 ± 0.15; 0.23 ± 0.21); and in the dry eye group, respective R values (CT-T; S-T) were R_0 (0.59 ± 0.23; 0.51 ± 0.19), R_{30} (0.52 ± 0.25; 0.22 ± 0.09), and R_{60} (0.51 ± 0.19; 0.21 ± 0.08). It was demonstrated in the dry eye group that R was diminished more by the S-T than by the CT-T in the time course of the measurement (P = 0.01). In the dry eye group alteration of R occurred within the first 30 seconds, and in this group significant correlation was found between R_0 and the S-T result ($r = 0.67$; $P = 0.05$), and between R_{60}- R_0 and the S-T result ($r = -0.81$; $P = 0.01$). Also, there was a significant correlation between R_{60}- R_0 and the S-T result in the normal group ($r = 0.71$; $P = 0.02$). There were no significant correlations between R_0 or R_{60}- R_0 and the CT-T results in either group.

Conclusions. These studies afford some insight into the dynamics of the Schirmer test, suggesting that wetting is influenced by the negative hydrostatic pressure within the tear meniscus. With the protocol used, no conclusion could be drawn about the relation between meniscus radius and wetting of the cotton thread. (*Invest Ophthalmol Vis Sci.* 2000;41:3748–3753)

An estimate of tear volume or secretion is regarded as an important aspect of dry eye diagnosis[1–3] and for this purpose, the Schirmer test[4] (S-T) and the cotton thread test (CT-T) are currently in use.[5–11]

The CT-T, incorporating phenol red as an indicator,[6,8–11] is commercially available in Japan. It is minimally invasive and stimulates little reflex tearing. Wetting length is measured at 15 seconds. A value of 10-mm wetting or less supports the diagnosis of dry eye. The CT-T largely reflects the basal tear volume in the inferior conjunctival sac.[10–12] To perform the test, the cotton thread is applied at a point between the lateral and middle one third of the lower lid margin for 15 seconds. The wetted part of the thread, indicated by the red color, is measured from the upper end. Several reports indicate the usefulness of this test.[6,8,11]

The S-T is believed to measure reflex tearing in response to irritation of the conjunctival surface by the inserted Schirmer strip. The Schirmer I test[1–4,13,14] measures tear secretion over 5 minutes while allowing natural blinking. The modified Schirmer I test,[14–16] which incorporates a drop of anesthetic, can be used to measure basal tear secretion but is less reliable, and the technique is less well standardized. The Schirmer I test with nasal stimulation[17] can be usefully applied in the more severe forms of dry eye. In Sjögren's syndrome, the reflex response to nasal stimulation is greatly diminished compared with patients with non-Sjögren's aqueous deficiency.

Reflective meniscometry[18] is a newly described test that provides the opportunity to measure tear meniscus curvature on-line. Because meniscus curvature is related to meniscus volume, it provides a tool with which to examine the dynamic features of the tear reservoir during the performance of the CT-T and S-T. In the present study, a new quantitative tech-

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nique, reflective meniscometry, was applied to elucidate the effect of the CT-T and S-T on the tear reservoir by evaluating the radius of tear meniscus curvature.

Subjects and Methods

Subjects

The control group comprised 11 healthy volunteers (six left eyes of 6 men, five left eyes of 5 women), aged 24 to 33 years (mean age, 27.7 ± 3.6 years [SD]) with no abnormalities of the cornea, conjunctiva, lacrimal system, or meibomian glands, when assessed by slit lamp examination.

In the control group, only the left eye was used for the study. The dry eye group comprised nine patients with dry eye (five left eyes of five women, four right eyes of four women), aged 38 to 66 years (mean age, 50.6 ± 10.4 years [SD]). There were six patients with Sjögren's syndrome and three without Sjögren's but with tear deficiency and dry eye. Sjögren's syndrome was diagnosed on the basis of the criteria of Fox et al.[19] All subjects enrolled in the dry eye group had successfully received punctal plugs (seven eyes; Ready-Set Punctum Plug-Small; FCI Ophthalmics, Issy-les-Moulineaux, France) or punctal surgery, including cauterization and upper and lower punctal suturing (two eyes). This group of patients with dry eye was specially selected to provide a model system in which the effects of uptake of tear into either the cotton thread or Schirmer strip could be tested with a minimal influence of reflex tearing and lacrimal drainage.

Before the punctal occlusion, the dry eye group showed the severest form of dry eye, with a diminished value in the Schirmer I test (0.67 ± 1.7 mm/5 min [SD]) and severe corneal fluorescein staining scores[20,21] (eight eyes, area [A]3:density [D]3 and one eye, A3D2, scored from 0 to 3, depending on the severity) and a rose bengal staining score[13] of 10 (8.2 ± 1.7 [SD]). Before the punctal occlusion, the patients had been treated with a combination of preservative-free artificial tears containing 0.1% KCl and 0.4% NaCl (Softsantear; Santen Pharmaceutical, Osaka, Japan) and hyaluronan ophthalmic solution (HyaleinMini; Santen) with little improvement. However, after the occlusion therapy, fluorescein staining scores improved to A0:D0 in all subjects, and all were treated with the same preservative-free artificial solution (Softsantear) four to six times per day. The dry eye group was assumed to have a low level of tear drainage and reflex tearing, so that the artificial tears provided a major proportion of the tear reservoir in the conjunctival sac, meniscus, and preocular film. On the day of the study, subjects in the dry eye group were requested not to use the artificial solution within 1 hour of the examination.

This research conformed to the tenets of the Declaration of Helsinki, and before the beginning of the study the procedures were fully explained to all subjects, and informed consent was obtained.

Video Meniscometry

Reflective meniscometry was first established by our group to observe tear meniscus behavior and to quantify the radius of tear meniscus curvature in a noninvasive manner.[18] In the original instrument a specular image of an illuminated target is photographed with the tear meniscus acting as a concave mirror. An additional instrument, the video meniscometer, was developed to capture such images using a video-recording system. In this study, a new video meniscometer was developed that differs from the old version because of the introduction of a half-silvered mirror that permits coaxial viewing of the meniscus. The coaxial alignment of the new video meniscometer permits the meniscus of either eye to be readily accessed. To obtain an image from the meniscus, the target with a series of black and white stripes (four black and five white; each 4 mm wide) is set in front of the objective lens. For the purpose of calculating the radius of tear meniscus curvature, selected meniscus images were printed out using a video printer (print magnification, ×70).

Measurement of Tear Meniscus Curvature during CT-T and S-T

For the CT-T and S-T, commercially available strips (Zone-Quick; Showa Yakuhin Kako, Tokyo, Japan; sterilized tear production measuring strips [scaled], Showa Yakuhin Kako) were used. In the present study, the tests were performed in a nonstandard manner for a period of 1 minute only. This is longer than the standard CT-T, and shorter than the standard S-T. Because the result obtained from a preliminary study showed that the CT-T had far less effect on tear meniscus curvature than had the S-T, the CT-T was performed first, followed by the S-T, after an interval of 10 minutes.

For both tests, images of the tear meniscus were captured in the region of the central part of the lower eyelid and were videotaped for periods of 10 seconds at baseline and at 30 seconds and 60 seconds. The subjects were allowed to blink naturally during the study. Adjustment of focus took approximately 10 seconds after the insertion of a strip, and a timer was started at the moment of the cotton thread insertion. During observation, images appeared very stable, although transient changes were seen at the time of blinking. Images at the selected time points were printed out using a video printer. The radius of tear meniscus curvature was calculated as described earlier at the center of each image at the three time points by an independent observer not involved in either meniscometry or tear testing.

In both CT-T and S-T, after the meniscometry, the wetted part of the strips were measured in millimeters in the normal way: For the CT-T, the wetting length was measured from the top of the thread and for the S-T, from the notch fold in the strip.

Statistical Analysis

The results were expressed as means ± SD. Meniscus radii between CT-T and S-T were compared using repeated-measures analysis of variance (ANOVA) separately for each subject group after confirming the normality of the data by plotting them on normal probability paper (Japanese Standards Association, Tokyo, Japan). A sphericity test was also used. In cases of significant sphericity, the Greenhouse–Geisser test and Huynh–Feldt test were used for further analysis by repeated-measures ANOVA. The meniscus radii before CT-T (R_0) were compared using an unpaired *t*-test between normal subjects and those with and dry eye. Pearson's correlation coefficient was used to compare the experimental and actual radius in the calibration of the meniscometer and to compare R_0 or (R_{60}–R_0) with the CT-T or S-T result. $P \leq 0.05$ was considered to be significant.

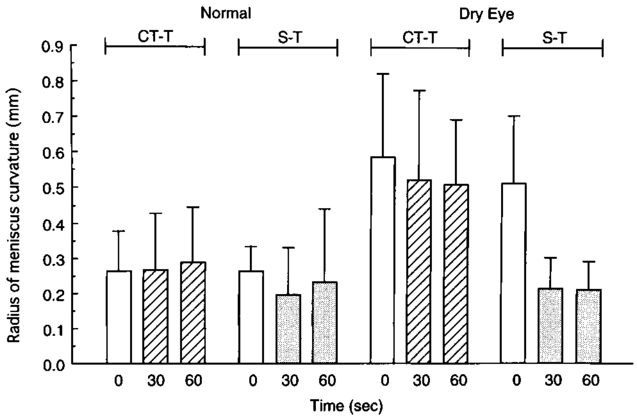

FIGURE 1. Radius of tear meniscus curvature before and at 30 seconds and 60 seconds during the CT-T and S-T tests. Values are mean ± SD.

RESULTS

Effect of CT-T and S-T on Radius of Tear Meniscus Curvature in Normal Subjects

The radii of tear meniscus curvature before (R_0) and during the tests at 30 seconds (R_{30}) and 60 seconds (R_{60}) for CT-T and S-T were calculated and are plotted in Figure 1. R_0, R_{30}, and R_{60} for CT-T were (in millimeters, mean ± SD) 0.26 ± 0.11, 0.27 ± 0.16, and 0.29 ± 0.15, respectively; and the corresponding values for S-T were 0.26 ± 0.07, 0.20 ± 0.13, and 0.23 ± 0.21. The radii data in normal subjects showed no notable skewness in their distribution, enabling us to assume normality. The sphericity of radii data, however, was significantly different ($\chi^2 = 9.29$, $df = 2$; $P = 0.01$). In view of this, the Greenhouse–Geisser test and Huynh–Feldt test were used for analysis by repeated-measures ANOVA. However, the result was the same as that without adjustment. We compared meniscus radii between CT-T and S-T, and we found no statistical difference in two-way interaction (test × time) by repeated-measures ANOVA ($P = 0.39$). Examples of an image of the meniscus in a normal subject exposed to the CT-T and S-T are shown in Figure 2.

Effect of CT-T and S-T on the Radius of Tear Meniscus Curvature in Patients with Dry Eye

The R_0, R_{30}, and R_{60} for CT-T and S-T were calculated and plotted (Fig. 1). R_0, R_{30}, and R_{60} for CT-T were 0.59 ± 0.23, 0.52 ± 0.25, and 0.51 ± 0.19, respectively. The respective values for S-T were 0.51 ± 0.19, 0.22 ± 0.09, and 0.21 ± 0.08. The radii data in patients with dry eye disclosed no notable skewness in their distribution, enabling us to assume normality. Also, the sphericity of radii data were not significantly different ($\chi^2 = 2.36$, $df = 2$; $P = 0.31$) in the radii data. We compared meniscus radii between CT-T and S-T, and we found a significant difference for two-way interaction (test × time) by repeated-measures ANOVA ($P = 0.01$), demonstrating that R was diminished by the S-T more than the C-T, during the time course of the measurement. R_0 before CT-T in the dry eye group was significantly larger than that in the normal group ($P = 0.00$). Images of the meniscus in a subject with dry eye exposed to the CT-T and S-T are shown in Figure 2.

Relation between R_0 and the Wetting Length for CT-T or S-T

The relation between R_0 for CT-T or S-T and respective wetting-length for CT-T or S-T was determined in both the normal and the dry eye groups. There was no significant correlation between R_0 for CT-T and the CT-T result ($r = 0.50$; $P = 0.12$) or between R_0 for S-T and the S-T result ($r = 0.13$; $P = 0.71$) in the normal group. Also, there was no correlation in the dry eye group between R_0 for CT-T and the CT-T result ($r = -0.07$; $P = 0.86$; Fig. 3); however, in the dry eye group, there was a significant correlation between R_0 for S-T and the S-T result ($r = 0.67$; $P = 0.05$; Fig. 3).

Relation between R_{60}– R_0 and the Respective Wetting Length for CT-T or S-T

The correlation between R_{60}- R_0 for CT-T or S-T and the respective wetting length for CT-T or S-T was investigated in the normal and dry eye groups. There was no correlation between R_{60}- R_0 and the CT-T result ($r = 0.57$; $P = 0.07$) in the normal group. However, there was a significant correlation between R_{60}- R_0 and the S-T result ($r = 0.71$; $P = 0.02$) in normal eyes. No significant correlation was found in the dry eye group between R_{60}- R_0 and the CT-T result ($r = -0.07$; $P = 0.86$; Fig. 4), but in this group, a significant correlation was evident between R_{60}- R_0 and the S-T result ($r = -0.81$; $P = 0.01$; Fig. 4).

DISCUSSION

Tests of tear secretion or volume are indispensable in the clinical diagnosis of dry eye.[1-3,13] The S-T[1-4,13,14] has been widely used to measure tear secretion, and the CT-T[5-11] is thought to reflect tear volume. This study was designed to elucidate the effect of these tests on the tear reservoir, by evaluating tear meniscus curvature.[12,18,22] In the present study, both tests were performed for the same periods (60 seconds) to compare their effects under similar conditions. Readings at 30 and 60 seconds were chosen to enable observation of change over time. In normal subjects, the volume of tears in the conjunctival sac is regulated chiefly by the balance between lacrimal secretion and tear drainage,[23] with evaporation playing a minor role. Evaporation is of increasing importance in the patient with dry eye.[3] In the severe forms of dry eye examined in this study, tear secretion was minimal (as indicated by the initial S-T results), and lacrimal drainage was completely obstructed by punctal occlusion. Thus, the volume of fluid on the ocular surface was determined chiefly by the accumulated artificial tears used by these patients. Because fluid is taken up from the tear meniscus into the cotton thread or Schirmer strip, this permitted us to use change in meniscus radius as an indicator of effect on the tear reservoir and to use the wetting length of the test material as an index of the effect of the hydrostatic pressure in the meniscus on wetting. It is an assumption of these studies that tear meniscus radius reflects meniscus volume and that this in turn is positively correlated with total tear volume (the sum of tear volume in the preocular film and the conjunctival sac). Thus, it is assumed that a lower volume of tears is present when the radius of the tear meniscus is small.

In this study, the results of neither the CT-T nor the S-T had a marked effect on the radius of curvature of the tear

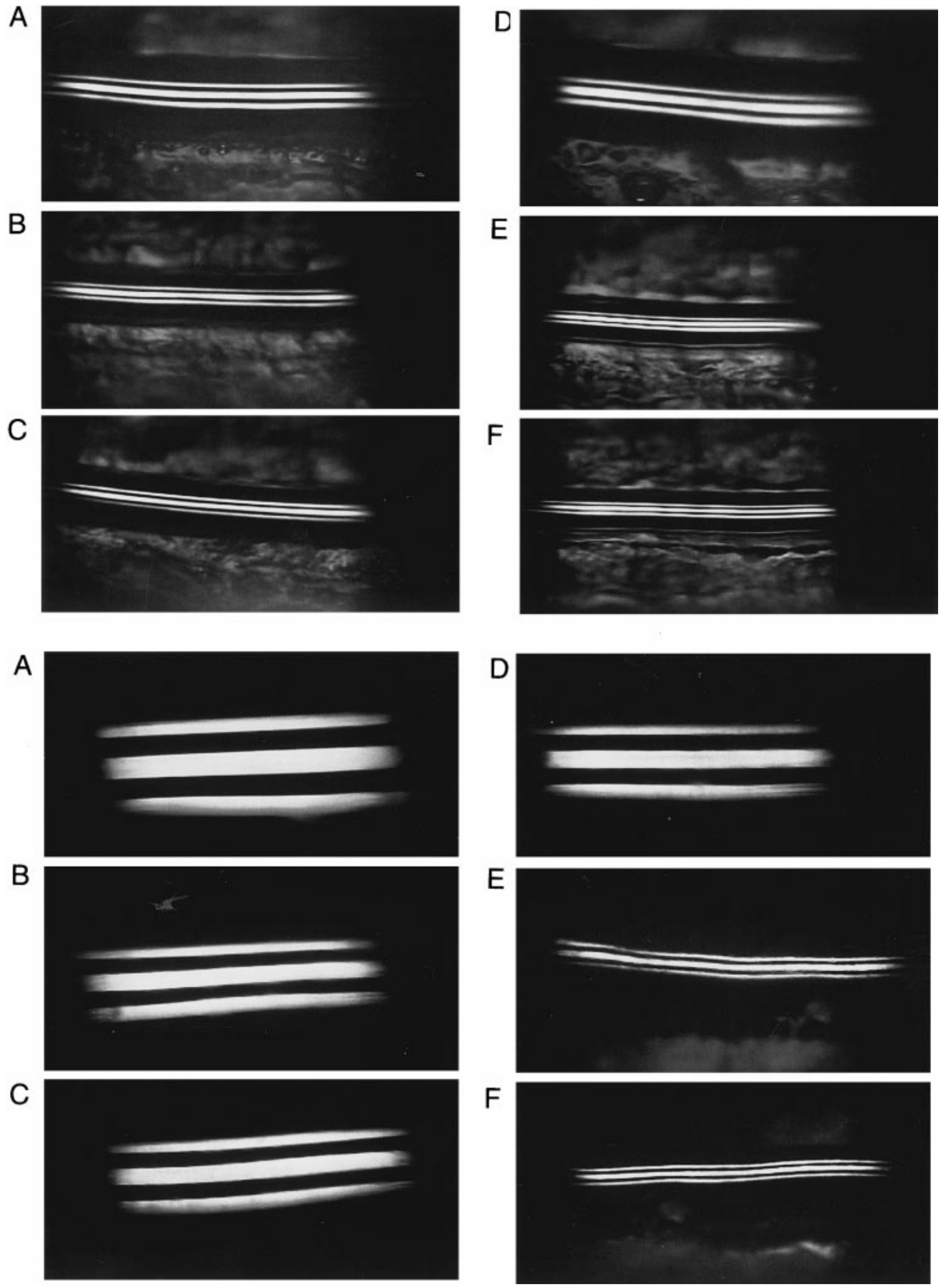

FIGURE 2. Change in meniscus images observed by meniscometry system. *Left*: CT-T (**A**) before test, (**C**) during test at 30 seconds, and (**E**) during test at 60 seconds. *Right*: S-T (**B**) before test, (**D**) during test at 30 seconds, and (**F**) during test at 60 seconds in normal (*top*, **A** through **F**) and dry eye (*bottom*: **A** through **F**).

meniscus in normal subjects, presumably because the amount absorbed into the strip was balanced by the addition of reflex tears to the tear pool. Lacrimal drainage may also have siphoned off excess tears. However, occasionally, when reflex tearing was sizable, an increase in the radius of curvature was noted, implying that tear absorption by the strip and increased lacrimal drainage were insufficient to deal with the increase in secretion. The dry eye group had a paradoxically larger baseline meniscus curvature than the normal group, probably because the patients with dry eye were receiving tear substitutes on a regular basis, so that meniscus volume reflected chiefly the persistence of the most recently instilled drop. Because the dry eye group was specially selected to provide a model system in which to observe the transfer of fluid from the tear reservoir into the test materials, this did not influence the interpretation of our results. It is not possible, however, to state whether differences in tear substitute constituents may have had a differential effect on the uptake of fluid into the test materials.

In patients with dry eye and occluded puncta, it was demonstrated that the uptake of tears into the Schirmer strip significantly reduced meniscus curvature. The meniscus radius had become significantly smaller by 30 seconds after the beginning of the test, indicating the greater absorbing power of the Schirmer strip. However, no additional effect on meniscus curvature was observed during the ensuing 30 seconds. We assume that wetting of the test strips continues until the suction effect of the strips is balanced by the negative hydrostatic effect of the tear meniscus.[24] Based on the capillary equation $P = 2T/r$, where P is the suction pressure of the fluid within the capillary, T is the surface tension of the fluid, and r

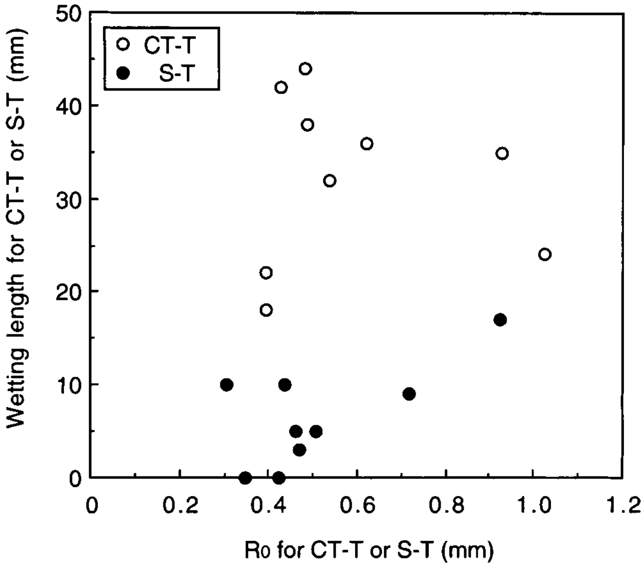

FIGURE 3. Relation between R_0 and the wetting length for the CT-T and the S-T in patients with dry eye. There was no significant correlation between R_0 (baseline) for CT-T and respective wetting length for CT-T ($r = -0.07$; $P = 0.86$), but there was significant correlation between R_0 for S-T and respective wetting length for S-T ($r = 0.67$; $P = 0.05$).

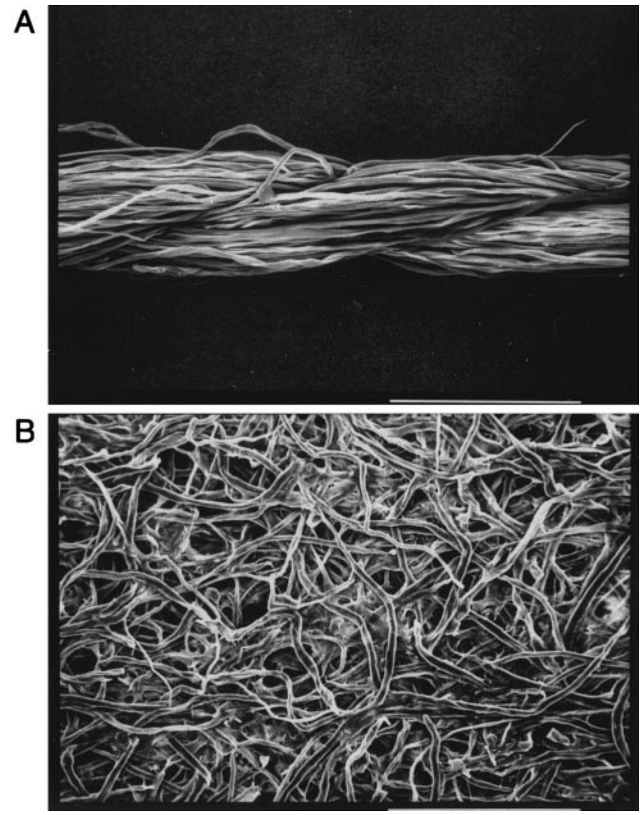

FIGURE 5. Microstructures of cotton thread (**A**) and Schirmer strip (**B**) observed by scanning electron microscopy. Bar, 500 μm.

is the radius of the capillary, it would be anticipated that the larger the radius of the tear meniscus, the lower the suction effect, so that a larger amount of tears would be absorbed by the strip. This hypothesis supports the findings in the dry eye group, where significant correlation was seen between the S-T result and the baseline meniscus radius (R_0), as well as with the total change in meniscus radius (R_{60}- R_0). This was not the case with the CT-T, in which there was no significant relation between either the wetting length and the baseline meniscus radius (R_0), or the wetting length and the change in radius (R_{60}- R_0). In fact, the CT-T result did not reduce the radius of the tear meniscus curvature in the dry eye group, which implies that the amount absorbed by the thread was insufficient to produce a detectable change in meniscus volume (and therefore meniscus curvature) even when the meniscus radius was large.

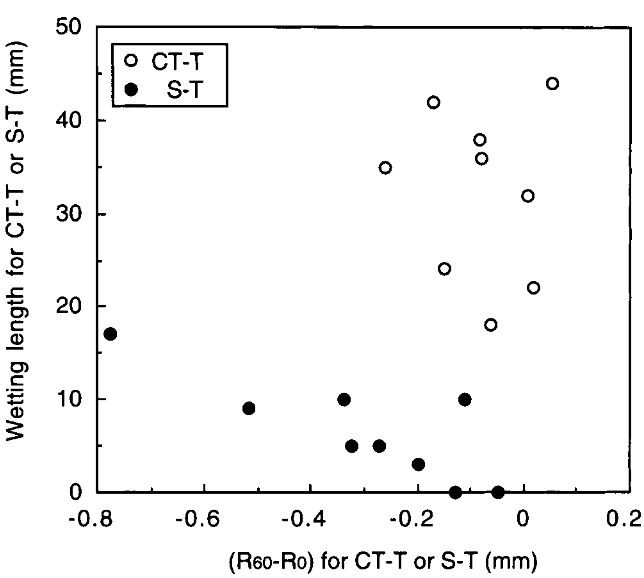

FIGURE 4. Relation between R_{60}- R_0 and wetting length for CT-T and S-T in patients with dry eye. There was no significant correlation between R_{60}- R_0 for CT-T and respective wetting length for CT-T ($r = -0.07$; $P = 0.86$), but there was significant correlation between R_{60}- R_0 for S-T and wetting length for S-T ($r = -0.81$; $P = 0.01$).

The difference in effect of the two tests on meniscus curvature over time is presumed to be due mainly to the greater mass of the Schirmer strip material and thus to its greater absorptive capacity. However, differences in the capillarity of the two materials may also have played a part, because under scanning electron microscopy it was found that the cotton thread was composed of a collection of longitudinally aligned threads, whereas the Schirmer strip exhibited a meshwork structure (Fig. 5). We surmise that the capillarity of the cotton thread may be lost as it absorbs water, because of swelling, whereas that of the Schirmer strip, with its more rigid meshlike structure, is more stable. We had expected, because the CT-T is regarded as an indicator of tear volume, that the wetting length would correlate with baseline meniscus radius. The reason that it did not do so in the present study is probably because, for technical reasons, we were obliged to make measurements at 30 and 60 seconds, whereas the CT-T is normally read at 15 seconds. It may be, that the suction effect of the meniscus is lost by 30 seconds so that the effect is not seen. In future studies we plan to re-examine this relationship by comparing baseline meniscus radius in a similar group of patients with dry eye with the wetting length at 15 seconds. Also, to exclude an effect of tear substitutes on uptake, a washout period with unpreserved saline will be used.

In conclusion, we have been able to explore the dynamics of the S-T in a selected group of patients with dry eye, in whom tear fluid was neither added to nor removed from the tear reservoir by secretion or drainage during the course of the test. In these circumstances the baseline meniscus curvature influenced the wetting length, which in turn correlated with the change in meniscus curvature during the period of study. This implies that as meniscus volume decreases in dry eye, the suction effect of the meniscus increasingly opposes entry of fluid into the test strip. This may explain why the S-T is especially useful in detecting the severest, most tear-deficient dry eye. With the present protocol it was not possible to draw conclusions about the dynamics of the CT-T. This will be the subject of further study.

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