Corneal Hydration Control in Diabetes Mellitus

Bonnie C. Weston,* William M. Bourne,* Kenneth A. Polse,† and David O. Hodge‡

Purpose. To assess the effects of diabetes mellitus on corneal structure and function.

Methods. The authors measured endothelial permeability to fluorescein and corneal deswelling for 7.5 hours after 2 hours of hypoxic contact lens wear in 20 patients with diabetes who had nonproliferative retinopathy and 21 age-matched control subjects. Central corneal endothelial photographs were also taken. Corneal deswelling rates, expressed as percent recovery per hour (PRPH), and open eye steady state (OESS) thickness were estimated by nonlinear regression techniques.

Results. The OESS thickness was greater in patients with diabetes than in controls (562 ± 35 μm versus 559 ± 24 μm, P = 0.02). During hypoxia, the diabetic corneas swelled less (7.7% ± 1.8% versus 9.9% ± 1.6%, P < 0.001) and had less endothelial permeability (3.55 ± 0.83 × 10⁻⁴ cm/min versus 4.14 ± 0.68 × 10⁻⁴ cm/min, P = 0.02) than the controls. During normoxia after contact lens removal, however, diabetic and control corneas had similar deswelling rates and permeabilities. Corneal autofluorescence was increased in the patients with diabetes (8.1 ± 3.1 versus 6.0 ± 1.9 ng/ml fluorescein equivalents, P = .005). The endothelial cells of the two groups were morphologically similar. Within the group with diabetes, however, those with moderate nonproliferative retinopathy had larger coefficients of variation of cell area and smaller percentages of hexagonal cells than those with mild nonproliferative retinopathy.

Conclusions. Although the diabetic corneas were thicker and more autofluorescent than control corneas, during hypoxia they swelled less and had decreased endothelial permeability. During normoxia, however, no difference was found in endothelial permeability or deswelling rate. The effects of diabetes on endothelial cell morphologic features appear to be related to the severity of the diabetes.


A number of investigators have reported abnormalities in corneal endothelial function in diabetes mellitus, both in animals1,2 and humans.3–5 A recent study by Keoleian and colleagues,6 however, failed to reveal any functional abnormality in the form of increased corneal thickness or change in endothelial permeability to fluorescein in humans with type 1 diabetes of long duration under fair control. The tests in that study6 were conducted on corneas in the resting, unstimulated state. After the surgical stress of phacoemulsification, however, the transfer coefficient for fluorescein is elevated in diabetic corneas compared to those of controls.7 Thus, diabetic corneas, despite a normal baseline barrier function, may not have sufficient functional reserve to handle the stress of induced corneal edema normally. For an in vivo test of corneal hydration control in the stressed state, corneal deswelling can be measured after contact lens-induced hypoxic edema.8 Investigators using a modification of this method have reported a decreased ability to recover from corneal edema in diabetic rabbits7 and humans.9 We sought to obtain more information about diabetic corneal hydration control by using this "stress" test in human patients with diabetes and simultaneously measuring the corneal endothelial permeability to fluorescein and cellular morphologic features.

METHODS

We recruited 20 patients (12 men, 8 women) with diabetes (15 with insulin-dependent type 1 diabetes

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and 5 with adult-onset type II diabetes) of at least 10 years' duration and 21 age-matched (≥6 years) and sex-matched (13 men, 8 women) control subjects from the local community for this study. One additional subject with diabetes was originally recruited but did not complete the protocol. Only patients with diabetes who had nonproliferative background retinopathy and diabetest of at least 10 years' duration were included. The protocol followed the tenets of the Declaration of Helsinki and was approved by our Institutional Review Board. After obtaining written informed consent, we performed a complete ophthalmic examination for screening on all potential subjects, including slit lamp biomicroscopy, applanation tonometry, and ophthalmoscopy. All subjects had clear corneas and anterior chambers without inflammation. Based only on the ophthalmoscopic examination, the retinopathy was graded according to the modified Airlie House classification, similar to the grades described by Stovlrijk et al. The control group had no ocular abnormalities. Any subject with a history of ocular surgery, photocoagulation, contact lens wear, trauma, inflammation, glaucoma, ptosis, or severe keratoconjunctivitis sicca was excluded. No subject was taking topical ocular medications or systemic medications that affect the eye.

All subjects, both patients with diabetes and controls, underwent identical procedures. On study day 1 for each subject, central corneal thickness was measured in each eye every half hour from approximately 1:30 to 4:30 PM (either six or seven measurements) with a modified Haag-Streit (Köniz, Switzerland) optical pachometer equipped with fixation lights and a potentiometer that allows direct entry of readings into computer memory. The operator was not aware of the individual thickness readings as they were recorded. Each measurement was the mean of 10 consecutive readings. The measurement was repeated if the standard deviation of the readings was 10 μm or more. The same investigator (BCW) performed all pachometry measurements, recalibrating the instrument daily. Before fluorescein was instilled for tonometry, the autofluorescence of the central cornea was measured with a two-dimensional scanning ocular fluorophotometer. Measurements were made separately with two wavelengths of the argon laser (457.9 nm and 488.0 nm) for excitation; the emission window accepted fluorescent wavelengths of 515 to 600 nm. The instrument has been fully described elsewhere.

The methods for data analysis have been described in detail elsewhere; a general description will suffice here. Based on the assumption that corneal deswelling is a first-order process operative only upon the amount of stromal swelling, i.e., the thickness in excess of the open eye steady state (OESS) thickness (the thickness that, once attained, is maintained in the open eye without further thinning), one can write the following differential equation:

\[ \frac{dq}{dt} = -D(q - B) \]

where \( q \) is corneal thickness, \( t \) is the deswelling time (in these experiments, the time in minutes since removal of the contact lens), \( D \) is the deswelling rate constant, and \( B \) is the OESS thickness. The solution to this equation was used as the model for corneal deswelling:

\[ q(t) = B + Se^{-Dt} \]

where \( q(t) \) is the measured corneal thickness at time \( t \), and \( S \) is the induced swelling (the thickness in excess of \( B \) at \( t = 0 \)). The measured experimental data were fit by nonlinear regression (S-PLUS, Statistical Systems, Seattle, WA) to this model to obtain the best-
The deswelling rate was calculated using nonlinear regression analysis. The six OESS thickness measurements obtained on the afternoon of study day 1 are shown at time zero; in the analysis, they are assumed to occur at \( t = 10,000 \) minutes. No measurements in the first 30 minutes after contact lens removal are used to calculate the deswelling curve.

The measurements of corneal thickness during the first 30 minutes after removal of the contact lenses were not used for this analysis to avoid the effects of decreased pH during this period. In addition to a possible effect on the deswelling rate, the lower pH decreases the fluorescence efficiency of fluorescein, which would produce an overestimate of endothelial permeability. A more clinically meaningful parameter, the percent recovery per hour (PRPH = \( 1 - e^{-60D} \times 100 \)), was used to describe the deswelling rate.

In conjunction with the corneal thickness values, we used the corneal and anterior chamber fluorescence measurements to calculate the endothelial permeability to fluorescein with two adjustments for the changes in corneal thickness. The basic method and assumptions of Jones and Maurice were used to calculate the mean permeability between each fluorescence measurement and the succeeding one:

\[
\text{Permeability} = k_{ca} q_t r_{ca}
\]

where \( k_{ca} \) is the cornea-to-anterior chamber mass transfer coefficient for fluorescein, \( q_t \) is the mean of the corneal thickness measurements at the beginning and end of the interval, and \( r_{ca} \) is the steady state distribution ratio for fluorescein between the cornea and the anterior chamber.

The distribution ratio was adjusted for the uncertainties introduced when the corneal thickness changes during the measurement interval. The adjustment assumes that the increase in thickness results from the addition of water to the stroma without additional protein or other compounds that bind fluorescein. The following relationship, reported incorrectly previously, was used:

\[
r_{ca}' = \frac{q_0(r_{ca} - 1)}{q_t} + 1
\]

where \( r_{ca}' \) is the steady state distribution ratio for fluorescein in human corneas of normal thickness, assumed to be 1.6, \( q_0 \) is the 8 AM thickness, and \( q_t \) is the mean of the thicknesses at the beginning and end of the measurement interval. The 8 AM thickness was not recorded in one control subject; we used the OESS thickness plus \( 4 \) \( \mu \)m (the mean difference between the 8 AM and OESS thicknesses) for \( q_0 \) in this subject.

A second adjustment for corneal thickness, fully and correctly described elsewhere, was made in each fluorescence reading. The adjustment corrects for the change in efficiency of the focal diamond of the fluorophotometer that occurs with changes in corneal thickness:

\[
C_c = \frac{C'_c}{m q + b}
\]

where \( C'_c \) is the uncorrected measurement of corneal fluorescein concentration, \( C_c \) is the corrected corneal fluorescein concentration, and \( q \) is the corneal thickness at the time of the fluorescence measurement. For the fluorophotometer used in this study, \( m = 0.86 \) mm\(^{-1}\) and \( b = 0.29 \).

We determined the permeability to fluorescein during two intervals. The first, termed the AM permeability, was calculated from the fluorescence measurements at 8 AM and 11 AM, just before contact lens insertion and approximately 30 minutes after contact lens removal. The 8 AM and 10:30 AM pachometry readings were used to correct for changing thickness. The second determination, termed the PM permeability, was calculated from the hourly fluorescence measurements from 11 AM to 6 PM.

To measure the morphologic features of the central corneal endothelial cells, we digitized the apices of 100 cells from images of the photographic negatives magnified 500 times. The mean and standard devia-
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TABLE 1. Results

| Measurement | 20 Patients With Diabetes | 21 Controls | P* | MDD\[\]
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>51.2 ± 14.2</td>
<td>50.9 ± 15.2</td>
<td>0.96</td>
<td>14.8</td>
</tr>
<tr>
<td>Intraocular pressure (mm Hg)</td>
<td>15.0 ± 3.9</td>
<td>13.9 ± 2.8</td>
<td>0.15</td>
<td>3.4</td>
</tr>
<tr>
<td>Deswelling rate constant, D (min⁻¹)</td>
<td>0.018 ± 0.006</td>
<td>0.017 ± 0.004</td>
<td>0.012-0.023</td>
<td>0.38</td>
</tr>
<tr>
<td>Percent recovery per hour, PRPH (%/hr)</td>
<td>64.0 ± 11.7</td>
<td>62.5 ± 7.6</td>
<td>0.63</td>
<td>9.9</td>
</tr>
<tr>
<td>Open eye steady state (OESS) thickness (µm)</td>
<td>562 ± 35</td>
<td>559 ± 24</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>8:00 AM thickness (µm)</td>
<td>566 ± 36</td>
<td>543 ± 26</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>Induced swelling (µm)</td>
<td>44 ± 9</td>
<td>54 ± 7</td>
<td>&lt;0.001</td>
<td>—</td>
</tr>
<tr>
<td>Induced swelling (%)</td>
<td>7.7 ± 1.8</td>
<td>9.9 ± 1.6</td>
<td>&lt;0.001</td>
<td>—</td>
</tr>
<tr>
<td>Endothelial permeability, AM (×10⁻⁶ cm/min)</td>
<td>3.55 ± 0.83</td>
<td>4.14 ± 0.68</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>Endothelial permeability, PM (×10⁻⁶ cm/min)</td>
<td>3.85 ± 0.60</td>
<td>4.03 ± 0.53</td>
<td>0.31</td>
<td>0.57</td>
</tr>
<tr>
<td>Corneal autofluorescence, 457.9 nm (ng/ml fluorescein equivalents)</td>
<td>8.1 ± 3.1</td>
<td>6.0 ± 1.9</td>
<td>0.005§</td>
<td>—</td>
</tr>
<tr>
<td>Corneal autofluorescence, 488.0 nm (ng/ml fluorescein equivalents)</td>
<td>4.1 ± 1.6</td>
<td>3.1 ± 0.9</td>
<td>0.007§</td>
<td>—</td>
</tr>
<tr>
<td>Endothelial cell density (cells/mm²)</td>
<td>2541 ± 361</td>
<td>2601 ± 353</td>
<td>0.59</td>
<td>361</td>
</tr>
<tr>
<td>Coefficient of variation of cell area (mean/SD)</td>
<td>0.33 ± 0.06</td>
<td>0.31 ± 0.07</td>
<td>0.52†</td>
<td>0.07</td>
</tr>
<tr>
<td>Hexagonal endothelial cells (%)</td>
<td>57.3 ± 7.6</td>
<td>60.4 ± 7.6</td>
<td>0.20</td>
<td>7.6</td>
</tr>
</tbody>
</table>

* Two-tailed Student’s t-test for means (except § below).
† N = 20 in each group (8:00 AM thickness not recorded in one control subject).
‡ Increase in thickness from 8:00—10:30 AM, from just before contact lens insertion to just after removal. N = 20 in each group (8 AM thickness not recorded in one control subject).
§ Wilcoxon rank sum test.
\[MDD = minimum detectable difference with 90% power (α = 0.05, β = 0.10).\]

...was recorded.

For statistical analysis, we compared values in the group with diabetes and the control group with a two-tailed Student’s t-test for means if the data were distributed normally. For data that were not distributed normally, we used the Wilcoxon rank sum test. Correlations between continuous variables were obtained with Pearson’s correlation coefficient (r) for normal data and Spearman’s rank correlation coefficient (rs) for non-normal data. One value, the mean of the values for the two eyes, was used for each subject. A two-tailed probability of 0.05 or less was considered statistically significant.

RESULTS

The mean values for each of the test parameters measured in the 20 patients with diabetes and 21 controls are listed in Table 1. The age range of the patients with diabetes was 22 to 72 years and of the controls was 20 to 71 years. The duration of diabetes ranged from 10 to 50 years (mean ± standard deviation 23.0 ± 12.6 years). There was no significant difference between the 15 patients with type I diabetes and the 5 patients with type II diabetes for any of the parameters we tested. Nonproliferative background diabetic retinopathy was present in all patients. Based upon the results of ophthalmoscopic examination in the worse eye, the retinopathy was graded as follows: 13 eyes, grade 2 (minimal nonproliferative retinopathy); 6 eyes, grade 3 (moderate nonproliferative retinopathy); and 1 eye, grade 4 (severe nonproliferative or preproliferative retinopathy). The 21 controls were all grade 1 (no retinopathy). The mean intraocular pressure was not significantly different between the patients with diabetes and the controls.

Total serum glycosylated hemoglobin levels, which approximate average blood glucose levels during the previous 2 to 3 months, were considered relative indicators of diabetic control. Although obtaining glycosylated hemoglobin levels was not part of the pro-
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protocol for this study, the values were recorded in the medical records within 2 months of this study in 10 subjects with diabetes (9 eyes grade 2 and 1 eye grade 3 retinopathy). The mean glycosylated hemoglobin value in these 10 subjects was 10.0% ± 1.3% (range, 8.0% to 12.1%), similar to the mean level of 10.0% in the 14 patients with diabetes in our previous study, which had similar entry criteria.6 The interpretation of total glycosylated hemoglobin values for our clinical laboratory is as follows: good diabetic control, <9%; fair control, 9 to 12%; and poor control, > 12%. Based on the assumption that the 10 patients with diabetes for whom glycosylated hemoglobin was recorded are representative of the entire group, the average level of glycemic control in the diabetic group was fair.

**Pachometry**

Each corneal thickness measurement was the mean of 10 consecutive readings. The mean of the standard deviations in 1410 stress thickness measurements (11 AM to 6 PM) in the 82 eyes was 5.2 μm, with no significant difference between the patients with diabetes (5.3 μm) and controls (5.1 μm, P = 0.21). Ninety-five percent of the standard deviations were <8.1 μm.

We found no difference between the PRPH values for the 20 patients with diabetes and 21 controls. Using 7 hours of corneal thickness recovery data beginning 30 minutes after the hypoxic contact lens was removed,18 we recorded mean PRPH values of 64.0% ± 11.7%/hour and 62.5 ± 7.6%/hour for the group with diabetes and the control group, respectively (P = 0.63). We can be 90% certain that the true difference, if any, in PRPH between these two groups is not more than 9.9% (Table 1). The PRPH decreased significantly with age (Fig. 2) in all 41 subjects (r = -0.48, P = 0.001). The correlation between PRPH and age was also significant in the 20 patients with diabetes (r = -0.60, P = 0.005) but was only suggestive in the 21 controls (r = -0.36, P = 0.10). The

**Fluorophotometry**

The endothelial permeability to fluorescein during the period of hypoxia, while the contact lens was in place (AM permeability, Fig. 5), was significantly decreased in the patients with diabetes compared to the controls (3.55 ± 0.83 × 10⁻⁴ cm/minute versus 4.14 ± 0.68 × 10⁻⁴ cm/minute, P = 0.02). After the contact lens was removed (PM permeability), there was no significant difference between the two groups. In the control group, the AM and PM permeabilities were similar. The PM permeabilities were calculated from the hourly fluorescence measurements from 11 AM

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**FIGURE 2.** Percent recovery per hour (PRPH) versus age. The least squares regression line for all subjects is y = 79.69 - 0.32x, where y = PRPH and x = age in years.

**FIGURE 3.** Open eye steady state (OESS) corneal thickness. The horizontal lines denote the mean values for the diabetic (562 μm) and control (539 μm) groups.

**FIGURE 4.** Swelling induced by 2 hours of hypoxic soft contact lens wear. The horizontal lines denote the mean values for the diabetic (7.7%) and control (9.9%) groups.

**FIGURE 5.** OESS thickness (Fig. 3) was significantly greater in patients with diabetes than in controls (562 ± 35 μm versus 539 ± 24 μm, P = 0.02), as was the 8 AM thickness (556 ± 36 μm versus 543 ± 26 μm, P = 0.02). The induced swelling (Fig. 4), however, was significantly less in patients with diabetes than in controls (7.7% ± 1.8% versus 9.9% ± 1.6%, P < 0.001). The peak corneal thickness measurements at 10:30 AM were invariably lower than the thickness predicted for t = 0 by the nonlinear regression, indicating that the deswelling rate was slower during the initial 30 minutes after contact lens removal.
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FIGURE 5. Hypoxic endothelial permeability (AM) during period of hypoxic contact lens wear. The horizontal lines denote the mean values for the diabetic (8.55 × 10^{-4} cm/minute) and control (4.14 × 10^{-4} cm/minute) groups.

to 6 PM in all except three patients with diabetes and two controls, who did not have a 6PM measurement; in these five subjects, the PM permeability was based on measurements from 11 AM to 5 PM. The corneal autofluorescence, corrected for corneal thickness, was significantly increased in the patients with diabetes at both excitatory wavelengths, 457.9 and 488.0 nm (Fig. 6). In the 13 patients with diabetes with grade 2 retinopathy, the mean autofluorescence at 457.9 and 488.0 nm, respectively, was 7.4 and 3.9 ng/ml fluorescein equivalents, and in the six patients with diabetes with grade 3 retinopathy, it was 9.7 and 4.6 ng/ml fluorescein equivalents (P > 0.05 for both).

Specular Microscopy

No significant differences in any endothelial morphologic values were found between the group with diabetes and the control group. There were significant morphologic differences, however, between the 13 patients with diabetes with grade 2 retinopathy and the 6 patients with diabetes with grade 3 retinopathy (Figs. 7 and 8). In those with grade 3 retinopathy, the coefficient of variation of cell area was greater (0.38 ± 0.40 versus 0.31 ± 0.06, P = 0.03), and the percentage of hexagonal cells was less (51.8 ± 3.0 versus 59.5 ± 8.1%, P = 0.04) than in those with grade 2 retinopathy. There were no significant differences between the two grades of retinopathy in age, endothelial cell density, PRPH, OESS thickness, induced swelling, AM or PM permeability, or autofluorescence at either excitatory wavelength.

DISCUSSION

Dermometry

The OESS thickness was significantly increased in the group with diabetes, confirming the results of others in humans and animals. We did not find increased thickness in a previous study, perhaps because that investigation lacked sufficient power to detect a similar difference. The present study does not explain the cause of increased corneal thickness in diabetes. There are several possible explanations, such as inhibition of the endothelial pump, increased stromal swell-
ing pressure for a given thickness, or increased endo-
thelial permeability. The finding of normal endo-
thelial permeability to fluorescein in this study makes the
last possibility unlikely. The first two possibilities could
result from the metabolic effects of diabetes, however.
Hyperglycemia is known to inhibit Na/K ATP-ase-de-
pendent transport.23 Altered stromal swelling pressure
could result from the accumulation of polyols or the
glycosylation of proteins, both known effects of diabe-
tes.24 Such a stromal alteration might also affect ϵe and,
thus, our estimate of endothelial permeability. Altered
stromal swelling pressure appears unlikely, however,
because Herse1 found no difference in swelling
pressure in diabetic rabbits.
By recording the corneal thickness hourly for
more than 7 hours after contact lens removal, we ob-
tained more reliable estimates of the OESS thick-
nesses. Mandell et al17 measured corneal deswelling
for at least 3 hours after contact lens removal and
combined the data by a coupled exponential model
with deswelling measurements on a different day after
the eye was patched shut all night; they included esti-
mates of OESS thickness with asymptotic standard er-
rors as large as 15 μm. Herse and Hooker4 measured
corneal deswelling for 3 hours after contact lens re-
moval only; they did not report the results of their
OESS thickness estimates or the asymptotic standard
errors they accepted. In contrast, the standard errors
of our OESS thickness estimates ranged from 0.8 μm
to 2.6 μm (mean = 1.3 μm) in the 20 patients with
diabetes and from 0.6 μm to 2.1 μm (mean = 1.2 μm)
in the 21 controls. If we excluded the direct OESS
thickness measurements from the first experimental
day and included only the thickness measurements
during the 7 hours after contact lens removal, the
standard errors of the OESS thickness estimates ranged
from 1.1 μm to 5.2 μm (mean = 2.0 μm) in the
patients with diabetes and from 0.9 μm to 3.8 μm
(mean = 1.8 μm) in the controls. When the first day's
measurements were excluded, the mean OESS esti-
mate changed little—from 562.2 μm to 561.5 μm in
the patients with diabetes and from 538.8 μm to 538.4
μm in the controls. Therefore, by measuring the cor-
neal thickness for 7 hours rather than for 3 hours after
removal of the stress lens, we eliminated the need
for a second day of measurements and obtained more
reliable estimates of OESS thickness in these subjects
with normal deswelling.
The stromal swelling induced by 2 hours of con-
tact lens-induced hypoxia was significantly less in the
patients with diabetes. If we ignore fluid exchanges
across the epithelium and limbus, which are likely to
be minor,25 this finding could only result from less
net fluid flow into the cornea across the endothelium
in the patients with diabetes than in the controls.
Three factors, alone or in combination, in the diabetic
corneas compared to the control corneas during the
hypoxic period would produce less swelling: less endo-
thelial permeability, less stromal swelling pressure,
and greater endothelial pump rate. The first factor
was indeed present—the measured permeability to
fluorescein was less (Table 1). The second factor was
probably present because the corneal thickness was
greater in the patients with diabetes, and Herse found
the same swelling pressure—hydration—thickness rela-
tionship in normal and diabetic rabbit corneas.1 In
addition, less osmotic swelling would have been in-
duced if the corneal epithelium of the patients with
diabetes produced less lactate.26 The third factor
could have been present if the endothelial pump were
less affected by epithelial hypoxia in the patients with
diabetes than in the controls. McNamara et al recently
reported that decreased stromal pH resulted in a de-
creased amount of hypoxic swelling.27 Our findings of
decreased endothelial permeability to fluorescein are
thus consistent with a lower stromal pH induced by
hypoxia in the patients with diabetes than in the con-
trols.
We found no difference in deswelling rate or
PRPH between the diabetic and control corneas. In a
recent study of 12 human patients with diabetes and
14 controls by Herse and Hooker,4 a difference in
PRPH of 27% was present. Our study could rule out
such a difference with greater than 90% confidence
(Table 1). We have no explanation for these disparate
results. If the diabetic corneas in the two studies were
truly different from each other, it could reflect a dif-
ference in the effects of diabetes in the two groups,
either from differences in the severity of the disease
or in its medical control. The PRPH decreased signifi-
cantly with age in our subjects (Fig. 2), confirming
the results of Porse et al.8 In the study by Herse and
Hooker,4 the patients with diabetes were older than
the controls, but it is unlikely that this difference ac-
counted for the decreased PRPH that they found.
Endothelial permeability and deswelling rates
were similar in the patients with diabetes and the con-
trols after contact lenses were removed. If the corneal
thicknesses were similar, this finding would suggest
that the endothelial pump rate is also similar in the
two groups. Because the diabetic OESS thickness was
greater, however, the swelling pressure may have been
less, requiring a lower pump rate to result in a similar
deswelling rate. Thus, this study has not ruled out a
decreased endothelial pump rate in the group with
diabetes. More study is needed to define these rela-
tionships better in human and diabetic corneas.

Fluorophotometry
We found no significant difference in endothelial per-
meability to fluorescein between the patients with dia-
betes and the controls from 11 AM to 6 PM. The
corneas were not hypoxic during this period; we consider the permeability measured over this interval to be the normal permeability for each cornea. This finding of no effect of diabetes on corneal endothelial permeability confirms the results of a previous study using similar techniques,6 of a study of human corneas in vitro,28 and of a study of diabetic rats and guinea pigs.39 In contrast, Lass et al9 found a significant 31% increase in diabetic endothelial permeability, but their findings may result from the maldistribution of stromal fluorescein that occurs with their method, as demonstrated by Carlson et al.16

The endothelial permeability from 8 to 11 AM, during the period of contact lens-induced hypoxia, was significantly less in the group with diabetes. This finding is consistent with the lower contact lens-induced swelling that occurred in the diabetic corneas. One possible explanation is that under these conditions, more edema occurs in the diabetic endothelial cells than in the normal cells, thus narrowing the paracellular pathway. Such a possibility is plausible because both metabolic30 and cytoskeletal abnormalities have been noted in diabetic corneal endothelial cells.

The diabetic corneas had significantly increased autofluorescence at both excitation wavelengths (λex), 457.9 and 488.0 nm. The range of emission wavelengths (λem) accepted by our fluorophotometer was 515 to 600 nm.15 This finding confirms the results of our previous study of patients with diabetes with λem = 488.0 nm6 and of the results of Stolwijk et al with λem = 430 - 490 nm and λem = 530 to 630 nm.10,32 Greater fluorescence resulted from excitation at 457.9 nm than at 488.0 nm, which is consistent with mitochondrial flavoproteins33 as its source, as suggested by others.34,35 Most of the fluorescence is likely to originate in the epithelium.36 The proportional increase in fluorescence in the patients with diabetes is the same for both excitation wavelengths (38%), suggesting that the fluorescence has the same source in both groups. The diabetic corneas may contain more of the same fluorophore or may exhibit local conditions that enhance its fluorescence, such as alterations in oxidative state,37 pH, temperature, light filtering characteristics of the overlying tissue, or interaction with other molecules.

The 38% increase in autofluorescence in the group with diabetes is similar to the 40% increase found by Stolwijk et al10 in patients with diabetes with minimal nonproliferative retinopathy (grade 2). The majority (13 of 20) of our patients had grade 2 retinopathy.

**Specular Microscopy**

We found no difference between the patients with diabetes and controls in central endothelial cell density, confirming many previous studies of the morphologic aspects of diabetic corneal endothelial cells.5,6,32-39 These same reports, however, also consistently demonstrated an increased coefficient of variation of cell area (polymegethism) and decreased percentage of hexagonal cells (pleomorphism) in the diabetic corneas, which were not seen in the present study. This discrepancy must result from one of two possibilities: either the endothelial cells of these patients with diabetes were not affected by the disease or our study could not demonstrate the effect. The former possibility would imply that these patients with diabetes are different from those in the former studies, who perhaps had milder or better controlled disease. The patients with diabetes in our previous investigation, however, in which a morphologic difference was present,6 appeared to be similar to those of the present study, as judged by the degree of retinopathy and the available glycosylated hemoglobin values. The latter possibility, that our study could not demonstrate a morphologic abnormality, is perhaps more plausible. The present study lacked sufficient statistical power to detect with 90% confidence (Table 1) morphologic differences of the size present in our previous study6 or in those of others. Our subjects were also older than those of former investigations; because polymegethism and pleomorphism increase with age,46 the differences between patients with diabetes and controls may be less in older subjects. Finally, the control group appears to have been morphologically, but not physiologically, atypical, decreasing our chances of finding statistically significant differences in the patients with diabetes. The present control group had substantially more polymegethism and pleomorphism than the controls in our former studies,6,16 more so than can be attributed to the difference in age. Therefore, although the current group with diabetes had more polymegethism (coefficient of variation of cell area 0.33 versus 0.29) and pleomorphism (percentage of hexagonal cells 57% versus 68%) than the patients with diabetes in our previous study,7 it was not statistically significantly different from the current control group.

Despite the lack of a morphologic difference from the control group, the six patients with diabetes with grade 3 retinopathy had significantly more polymegethism and pleomorphism than the 13 patients with diabetes with grade 2 retinopathy (Figs. 7 and 8). This finding suggests a positive correlation between the severity of the diabetes and its morphologic effects on endothelial cells. A direct correlation between diabetic control and endothelial morphologic abnormalities has been demonstrated in diabetic dogs,40 although such a correlation was not found in humans.40 For several reasons, more data are required to confirm this suggested relationship. First, the present study contained small numbers for stratification into
groups. Second, the grading of retinopathy was based on clinical ophthalmoscopic examination rather than photographic evaluation. Third, no significant differences between retinopathy grades were noted for any of the other measures such as PRPH, OESS thickness, induced swelling, endothelial permeability, or corneal autofluorescence.

Key Words
corneal endothelial permeability, corneal deswelling, diabetic cornea, corneal thickness, corneal endothelial morphology, corneal endothelial pump

References

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