Objective: To evaluate the correlations of the fluorescein clearance test (FCT) and the Schirmer 1 test with the severity of corneal epithelial and eyelid disease in normal patients and patients with tear film disorders due to meibomian gland disease (MGD) and/or aqueous tear deficiency (ATD).

Methods: Nineteen normal control subjects, 16 patients with MGD associated with rosacea, 21 patients with noninflammatory atrophic MGD, and 43 patients with ATD were enrolled. There was a similar age and sex distribution in each group. Each patient completed a symptom severity questionnaire that consisted of 11 questions and then underwent a panel of diagnostic tests in the following order: assessment of corneal and conjunctiva sensation with the Cochet-Bonnet esthesiometer, FCT, assessment of corneal fluorescein staining, Schirmer 1 test (5 minutes without anesthesia), and biomicroscopic examination of the eyelid margins and meibomian glands. The FCT was performed with a fluorophotometer by measuring the fluorescein concentration in minimally stimulated tear samples collected from the inferior tear meniscus. By studying the best area under the receiver operating characteristic curves, we developed a formula that combined the FCT and Schirmer test results, which we termed the FCT corrected by Schirmer test.

Results: The FCT showed stronger correlation with ocular irritation symptoms ($r=0.35$, $P<.001$), corneal fluorescein staining ($r=0.54$, $P<.001$), and meibomian gland and eyelid pathologic signs than the Schirmer 1 test. A correction factor that was based on the best area under the receiver operating characteristic curves, added to the FCT score, improved its correlation with ocular irritation symptoms, eyelid margin and meibomian gland pathologic signs, and ocular surface sensitivity scores.

Conclusions: Corneal epithelial disease is correlated with decreased aqueous tear production and delayed tear clearance, whereas eyelid and MGD are correlated with delayed tear clearance. The FCT corrected by Schirmer 1 test improves the correlations of the FCT with ocular irritation symptoms, corneal epithelial and eyelid pathologic signs, and corneal and conjunctival sensitivity for patients with MGD and ATD.

Arch Ophthalmol. 2000;118:1632-1638
PATIENTS AND METHODS

This research was conducted by medically qualified personnel in strict accordance with the guidelines of the University of Miami School of Medicine, Miami, Fla, institutional review board and the tenets of the Declaration of Helsinki.

The enrolled patients had no history of ocular surgery, contact lens use, punctal occlusion, or eyedrop use (other than nonpreserved artificial tears) for at least 1 month. Subjects did not instill any teardrops in their eyes on the day they were evaluated. Adult patients who had complaints of ocular irritation were evaluated by the 2 investigators (A.M. and S.C.P.) at the Ocular Surface Center (Bascom Palmer Eye Institute, University of Miami School of Medicine). Each subject was first asked to complete a symptom questionnaire that consisted of 11 questions describing the severity and the nature of their irritation symptoms (Table 1).

The patients then underwent a panel of diagnostic tests that were performed in the following order: corneal and conjunctival sensation, FCT, corneal fluorescein staining, Schirmer 1 test, and biomicroscopic examination of the eyelid margins and meibomian glands. The patients were classified into 1 of 3 groups. The ATD group included patients who had a Schirmer 1 test result of 5 or lower in at least 1 eye and a questionnaire score of greater than 5. The MGD group included patients who had a Schirmer 1 test result of greater than 5 in both eyes and a symptom questionnaire score of greater than 5. These patients were classified as having rosacea-associated (inflammatory) MGD or noninflammatory meibomian gland atrophy. Diagnosis of rosacea was based on previously reported criteria and required the presence of at least 2 facial signs of rosacea (which include rhinophyma, telangiectasia, persistent erythema, papules, pustules, and hypertrophic sebaceous glands in facial flush areas) and hyperemia of lid margins (brush marks) and/or conjunctival hyperemia. Atrophic MGD was defined as at least 30% atrophy of meibomian gland acini in the lower lid (determined by transillumination of the lower lid as described herein), no facial signs of rosacea, and mild or no hyperemia of the lid margins or conjunctiva. The normal group consisted of 19 healthy subjects of similar age and sex distribution. Subjects were considered normal if they had no history of ocular irritation (symptom score ≤5), no use of eyedrops, and a Schirmer 1 test result of more than 10 mm.

FLUORESCIN CLEARANCE TEST

The FCT was performed with a fluorophotometer (CytoFluor II, PerSeptive Biosystems, Framingham, Mass) by measuring the fluorescein concentration in minimally stimulated tear samples collected from the inferior tear meniscus 15 minutes after instillation of 5 µL of 2% sodium fluorescein as previously reported.2

CORNEAL FLUORESCIN STAINING

The ocular surface was examined with a biomicroscope and the ×10 objective under blue light illumination 2 minutes after fluorescein instillation into the tear film. The density of corneal fluorescein staining was assessed as previously described2 in each of 4 quadrants on the cornea (temporal, nasal, superior, and inferior) using a standardized 4-point scale (0=none, 1=mild, 2=moderate, 3=severe). The staining scores ranged from 0 to 12.

SCHIRMER 1 TEST

Without previously instilling anesthetic drops, Schirmer paper test strips (Alcon Laboratories Inc, Fort Worth, Tex) were placed over the lid margin at the junction of the

Continued on next page

Table 1. Symptom Questionnaire*

<table>
<thead>
<tr>
<th>A. Have you experienced any of the following during the last week?</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Light sensitivity</td>
<td></td>
</tr>
<tr>
<td>2. Gritty or scratchy sensation</td>
<td></td>
</tr>
<tr>
<td>3. Burning or stinging</td>
<td></td>
</tr>
<tr>
<td>4. Vision that fluctuates with blinking</td>
<td></td>
</tr>
<tr>
<td>5. Vision that improves with artificial tears</td>
<td></td>
</tr>
<tr>
<td>6. Tearing</td>
<td></td>
</tr>
<tr>
<td>7. Pain or burning in the middle of the night or on awakening in the morning</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Have problems with your eyes limited you in performing any of the following during the last week?</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>8. Reading or driving a car for a long period</td>
<td></td>
</tr>
<tr>
<td>9. Watching television or working on a computer for an extended period</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Have your eyes felt uncomfortable in any of the following situations during the last week?</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10. Windy conditions</td>
<td></td>
</tr>
<tr>
<td>11. Places with low humidity such as air-conditioned or heated buildings or airplanes</td>
<td></td>
</tr>
</tbody>
</table>

* Scores ranged from 0 to 48. In sections A and C, the score for each row ranged from 0 to 4 (none of the time, some of the time, half of the time, most of the time, all of the time). In section B, the score for each row ranged from 0 to 6 (none of the time, seldom, some of the time, half of the time, most of the time, almost all of the time, always).
lateral and middle thirds of the lower eyelid for 5 minutes. The length of strip wetting in millimeters was recorded.

**CORNEA AND CONJUNCTIVA SENSATION**

Cornea and conjunctiva sensation were assessed using the Cochet-Bonnet esthesiometer. The stimulus from the Cochet-Bonnet instrument consists of a nylon filament that can vary in length from 0 to 6 cm. The procedure for measuring corneal surface sensitivity was as follows. Under visual control, the nylon filament of the Cochet-Bonnet instrument was approached smoothly and perpendicularly toward the center of the cornea. Contact was detected by the slightest bend of the nylon; sensitivity was taken as the length of the filament that gave a 50% positive response from a minimum of 4 stimulus applications. Subject reliability was tested by bringing the filament close to the cornea without actually touching. The same procedure was used to test conjunctival sensation, with the stimulus applied to the middle of the exposed temporal-bulbar conjunctiva.

**EYELID MARGIN AND MEIBOMIAN GLAND EXAMINATION**

The meibomian gland orifices were examined by slitlamp biomicroscopy for the presence of metaplasia (abnormal growth and keratinization of duct epithelium manifesting as white shaft protruding from the orifices). The inferior tarsus was transilluminated with a halogen Finhoff transilluminator (Welch Allyn Inc., Schenectady, NY), and the percentage of meibomian gland acinar dropout was measured as previously reported.

The presence of irregularity of the lid margin and anterior migration of the Marx line was evaluated by slitlamp biomicroscopy. Anterior migration of the Marx line was determined using criteria described by Norn.

**FCT CORRECTION FACTOR BASED ON THE SCHIRMER TEST**

The following equation was used to correct the results of the FCT by the Schirmer test:

$$ \text{FCT (Corrected)} = \text{FCT} + (\text{Schirmer Score} \times y) $$

The coefficient $y$ was calculated by looking for the best corresponding area under the receiver operating characteristic curves according to previously described methods.

All statistical calculations were performed with GraphPad Prism 2.0 Software (GraphPad Software, Inc, San Diego, Calif.).

**STATISTICAL ANALYSIS**

For statistical evaluation, only the right eye was considered for each patient. Data distribution was analyzed. If data were normally distributed, then parametric statistical tests were used; otherwise nonparametric tests were used. The differences in age among the 3 groups (normal controls, patients with MGD, and patients with ATD) were studied by means of 1-way analysis of variance and the Bartlett test for equal variances. The difference in sex among the 3 groups was studied using the Kruskal-Wallis statistic.

Correlation coefficients were calculated between the FCT and Schirmer 1 test, corneal fluorescein staining score, anterior migration of the Marx line, percentage of meibomian gland acinar loss, presence of orifice metaplasia (0=absent, 1=present), and cornea and conjunctiva sensitivity scores. If data distribution was normal, then the Pearson correlation coefficient was used, otherwise the Spearman rank correlation coefficient was calculated. Furthermore, the correlation coefficients between symptom questionnaire score and FCT, Schirmer test, and corneal fluorescein staining scores were evaluated for each group of patients (MGD patients, ATD patients, and normal controls).

This study evaluated the correlations of the FCT and the Schirmer test with the severity of corneal epithelial and eyelid disease in normal subjects and patients with tear film disorders due to MGD and/or ATD. These studies were performed to gain a greater understanding regarding the factors that cause ocular surface disease. The results of these studies may improve the ability of clinicians to identify a tear film disorder as the cause of a patient's ocular surface disease.

In a previously reported study, we found that the FCT showed greater correlation with irritation symptoms than the Schirmer 1 test, whereas the Schirmer 1 test showed slightly better correlation with the severity...
of corneal fluorescein staining. In the present study performed on a different patient population, the FCT showed better correlation with both irritation symptoms and corneal fluorescein staining scores than the Schirmer 1 test. These findings indicate that clearance of tears from the ocular surface is a key factor in the pathogenesis of keratoconjunctivitis sicca. These correlations were further improved by adding a correction factor for the Schirmer test result to the FCT score. This suggests that the correlation of decreased aqueous tear production with ocular irritation and ocular surface disease is not solely due to its effect on reducing tear clearance. Indeed, secretory dysfunction of the lacrimal gland may promote ocular surface inflammation and decrease delivery of protective proteins and growth factors needed to maintain ocular surface homeostasis. We have reported that in patients with Sjogren syndrome as aqueous tear production decreases the levels of inflammatory cytokines in the conjunctival epithelium and tear fluid increase and that the concentration of epidermal growth factor in the tear fluid decreases.

The corrected FCT is a relatively easy test for clinicians to perform. Unlike tear osmolarity, a diagnostic technique touted as a gold standard for diagnosis of dry eye, which requires expensive instrumentation, tear fluorescein clearance can be assessed in an inexpensive fashion by practitioners. Our group and others have reported the use of colorimetric techniques to evaluate tear clearance by collecting fluorescein-stained tear fluid on a Schirmer test strip. We have developed a 7-point technique touted as a gold standard for diagnosis of dry eye, which requires expensive instrumentation, tear fluorescein clearance can be assessed in an inexpensive fashion by practitioners. Our group and others have reported the use of colorimetric techniques to evaluate tear clearance by collecting fluorescein-stained tear fluid on a Schirmer test strip. We have developed a 7-point vi-

Table 2. Statistical Comparison of Normal Control, Meibomian Gland Disease (MGD), and Aqueous Tear Deficiency (ATD) Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Corneal Fluorescein Staining Score</th>
<th>Schirmer 1 Test Result, mm</th>
<th>Meibomian Gland Atrophy, %</th>
<th>Meibomian Gland Orifice Metaplasia</th>
<th>FCT, Fluorophotometric U/µL</th>
<th>Corrected FCT, Fluorophotometric U/µL</th>
<th>Corneal Sensation</th>
<th>Conjunctival Sensation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control subjects</td>
<td>0.0 ± 0.0</td>
<td>21.7 ± 5.1</td>
<td>2.6 ± 11.5</td>
<td>0.1 ± 0.2</td>
<td>95.4 ± 80.4</td>
<td>182.1 ± 68.5</td>
<td>5.7 ± 0.2</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>MGD (n = 37)</td>
<td>1.9 ± 2.3</td>
<td>17.0 ± 7.5</td>
<td>62.6 ± 27.8</td>
<td>0.9 ± 0.4</td>
<td>520.3 ± 1356.2</td>
<td>666.3 ± 1350.2</td>
<td>4.2 ± 1.4</td>
<td>1.3 ± 0.8</td>
</tr>
<tr>
<td>Differences between normal and MGD patients</td>
<td>t = 2.4</td>
<td>U = 38.0</td>
<td>U = 65.5</td>
<td>U = 286.5</td>
<td>U = 274.0</td>
<td>U = 94.0</td>
<td>P &lt; .001</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>ATD patients (n = 43)</td>
<td>2.6 ± 3.0</td>
<td>1.8 ± 1.7</td>
<td>33.7 ± 33.3</td>
<td>0.5 ± 0.4</td>
<td>1329.8 ± 2348.2</td>
<td>1446.9 ± 2412.0</td>
<td>3.7 ± 1.6</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td>Differences between U = 669.5</td>
<td>t = 12.9</td>
<td>U = 286.0</td>
<td>U = 485.5</td>
<td>U = 380.5</td>
<td>U = 524.0</td>
<td>t = 1.25</td>
<td>t = 2.0</td>
<td></td>
</tr>
<tr>
<td>MGD and ATD patients</td>
<td>P = .22</td>
<td>P &lt; .001</td>
<td>P = .002</td>
<td>P = .002</td>
<td>P = .008</td>
<td>P = .21</td>
<td>P = .048</td>
<td></td>
</tr>
<tr>
<td>Differences among the 3 groups</td>
<td>P = .04</td>
<td>P &lt; .001</td>
<td>P &lt; .001</td>
<td>P &lt; .001</td>
<td>P &lt; .001</td>
<td>P &lt; .001</td>
<td>P &lt; .001</td>
<td></td>
</tr>
</tbody>
</table>

*Values are presented as mean ± SD. FCT indicates fluorescein clearance test; t, 2-tailed unpaired t test; U, Mann-Whitney U test; K, Kruskal-Wallis statistic; and F, 1-way analysis of variance.

Table 3. Correlation Among Questionnaire Score, Fluorescein Clearance Test (FCT), Corrected FCT, Schirmer Test, and Corneal Fluorescein Staining Scores

<table>
<thead>
<tr>
<th>Questionnaire Score</th>
<th>FCT</th>
<th>Corrected FCT</th>
<th>Corneal Fluorescein Staining</th>
<th>Schirmer Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>r = 0.07, P &gt; .1</td>
<td>r = 0.02, P &gt; .1</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>MGD patients</td>
<td>r = 0.35, P = .04</td>
<td>r = 0.36, P = .03</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ATD patients</td>
<td>r = 0.53, P &lt; .001</td>
<td>r = 0.54, P &lt; .001</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>All patients</td>
<td>r = 0.37, P &lt; .001</td>
<td>r = 0.47, P &lt; .001</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*MGD indicates meibomian gland disease; ATD, aqueous tear deficiency; r_P, Pearson correlation coefficient; and r_s, Spearman correlation coefficient.

Figure 1. Questionnaire score vs corneal fluorescein staining score and Schirmer 1 test score (in millimeters) (see Table 2).

Figure 2. Questionnaire score vs fluorescein clearance test (FCT) and FCT corrected by the Schirmer test (FCT Corrected) scores (see the “Patients and Methods” section and Table 2).
suval scale to assess tear fluorescein clearance that shows very strong correlation with the results of the fluorometric technique used in the present study.\textsuperscript{16}

It is well recognized that anesthesia of the ocular surface decreases aqueous tear production.\textsuperscript{17} The results of our study support recently proposed unified concepts for development of dry eye where perturbations on the ocular surface affect tear fluid secretion by the lacrimal glands by inhibiting afferent sensory to efferent cholinergic neural reflex loops.\textsuperscript{18,19} Both decreased aqueous tear production and delayed tear clearance are associated with decreased ocular surface sensation.\textsuperscript{4,7} Decreased ocular surface sensation could explain why patients with MGD were noted to have significantly reduced aqueous tear production compared with a control group of similar age and sex (Table 1). Patients with MGD and lipid tear deficiency have been noted to have increased tear film evaporation and a decreased tear volume.\textsuperscript{20} These factors alone could be responsible for the delayed tear clearance and the secondary changes to the eyelid and ocular surface that occur in MGD. Regardless of the mechanism by which tear clearance decreases in MGD, delayed tear clearance leads to decreased ocular surface sensation and decreased sensory stimulation of lacrimal gland tear secretion. This creates a viscous, self-perpetuating cycle on the ocular surface.

An intriguing finding of this study was that the FCT and to a greater degree the FCT corrected by the Schirmer

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**Figure 3.** A, Log fluorescein clearance test (FCT) vs corneal fluorescein staining score for patients with aqueous tear deficiency (Spearman \( r = -0.34, P < .001 \)). B, Log FCT corrected by the Schirmer test vs corneal fluorescein staining score for patients with aqueous tear deficiency (Spearman \( r = -0.34, P < .001 \)).

**Figure 4.** Schirmer test (wetting in millimeters) vs corneal fluorescein staining score for patients with aqueous tear deficiency (Spearman \( r = -0.38, P = .01 \)).

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**Table 4.** Correlation Coefficients and Statistical Significance Among Fluorescein Clearance Test (FCT), Corrected FCT, Schirmer Test, Corneal Fluorescein Staining Score, Anterior Migration of the Marx Line, Percentage of Meibomian Gland Acinar Dropout (MGAD), Orifice Metaplasia Score, and Cornea and Conjunctiva Sensitivity Score in All Patients\textsuperscript{*}

<table>
<thead>
<tr>
<th></th>
<th>FCT</th>
<th>Corrected FCT</th>
<th>Schirmer Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schirmer test</td>
<td>( r_s = -0.57, P &lt; .001 )</td>
<td></td>
<td>( r_s = -0.41, P &lt; .001 )</td>
</tr>
<tr>
<td>Corneal fluorescein staining score</td>
<td>( r_s = 0.54, P &lt; .001 )</td>
<td>( r_s = 0.47, P = .003 )</td>
<td>( r_s = 0.58, P &lt; .001 )</td>
</tr>
<tr>
<td>Anterior migration of the Marx line</td>
<td>( r_s = 0.23, P = .02 )</td>
<td>( r_s = 0.28, P = .004 )</td>
<td>( r_s &lt; 0.01 )</td>
</tr>
<tr>
<td>% of MGAD</td>
<td>( r_s = -0.30, P = .002 )</td>
<td>( r_s = -0.38, P &lt; .001 )</td>
<td>( r_s &lt; 0.001 )</td>
</tr>
<tr>
<td>Orifice metaplasia score</td>
<td>( r_s = 0.34, P &lt; .001 )</td>
<td>( r_s = 0.37, P &lt; .001 )</td>
<td>( r_s &lt; 0.001 )</td>
</tr>
<tr>
<td>Cornea sensitivity</td>
<td>( r_s = -0.34, P &lt; .001 )</td>
<td>( r_s = -0.35, P &lt; .001 )</td>
<td>( r_s &lt; 0.001 )</td>
</tr>
<tr>
<td>Conjunctiva sensitivity</td>
<td>( r_s = -0.34, P &lt; .001 )</td>
<td>( r_s = -0.35, P &lt; .001 )</td>
<td>( r_s = 0.36, P &lt; .001 )</td>
</tr>
</tbody>
</table>

\*\( r_s \) indicates Spearman rank correlation coefficient.

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**Table 5.** Correlations Among Fluorescein Staining Score and Tear Diagnostic Tests for Patients With Meibomian Gland Disease (MGD) and Aqueous Tear Deficiency (ATD)\textsuperscript{*}

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Schirmer Test</th>
<th>FCT</th>
<th>Corrected FCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGD</td>
<td>( r_s = -0.06, P &gt; .1 )</td>
<td>( r_s = 0.47, P = .003 )</td>
<td>( r_s = 0.58, P &lt; .001 )</td>
</tr>
<tr>
<td>ATD</td>
<td>( r_s = -0.38, P = .01 )</td>
<td>( r_s = 0.43, P = .004 )</td>
<td>( r_s = 0.54, P &lt; .001 )</td>
</tr>
<tr>
<td>All</td>
<td>( r_s = -0.41, P &lt; .001 )</td>
<td>( r_s = 0.54, P &lt; .001 )</td>
<td>( r_s = 0.63, P &lt; .001 )</td>
</tr>
</tbody>
</table>

\* The formula for the Schirmer corrected FCT was FCT (corrected) = FCT + (Schirmer score \times 4). \( r_s \) indicates Spearman rank correlation coefficient.
test showed very strong correlation with the pathologic changes of the eyelid margin that develop in patients with MGD (Table 3). It is possible that the biochemical changes in the tear fluid that accompany delayed tear clearance, such as an increased concentration of the inflammatory cytokine interleukin 1 and increased activity of the matrix-degrading enzyme matrix metalloproteinase 9, could be responsible for these changes.21 These changes could also explain the increased prevalence of MGD in patients with severe ATD, a condition where tear clearance is markedly decreased.2,22

Future research may identify the mechanism(s) by which delayed tear clearance decreases ocular surface sensation and promotes corneal epithelial and eyelid disease. Our results indicate that the corrected FCT appears to be an even better tool to study these mechanisms than the FCT alone.

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REFERENCES


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**Answer to Crossword Puzzle**

Ocular Pun-ditions
William H. Schutten, MD

This is the answer to the November crossword puzzle (Arch Ophthalmol. 2000;118:1597).

```
TAV M I T R A L O R A L S P A M
O R A A R E O L E P A S O C E L L O
B A N D K E R A T O P A T H Y A G A I N
A B I D E C R O E L E S T R A N D T
C I T E W E E P A E Y E L A S H
C A Y V I T R E O U S H U M O R
O N P E D N O E U P E N A R C S
M A N O R I D L E R S E L I H U
N A R R O W A N G L E S B O B B E R
I L E U M C O M E T S T I E S E G
C A D S R E M A S T I C A T E D A R E
K D A E D S I N R U N E E L E R
E D G E R S A S T E R O I D B O D Y
L I N E N E S C H E W W R I T E
S N U G G Y N E C G U I F S R A
E L E V A T O R P A L S Y C U L
A S S Y R I A A I R A H G O B I
R E T I N A F E E T W A S K O R E A
D R A P E E L S C H N I G S P E A R L S
O G R E S F E P C Ü N O I L Y L A E
N E T S G A Y E M O N E Y S L A S
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