The Use of Vitreous Fluorophotometry to Distinguish between Diabetics with and without Observable Retinopathy: Effect of Vitreous Abnormalities on the Measurement

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Ten age-matched normals, diabetics with retinopathy, and diabetics without observable retinopathy were evaluated by vitreous fluorophotometry (VFL) using a 0.15 mm and a 0.45 fiberoptic probe in a photomultiplier system as well as a commercially available photodiode instrument to determine whether differences in intraocular sodium fluorescein levels could be detected among the three groups. Each subject was injected in the antecubital vein with 7 mg/kg of sodium fluorescein (25% solution) and measurements were taken 1 hr postinjection at 4.5 mm and 7.5 mm from the retina. The influence of choroidal fluorescein and ocular pigmentation are reduced at these locations. We found that a breakdown in the blood-ocular barrier may not be present early in the course of diabetes. Furthermore, no significant difference was found between normals and diabetics without retinopathy. Although the mean value for vitreous fluorescein was significantly higher in diabetics with retinopathy compared to normals, several of the diabetics with retinopathy had values in the normal range. These results differ from those previously reported in the literature. However, our studies took into consideration several factors not considered by other investigators, such as ocular pigmentation, choroidal fluorescence, slit width, and vitreous changes, that may have significant effects on the fluorophotometry values. Invest Ophthalmol Vis Sci 24:57-65, 1983

Vitreous fluorophotometry (VFL) is a quantitative method of detecting a breakdown in the blood-ocular barriers as measured by the concentration of sodium fluorescein at various intraocular locations. Since this method was first introduced by Maurice1 in 1963 and further refined by Waitman and Kaufman2 in 1970, numerous studies have been conducted among those populations with compromised blood-ocular barriers. Juvenile3,4 and adult-onset5-9 diabetic patients, with and without observable retinopathy, have been studied most intensely. Some laboratories have reported that diabetics who demonstrate intact retinal vasculature and pigment epithelium by fluorescein angiography and ophthalmoscopy have significantly higher VFL readings than normals.3,5-7 Although these studies suggest that elevated VFL readings may represent the earliest abnormality in diabetic retinopathy, they did not take into consideration the effect of recently identified factors that might influence the results. These include choroidal fluorescein,10 ocular pigmentation,10,13 vitreous change,12 and specific intraocular locations.10,13

In this report we compare VFL readings in normals and age-matched diabetics with and without observable retinopathy (ie, not exceeding an approximate total of 30 microaneurysms, hemorrhages, and soft
exudates). A photomultiplier system using either a 0.15-mm or 0.45-mm probe is also compared with a commercially available system using an array of photodiodes.

Another purpose of this study is to determine the consequences of including subjects with observed vitreous change on group means, standard deviations (SD), and Analysis of Variance F values. The consequences of including these individuals will be evaluated for two intraocular locations, 4.5 mm and 7.5 mm from the retina, and for the several vitreous fluorophotometers.

Materials and Methods

Apparatus

Photomultiplier system: A Haag Streit Model 360 slit lamp was slightly modified by replacing the right ocular with an eyepiece containing either a 0.15-mm or 0.45-mm fiberoptic probe (Gamma Scientific, San Diego, CA). The light-gathering probe was superimposed onto the center of a slit beam 1.00 mm × 0.75 mm for the 0.15-mm probe or 1.00 mm × 0.30 mm for the 0.45-mm probe. Specially constructed glass sandwiches (Optical Instrument Laboratory, Houston, TX) were used as standards to check the linearity of the instrument. The selected slit widths maintained a linear relationship among the glass analogs, which were equivalent to 10⁻⁹⁻⁻⁶ g/ml of sodium fluorescein. Thinner slit widths would have resulted in a photomultiplier signal that was obscured by dark current noise. This was especially true for the weaker dilutions of sodium fluorescein (1 × 10⁻⁹ g/ml).

Averaging multiple samples (three) markedly reduces the affect of dark current noise on the resultant data. When using the 0.45-mm probe, the absolute range of dark current noise for three samples was 0.33 ng/ml, although the average midpoint of the range was not far from baseline, ±0.02 ng/ml, ±0.08 ng/ml SE. For the 0.15-mm probe the average range of dark current noise was 1.63 ng/ml, with a typical midpoint of ±0.10 ng/ml above baseline, ±0.42 ng/ml SE. Thus, by taking the mean value of multiple measurements as data, much of the effect of dark current noise may be eliminated. If 3 SD above baseline is used to measure sensitivity, we estimate sensitivity with the 0.45-mm probe and 0.30-mm slit beam width to be 2.2 × 10⁻¹⁰ g/ml Nafl. The 0.15-mm probe with a 0.75-mm slit width has an estimated sensitivity of 1.1 × 10⁻⁹ g/ml of Nafl.

To re-establish the same slope and intercept for the concentration curve, luminance output (2.65 × 10⁻³ watts/cm² or 6.08 × 10⁴ lux) was kept invariant (compensating for bulb grayout) by means of a variable output transformer set at approximately 5.5 volts. Excitation light peaked at 490 nm (SE40R Spectrotech Filter, Lincoln, MA). Light gathered by the fiberoptic probe was directed to a photomultiplier (EG aG Model 555-32) via a fiberoptic cable with a Spectrotech barrier filter, SB50R, located at the interface between the cable and the photomultiplier. Current from the photomultiplier was displayed on a digital output photometer/radiometer (EG aG Model 550-1). This information was fed into an X-Y plotter for a permanent record. (For a more detailed description of apparatus and procedure see Prager et al, 1981.)

Photodiode system: A Metricon Model 120 fluorophotometer was affixed to a Haag Streit 900 slit lamp. The left ocular was replaced by a light detector containing an array of 128 photodiodes, averaging five readings over four diodes to detect fluorescein at 32 points from the retina to the lens. Using A-scan ultrasonography to measure the distance between the retina and lens, we estimate the distance between the points to be 0.834 mm ± 0.088 SE. The detector could be activated by a foot switch, and the resultant information was displayed on a remote control console in both graphical and numerical formats. The recommended slit dimensions were 2.5 mm × 0.45 mm. Glass sandwiches were used to calibrate the instrument. A potentiometer located at the back of the control console could be adjusted to correct for bulb output decay. (For a technical evaluation of the Metricon, see Zeimer and Cunha-Vaz.)

Patient Population

Normals: Ten subjects (average age 36.7 ± 13.13 years SD) with no observable retinopathy or vitreous change (by slit-lamp examination with a Hruby lens) and no history of hypertension served as normals. Two additional nondiabetic subjects had normal appearing retinas, but were observed to have vitreous changes (ie, lacunae and vitreous detachments). Consequently they were not included in the initial analysis.

Diabetics without observable retinopathy: This group included ten diabetics (average age 29.9 ± 13.27 years SD) with no observable retinopathy or vitreous change. The average duration of diabetes was 5.8 ± 3.6 years (SD), with eight insulin-dependent patients and two patients treated by diet alone. None of these subjects had a history of hypertension. An additional diabetic with no retinopathy but whose vitreous contained syneresis was also examined but was not included in the initial analysis.

Diabetics with background retinopathy: Ten patients (average age 33.3 years ± 12.60 SD) with background retinopathy were also studied. The majority of these patients (80%) had a total of less than 30
microaneurysms, hemorrhagic spots, and soft exudates, while 30 of the patients had less than ten observable lesions. No patient had neovascularization, and none was found to have vitreous change. The average duration of diabetes was 11.8 ± 5.5 years (SD), with eight patients receiving insulin, one patient treated with oral hypoglycemics, and one patient managed by diet alone.

Only those individuals free of vitreous change were included in the first analysis. When collecting the data for the initial analysis, three people had been eliminated due to an abnormal vitreous. Detection was enhanced by using Tolentino's whiplash technique where the subject quickly flicks his eyes (eg, up) and then returns to the primary position of gaze. Two normals with a clear retina and no history of diabetes or hypertension were found to have changes in the vitreous gel. One individual had a V-shaped vitreous detachment, the other exhibited several lacunae. Also one diabetic without observable retinopathy demonstrated fibril strands, ie, syneresis, within the vitreous. None of the diabetics with background retinopathy evidenced vitreal change. To demonstrate the effect of including several subjects with abnormal vitreous on group means, SD, and F-test analyses these individuals were substituted for subjects with normal vitreous. In other words, for the second data analysis, the normal group now contained eight subjects with no vitreous change and two subjects with vitreous change. The diabetics without observable retinopathy group had nine patients with no vitreous change and one patient with syneresis. All ten patients with minimal retinopathy had a normal appearing vitreous.

A one-way analysis of variance (ANOVA) showed that the age among the various groups did not differ significantly.

**Patient Procedure**

Prior to testing, each subject read and signed a consent form. Visual acuity was assessed, and the eyes were dilated (Mydriacyl 0.5% and Phenylphrine 2.5%). Each subject was injected in the antecubital vein with 7 mg/kg of sodium fluorescein 25%. One-hour postinjection topical anesthetic drops were applied, and an Engel contact lens coupled with Methy cellulose was placed on the right eye. The center of the slit beam was positioned over the foveola. The slit beam was returned to the primary position of gaze. Two passes were made, and the average value at 4.5 mm and 7.5 mm from the retina was recorded as data.

It has been demonstrated for the photomultiplier system using slit widths of 0.30 mm and 0.75 mm that measurements taken at 4.5 mm from the retina are influenced very little (<5%) by the effects of choroidal fluorescein and varying ocular pigmentation. The influence of natural fluorescence and backscatter from the lens is minimal at 7.5 mm: from the retina. The contribution of choroidal fluorescein is less at this location than at 4.5 mm from the retina. Readings were taken at both 4.5 and 7.5 mm from the retina to determine the best position for data collection. Readings at 4.5 mm from the retina are approximately midposterior vitreous and measurements at 7.5 mm from the retina are at the midvitreous.

**Statistical Analysis**

Since each subject contributed more than one score and each subject could not be in each group, the data are analyzed by a subject nested in group × trial Analysis of Variance (ANOVA). The group × trial (position) interaction is used to determine whether one intraocular position separates groups better than another position. A Scheffe subsequent test on the simple effects allows the comparison of normals to diabetics without retinopathy, normals to diabetics with retinopathy, and diabetics without retinopathy to diabetics with retinopathy.

**Results**

**I. Normals, Diabetics with and without Retinopathy, Free of Vitreous Abnormalities**

Table 1 (column 1) summarizes the means and SD for normals and diabetics with and without observable retinopathy obtained at both 4.5 mm and 7.5 mm from the retina with the 0.15-mm and 0.45-mm probes in the photomultiplier system as well as the photodiode instrument. None of these subjects had any observable vitreous change.

Figure 1 presents the mean of the vitreous fluorescein levels ± 1 SE for the normals, diabetics without retinopathy, and diabetics with retinopathy. The values in nanograms per milliliter for the three groups are plotted as a function of the distance from the retina. Figure 1A depicts data from the photomultiplier with the 0.45-mm probe, 1B with the 0.15-mm probe, and 1C with the Metricon photodiode system.

# Plasma fluorescein levels were not determined because previous unpublished data demonstrated no statistical difference in sodium fluorescein concentration between normals and diabetic populations. Despite equivalent plasma fluorescein concentrations, it is possible that protein binding of the dye might be different between the diabetics and normals and any such difference could significantly affect the results.
Table 1. The effect of including two normals and one diabetic without observable retinopathy, but detectable vitreous change, on group means. VFL readings were taken at 4.5 mm (A) and 7.5 mm (B) from the retina. Means, in nanograms/ml (SD), and percent change of group means, are noted for normals, diabetics without retinopathy (s), and diabetics with retinopathy (c). In each group N = 10.

<table>
<thead>
<tr>
<th></th>
<th>Normal vitreous group</th>
<th>Abnormal vitreous group</th>
<th>% Change in group x</th>
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<tr>
<td></td>
<td>x (SD)</td>
<td>x (SD)</td>
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<tr>
<td><strong>A (4.5 mm)</strong></td>
<td></td>
<td></td>
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<tr>
<td>PM 0.15-mm probe</td>
<td>Normal</td>
<td>4.43 (1.17)</td>
<td>4.60 (1.51)</td>
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<td></td>
<td>Diabetic s</td>
<td>4.74 (1.49)</td>
<td>4.89 (1.57)</td>
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<td>Diabetic c</td>
<td>6.48 (2.26)</td>
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</tr>
<tr>
<td>PM 0.45-mm probe</td>
<td>Normal</td>
<td>1.95 (0.40)</td>
<td>2.62 (2.12)</td>
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<tr>
<td></td>
<td>Diabetic s</td>
<td>1.52 (0.27)</td>
<td>1.84 (0.93)</td>
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<tr>
<td></td>
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<td>2.36 (1.05)</td>
<td>—</td>
</tr>
<tr>
<td>Metricon</td>
<td>Normal</td>
<td>6.62 (6.51)</td>
<td>7.17 (6.38)</td>
</tr>
<tr>
<td></td>
<td>Diabetic s</td>
<td>6.65 (3.61)</td>
<td>8.00 (4.80)</td>
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<tr>
<td></td>
<td>Diabetic c</td>
<td>16.84 (15.42)</td>
<td>—</td>
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<tr>
<td><strong>B (7.5 mm)</strong></td>
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<td>PM 0.15-mm probe</td>
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<td>2.09 (0.65)</td>
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</tr>
<tr>
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<td>1.22 (0.40)</td>
<td>3.17 (5.32)</td>
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<td>Diabetic s</td>
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</tr>
<tr>
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<td>3.37 (2.83)</td>
<td>4.29 (2.98)</td>
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<td></td>
<td>Diabetic s</td>
<td>3.08 (2.23)</td>
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<tr>
<td></td>
<td>Diabetic c</td>
<td>6.13 (4.43)</td>
<td>—</td>
</tr>
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</table>

Note that the scale on the ordinate is different for each of the systems, probably due to differences in probe sensitivities, slit-beam widths, and the volume of vitreous sampled.

Figure 2 depicts the individual data points, in nanograms per milliliter, at 7.5 mm from the retina for normals, diabetics without retinopathy, and diabetics with background retinopathy. Individual data points for these three groups are shown for the 0.15-mm probe, 0.45-mm probe, and the Metricon fluorophotometer. The horizontal bar represents the group mean. Considerable overlap between normals and diabetics with retinopathy was found with all three instruments.

To test whether the group means differed significantly from one another, a separate subject nested in group X trial ANOVA was performed on the data gathered from each of the three VFL systems. Table 2 (column 1) summarizes the F values and significance levels for groups, intraocular position, and the group by position interaction. The main effect for groups was significant at the 0.05 level of significance for all the systems: 0.45-mm probe, F = 5.25; 0.15-mm probe, F = 5.37; and Metricon, F = 3.42.

In the preceding analyses the data were collapsed over the two intraocular positions and only groups were considered. The group × trial (position) interaction gives information about whether the groups were more widely separated at one position than at the other. For both the 0.45-mm and 0.15-mm probes, the separation between groups did not differ significantly for either intraocular location, which suggests that 4.5 mm or 7.5 mm from the retina offers similar information one hour postinjection. This was not so for the Metricon unit; the group × trial interaction was significant: F = 3.56, P < 0.05. An inspection of the data (Fig. 1C) shows the separation at 4.5 mm to be greater than that achieved at 7.5 mm from the retina. Thus, collecting data at 4.5 mm will tend to optimize the results with this system.

A Scheffe subsequent test was performed on the simple effects (position 4.5 mm and 7.5 mm for each instrument) to contrast normals to diabetics without retinopathy. At 4.5 mm and 7.5 mm from the retina neither the photomultiplier with the 0.45 mm probe nor the 0.15 mm probe nor the photodiode system could statistically separate these two populations. In no case did the F value approach significance.

The results of the Scheffe test between normals and diabetics with observable retinopathy showed the
Fig. 1. The mean of the vitreous fluorescein levels ± 1 SE in nanograms per milliliter is depicted for normals (---), diabetics without retinopathy (-- - - ), and diabetics with retinopathy (O O O O O). Ten subjects were in each group. Values for the three groups are plotted against position (4.5 and 7.5 mm from the retina). Figure A illustrates data from the photomultiplier with the 0.45-mm probe, B with the 0.15-mm probe, and C the Metricon, photodiode system.

The photomultiplier system to be more capable of differentiating populations than the photodiode system (Metricon). At 4.5 mm from the retina, only the difference for the 0.15 mm probe was significant: $F = 3.65, P < 0.05$. Both probes used in the photomultiplier system separated normals from diabetics with retinopathy at 7.5 mm from the retina: 0.45-mm probe, $F = 3.39, P < 0.05$; 0.15-mm probe, $F = 4.17, P < 0.05$.

The Metricon was unable to separate populations at either intraocular location as assessed by the Scheffe subsequent test, although the main effect for groups was previously reported to be significant. To explain this difference between test results, we note that the Scheffe test, while the most appropriate test of subpopulations, is less powerful than the main effect ANOVA for groups.

A Scheffe test comparison was run between dia-
betics without observable retinopathy and diabetics with background retinopathy. For the 0.15-mm probe and the Metricon there was no significant difference between the two groups at either intraocular position. However, the 0.45-mm probe showed significance at both 4.5 mm (F = 4.01, P < 0.05) and 7.5 mm from the retina (F = 5.03, P < 0.05). This result may be attributed to the mean value at both intraocular locations being lower for the diabetics without retinopathy group than for the normals (Table 1, column 1) when using the 0.45-mm probe.

II. The Effect of Vitreous Change on Separating Diabetics with and without Retinopathy from Normal Populations

Table 1 (columns 2 and 3) details group means, SD and percent change in group means at 4.5 mm (A) and 7.5 mm (B) from the retina when the groups are composed of several subjects with an abnormal vitreous. Values are listed for the photomultiplier system (PM) with the 0.15-mm probe, 0.45-mm probe, and the Metricon photodiode system.

Table 2 (column 2) summarizes the F values and probability levels for groups, intraocular position, and group X position interaction. The Scheffe subsequent test on simple effects contrasts: normals to diabetics without retinopathy; normals to diabetics with background retinopathy; and diabetics without retinopathy to diabetics with retinopathy at 4.5 mm and 7.5 mm from the retina. The effect of including several subjects with vitreous change on F values and significance levels may be compared by noting the results of the normal vitreous group relative to the abnormal vitreous group.

In this small sample population, n = 10 for each group, there was a significant difference among groups for all three systems when all the individuals had a normal appearing vitreous (Table 2, column I). With the inclusion of three subjects who had vitreal abnormalities there was no longer a significant difference among groups (column 2), a finding that holds true for all three instruments. In this analysis, the main effect for groups has been collapsed over both intraocular locations. The Scheffe subsequent tests and the percentage change in group means between normal and abnormal test comparison at both intraocular locations provides additional information about the influence of vitreous change in separating populations.

For each of the three systems at both intraocular locations, the inclusion of several subjects with vitreous change resulted in an increase in group means (Table 1). After collapsing data over fluorophotometers, the average percentage change (Table 1) induced by including the several subjects with vitreous change was 15% at 4.5 mm and 51% at 7.5 mm. The average increase in group means at 4.5 mm from the retina was greatest for the 0.45-mm probe (27.5%) and least for the 0.15-mm probe (3.5%). The Metricon data fell in the middle (14%). The average increase was greater at 7.5 mm than at 4.5 mm (0.45-mm probe 94%; 0.15-mm probe 40.5%; and Metricon 19.5%). It seems that the effect of vitreal anomalies is more pronounced at locations closer to the anterior segment.

The Scheffe subsequent test comparisons show a general trend toward lower F values (less chance of significance), regardless of instrument, once the groups contain individuals with vitreous abnormalities. The Scheffe subsequent tests and the percentage change in group means between normal and abnormal test comparison at both intraocular locations provides additional information about the influence of vitreous change in separating populations.

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The Scheffe subsequent test comparisons show a general trend toward lower F values (less chance of significance), regardless of instrument, once the groups contain individuals with vitreous abnormalities (Table 2). None of the subsequent test comparisons approached significance.

Discussion

Using three fluorophotometry systems that were roughly equal in sensitivity and were capable of measuring sodium fluorescein concentration down to $1 \times 10^{-9}$ g/ml, it was found that:

1. The majority of the diabetics without observable retinopathy had values that fell within the range of normal values. We can not separate diabetics with-
out retinopathy from normals. None of the instruments could statistically separate normals from age-matched diabetics without background retinopathy. The two group means were so close on all three fluorophotometers that none of the F values even showed a trend in the direction of significance. In some instances (Table 1) the group means for diabetics without retinopathy were less than for normals. This was found at both intraocular locations. Thus, a breakdown in the blood-ocular barrier may not be present at the onset of diabetes.

2. Not all diabetics with retinopathy have increased readings. Some of the diabetics with background retinopathy had VFL values that fell within the range of normal values. Consequently, we can identify groups of diabetics, but not individuals within each group. While diabetics with retinopathy as a group could be separated from normals, only probability statements can be made regarding abnormality for each individual.

3. Not only are these negative findings different from other reports that have found a significant difference between normals and diabetics without retinopathy, but our values appear to be lower than...
those found in other laboratories. However, in the other studies the exact intraocular location has not been specified.

In this experiment several factors that have been shown to add variability and consequently affect the resultant data have been taken into account. Ocular pigmentation acts as a variable density screen to choroidal fluorescein. A lightly pigmented fundus will also produce more reflected light than a darkly pigmented retina, which results in greater influence of choroidal fluorescein in the posterior vitreous. The extent of the chariodal influence, or so called tailing, has been demonstrated in model eyes to be positively related to the width of the slit-lamp beam. The relative contribution of pigmentation and choroidal fluorescein appears to be negligible 4.5 mm anterior to the retina, even with slit beams as wide as 1.25 mm.

The absence of vitreous change in the patient populations was probably a significant factor accounting for low group mean values and for our inability to separate normals from diabetics without observable retinopathy. All subjects in the initial analysis had normal appearing vitreous, free of syneresis, lacunae, or vitreous detachments. While Kohner demonstrated that vitreous detachments may influence VFL reading, a previous study from our laboratory has shown that in normals and retinitis pigmentosa patients whose angiograms demonstrated no leakage of fluorescein, the degree of severity of vitreous change correlated significantly with midvitreous values. We found that 95% of 28 retinitis pigmentosa patients with vitreous change had the highest readings in the anterior midvitreous (7.5-12.2 mm from the retina). In this study the addition of just three subjects with subtle vitreous change produced an increase in group means at both intraocular locations. The increasing VFL values were greater at 7.5 mm than at 4.5 mm from the retina, with the 0.45-mm probe data being most affected by vitreous change.

Although the larger sampling volume associated with the 0.75-mm beam probably explains the increase in VFL values over the 0.30-mm beam, in this experiment the 0.15 mm probe with a relatively wide slit width (0.75 mm) was somewhat better able to separate normals from diabetics with retinopathy than the 0.45 mm probe with a narrow slit width (0.30 mm). The thinner slit beam results in more local sampling than with the wider slit-lamp beam. Since the vitreous changes in the three subjects in this study were most prominent in the midvitreous, a small sampling area would be more readily affected by syneresis and lacunae than a wider sampling volume that not only detects the fluorescein in abnormal vitreous, but is also influenced by the fluorescein content in the adjacent normal vitreous. This study suggests that a narrow slit-beam width may better avoid the contaminating effects of vitreous change at more remote locations due to local sampling than a wider slit beam, but produces higher readings in the presence of vitreous change. Therefore, an undetected vitreous change would influence the data more when using a very narrow slit beam. Regardless of slit-lamp beam width, the net effect of undetected vitreous change is to increase group means.

It is interesting to note the effect of including just one subject with subtle vitreous change on group means. If all the subjects in this study had had normal vitreous except the one diabetic without retinopathy but syneresis, the diabetic without retinopathy group mean (abnormal vitreous group) would have been artifactually elevated at both intraocular locations for all three instruments. Since increasing degrees of vitreous change correlate with increasing VFL readings, the inclusion of individuals with vitreous detachments would be expected to have a more pronounced effect on the data than subjects with syneresis and small lacunae.

While the problems associated with vitreous change may be avoided by excluding those individuals with vitreal abnormalities, it should be recognized that 58% of normals have vitreous detachments by age 50. This increases to 65-75% by age 65 with incipient changes beginning in the second or third decade particularly in high myopia. Since VFL is conducted in the vitreous, the consequence of even subtle vitreal changes may be a limiting factor in the general application of this technique.

In a recent article by Kernell et al, hyperglycemic diabetics without retinopathy were found to have increased midvitreous VFL values, whereas in our study diabetics without retinopathy and free of vitreous change had VFL values within the normal range. Although the two articles appear to be at variance, it may be that both conclusions are correct given the experimental constraints. Kernell did not look at vitreous change and we did not consider degree of blood glucose control. While none of the ten diabetics without retinopathy in our study had abnormal VFL values, we do not mean to imply that this finding will be true in every case. However, once patients with vitreal change have been removed from the data, perhaps the number of diabetics exhibiting abnormal VFL readings will be reduced. The points raised in this paper do not necessarily invalidate previously published data, however, future VFL studies might consider the problems that result from vitreous change and intraocular location.
Key words: vitreous fluorophotometry, diabetes, diabetic retinopathy, vitreous change, intraocular position, photomultiplier, photodiode, slit width

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References