In Vivo Measurement of Iridial Circulation Using Laser Speckle Phenomenon

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PURPOSE. To evaluate the use of the laser speckle phenomenon for noninvasive in vivo consecutive measurement of the iridial circulation.

METHODS. A pigmented rabbit iris was illuminated using a diode laser, and the normalized blur of the resulting laser speckle pattern, \( NB_{\text{iris}} \), was determined as a quantitative index of blood velocity in the iridial tissue. The authors compared data on positional variation, reproducibility, and correlation to iridial blood velocity derived with this technique with the blood flow rate simultaneously determined by the microsphere technique. They also evaluated the effects on iridial circulation of ocular perfusion pressure (OPP) change, rectus muscle excisions, and instillation of topical timolol or betaxolol.

RESULTS. The \( NB_{\text{iris}} \) increased gradually from the pupil margin to the periphery; the coefficient of variation of \( NB_{\text{iris}} \) was lowest at the center of this area. The coefficient of reproducibility of two \( NB_{\text{iris}} \) measurements at 5-minute intervals was 8.8%; at 24-hour intervals, it was 14.1%. The \( NB_{\text{iris}} \) correlated well with the microsphere technique measurements of blood flow rate at several intraocular pressures (IOP) \( (r = 0.61, P = 0.0002, n = 40) \) and with the comparison of preinstillation and postinstillation unoprostone \( (r = 0.93, P = 0.0008, n = 8) \). The \( NB_{\text{iris}} \) decreased with OPP reduction, decreased temporarily after excision of the superior or inferior rectus, and showed no significant change after excision of the medial or lateral rectus. Instillation of timolol caused a significant decrease in IOP but did not significantly change the \( NB_{\text{iris}} \). Topically applied betaxolol decreased IOP and increased \( NB_{\text{iris}} \) at 2.5 hours after instillation in an ipsilateral eye.


Iridial circulation is vital for maintenance of the normal physiology of the anterior part of the eye. Many previous studies of iridial circulation have used the microsphere technique, 1-11 which is applicable only for animal experiments. Iris fluorescein angiography, 12-16 corneal temperature measurement with ocular thermography, 17-19 and laser Doppler flowmetry 20-21 have been used for noninvasive evaluation; however, iris fluorescein angiography and corneal temperature measurement give qualitative rather than quantitative information. A noncontact, quantitative method of evaluating iridial hemodynamics without requiring any exogenous substances, thereby permitting safe application in the clinical setting, would be of greater value. Although laser Doppler flowmetry can be useful in the analysis of iridial or ciliary circulation in humans, 20 it does have some disadvantages. It is difficult to identify the target site precisely, and the size of the measuring area is limited.

The speckle phenomenon is an interference phenomenon of coherent light sources, such as lasers. 22 When tissue is illuminated by laser radiation, a speckle pattern appears and changes rapidly in response to changing blood flow in the tissue. 22-23 We have recently designed a device for noncontact, two-dimensional measurement of ocular fundus tissue circulation using the laser speckle phenomenon, 24-25 which provides a quantitative index of the tissue blood flow velocity in a defined measurement field. This index, the normalized blur, was measured with \( \pm 10\% \) reproducibility in fundus tissues 24-25.

The present report describes our investigation of the laser speckle method in monitoring changes in iridial circulation, quantitatively and with reasonable reproducibility. We compared the in vivo results obtained using this technique with those obtained by the microsphere technique and also studied the effects of changes in ocular perfusion pressure (OPP), the excision of extraciliary muscles, and the topical application of \( \beta\)-adrenergic blocking agents on iridial circulation.

METHODS

Apparatus

The apparatus 24-25 used in this study included a fundus camera (TRC-WT3; Topcon, Tokyo, Japan) equipped with a diode laser (wavelength, 808 nm; maximum power, 5 mW on the corneal surface) and an image sensor (100 \( \times \) 100 pixels; BASIS, Canon, Tokyo, Japan). To measure the iridial circulation, the maximum laser output was increased from 2 mW on the...
FIGURE 1. Normalized blur measurement sites. Field size: 1.07 X 1.07 mm². Sites are located at the pupil margin, midpoint, and periphery of equidistant radials (A-H) around the iris. The midtemporal site (I in C) was selected for the experiments.

Measurement Positions
After the induction of general anesthesia, four consecutive measurements of NBiras were made at each of 24 sites at the pupillary margin, the midpoint, and the periphery of eight equidistant radials around the iris (Fig. 1). The average and the coefficient of variation of the measurements at each site were calculated.

Reproducibility of In Vivo Measurements
The NBiras of the midtemporal site (Fig. 1; striped box in C) was measured twice with a 5-minute interval. After photographing the anterior segment for identification of the measurement field, the animals were allowed to recover from anesthesia. The NBiras at this site was obtained again after a 24-hour interval. The coefficient of the reproducibility of the in vivo measurements was determined by the equation:

\[ \text{Coefficient of Reproducibility} = \frac{|NB_{iras,1} - NB_{iras,2}|}{(NB_{iras,1} + NB_{iras,2})/2} \]

where NB_{iras,1}, NB_{iras,2}, and NB_{iras,3} are the NBiras values of the first, second, and third measurements, respectively.

Comparison of NBiras and Microsphere Data
Intraocular Pressure Changes. The microsphere study was conducted, after the 3-week adaptation period, by controlling the intraocular pressure (IOP) manometrically through needle infusion sets inserted into the anterior chamber\(^4\) with IOP elevations from 20 to 30 mm Hg ($n = 4$), from 20 to 40 mm Hg ($n = 4$), from 30 to 50 mm Hg ($n = 2$), from 30 to 40 mm Hg ($n = 4$), and from 40 to 50 mm Hg ($n = 2$). The carotid artery and the ipsilateral femoral artery were cannulated. Immediately after NBiras measurements in the eye contralateral to arterial cannulation at the first IOP level, a 0.10-ml suspension of nonlabeled red microspheres ($15 \pm 0.3 \text{ mm}, 10^7 \text{spheres/ml} \text{ E-Z TRAC, Los Angeles, CA}$) was injected into the left ventricle cannulated through the carotid artery. A reference blood sample
was obtained from the cannulated femoral artery. The IOP was then elevated to the second level, and \( NB_{iris} \) measurements and the microsphere injection were repeated using blue microspheres. Systemic parameters (blood pressure; pulse rate; \( P_{O_2} \), \( P_{CO_2} \), and \( pH \) of arterial blood; and body temperature) were monitored with a \( pH \)-blood gas analyzer (Model 170; Corning Glass, Corning, NY). Body temperature was monitored with a rectal thermometer (MGA-3219; Shibaura Densi Seisakusyo, Tokyo). The animals were then killed by injections of intravenous sodium pentobarbital. The eyes were enucleated and bisected 2 mm posterior to the limbus, and the retina and choroid were excised. The iris-ciliary body complex was excised and divided into the iris, the iridial process, and the ciliary process. The colored microspheres in the excised tissues and reference blood sample were counted by examiners masked to the IOP levels and those investigators who counted the microspheres in the tissues or the blood sample were counted to determine the iridial blood flow rate. The investigators who measured the \( NB_{iris} \) and IOP levels were masked to the treatment, and those investigators who counted the microspheres were masked to the treatment and the results of \( NB_{iris} \) and IOP measurements.

**Topical Unoprostone**

Comparison of \( NB_{iris} \) and microsphere measurements was made by artificially changing the iridial blood flow with a pharmacologic agent. Unoprostone, a prostaglandin-related compound developed as a new ocular hypotensive agent,29 disrupts the blood-aqueous barrier in rabbit eyes,30 but not in human eyes.31 Changes of \( NB_{iris} \) and blood flow rate, measured simultaneously with the microsphere technique, were compared before and after the application of topical unoprostone.

Rabbits were prepared in a manner similar to that described in the above IOP change experiment, except that the needle infusion sets, inserted into the anterior chamber for manometrical IOP control, were not used. Immediately after \( NB_{iris} \) measurements in the eye, contralateral to arterial cannulation, nonlabeled red microspheres were injected into the left ventricle and a reference blood sample was taken. After this, 20 \( \mu l \) 0.12% isopropyl unoprostone (Rescula; Fujisawa Pharmaceutical, Osaka) was instilled into the eye, contralateral to arterial cannulation. Two hours after instillation, the \( NB_{iris} \) of the unoprostone-treated eye was measured, and blue microspheres were injected. The IOP and pupil diameter of the treated eye were also measured with a calibrated applanation pneumotonometer (Alcon, Fort Worth, TX) and calipers. Systemic parameters were monitored as above.

For controls, eyes that were contralateral to the arterial cannulation, in another group of rabbits prepared in a similar manner, were administered physiological saline. Control and experiment rabbits were then killed; the eyes treated with unoprostone and with physiological saline were enucleated, and microspheres in the tissues or the blood sample were counted to determine the iridial blood flow rate. The investigators who measured the \( NB_{iris} \) and IOP levels were masked to the treatment, and those investigators who counted the microspheres were masked to the treatment and the results of \( NB_{iris} \) and IOP measurements.

**Effect of Ocular Perfusion Pressure Change**

To study the effect of OPP changes on the \( NB_{iris} \), after the induction of general anesthesia, the femoral artery was cannulated and systemic parameters and body temperature were monitored as above. The IOP was manometrically adjusted at 20, 30, 40, 50, and 60 mm Hg,24 as above, and \( NB_{iris} \) was measured at each IOP level that was maintained for 5 minutes. OPP was calculated as31 follows:

\[
\text{OPP} = \text{FABP}_m - \text{IOP} - 14, \tag{2}
\]

where \( \text{FABP}_m \) is the mean femoral arterial blood pressure. \( \text{FABP}_m \) was calculated as:

\[
\text{FABP}_m = \text{FABP}_d + \frac{1}{3}(\text{FABP}_s - \text{FABP}_d), \tag{3}
\]

where \( \text{FABP}_d \) and \( \text{FABP}_s \) were the diastolic and systolic femoral arterial blood pressures, respectively.

**Effects of Excision of Extraocular Muscles**

After the induction of general anesthesia, 0.1% diclofenac sodium (Diclod; Wakamoto Pharmaceutical, Tokyo, Japan) was applied topically three times, at 5-minute intervals. The conjunctiva covering the insertion of the superior rectus muscle was removed to expose the muscle. After 5 seconds of serial \( NB_{iris} \) measurements at the peripheral site nearest the insertion, the muscle was released using surgical ophthalmic scissors. \( NB_{iris} \) recordings were taken at 5, 30, and 60 seconds and each minute for the 5 minutes immediately after the detachment. The patterns of \( NB_{iris} \) change after excision of the inferior rectus, lateral rectus, and medial rectus muscles were
After the induction of general anesthesia, one randomly chosen eye of each animal received 20 µl 0.5% timolol or 0.5% betaxolol at 6:00 PM (timolol-betaxolol group, n = 8). To provide a control for each drug, both eyes of each rabbit in another group that were also anesthetized (but did not receive timolol or betaxolol) were administered 20 ml physiological saline at 6:00 PM (timolol-betaxolol group, n = 8). The NBirisi, NB iris2, and pupil diameter in the treated eye in the timolol and betaxolol groups and one randomly chosen eye of each rabbit in the control group were monitored before instillation and at 0.5, 1, 1.5, 2, and 2.5 hours after instillation. IOP and pupil diameter were monitored with a calibrated applanation pneumotonometer and calipers. All measurements were carried out by an investigator masked to the treatment.

**RESULTS**

**Positional Variation of Normalized Blur**

The averages of four consecutive NBirisi values from each site are shown in Figure 2. There were no significant differences in NBirisi when comparing the data of the eight radials (A–H) (P = 0.369, multiple analysis of variance). Significant differences were found, however, when comparing the pupil margin, midpoint, and peripheral sites of each radial (P = 0.000001, multiple analysis of variance). The average NBirisi value at the periphery was significantly greater than at the midpoint, which was again significantly greater than at the pupil margin (P < 0.000001, paired t-test).

The coefficient of variation of the calculated averages of NBirisi at each point (pupil margin, midpoint, periphery) on the eight radials (A–H), was 18.0%, 8.8%, and 11.0%, respectively. This identified the midpoint as the site with the least positional variation induced by circumferential displacement, so the mid-temporal site (Fig. 1; striped box in C) was selected for the following experiments, with the exception of the rectus excision.

**Reproducibility of In Vivo Measurements**

The coefficients of reproducibility for 5-minute- and 24-hour-interval NBirisi measurements were 8.8 ± 2.0% and 14.1 ± 0.3%, respectively (mean ± SEM, n = 10). No significant differences were observed in NBirisi1, NBirisi2, and NBirisi3 (P = 0.5293, ANOVA).

**Comparison of NBirisi and Microsphere Measurements**

Only rabbits with systemic parameters in the normal range were used in this study (Tables 1, 2).

**IOP Change.** There were no significant changes in pupil diameter during the experiment. Comparison of NBirisi and iridial blood flow rates determined by the microsphere technique is shown in Figure 3. The NBirisi correlated significantly with microsphere technique data; the correlation coefficient for data obtained by each method was 0.61 (P = 0.00021, n = 40).

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### Table 1. Systemic Parameters at Time of Microsphere Injection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>FABP&lt;sub&gt;mu&lt;/sub&gt; (mm Hg)</td>
<td>85.1 ± 1.5</td>
<td>99.6 ± 1.5</td>
<td>87.7 ± 1.8</td>
<td>79.8 ± 3.3</td>
</tr>
<tr>
<td>OPP (mm Hg)</td>
<td>51.1 ± 1.5</td>
<td>55.6 ± 1.5</td>
<td>33.6 ± 1.8</td>
<td>15.8 ± 3.3</td>
</tr>
<tr>
<td>Pulse rate (per minute)</td>
<td>290 ± 3</td>
<td>305 ± 3</td>
<td>300 ± 4</td>
<td>295 ± 5</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>36.7 ± 0.1</td>
<td>36.9 ± 0.1</td>
<td>36.6 ± 0.1</td>
<td>36.5 ± 0.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.35 ± 0.01</td>
<td>7.30 ± 0.01</td>
<td>7.40 ± 0.01</td>
<td>7.30 ± 0.01</td>
</tr>
<tr>
<td>Pco2 (mm Hg)</td>
<td>29.7 ± 0.4</td>
<td>33.9 ± 0.4</td>
<td>30.8 ± 0.4</td>
<td>28.7 ± 0.7</td>
</tr>
<tr>
<td>Po2 (mm Hg)</td>
<td>84.4 ± 2.1</td>
<td>80.2 ± 1.0</td>
<td>87.6 ± 1.7</td>
<td>90.2 ± 3.0</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 10, 12, 10, and 8 at the IOPs of 20, 30, 40, and 50 mm Hg (respectively). IOP, intraocular pressure; FABP<sub>mu</sub>, mean femoral arterial blood pressure; OPP, ocular perfusion pressure.

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### Table 2. Systemic Parameters before and after Topical Instillation of Unoprostone or Normal Saline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unoprostone Before</th>
<th>Unoprostone After 2 hours</th>
<th>Control Before</th>
<th>Control After 2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>FABP&lt;sub&gt;mu&lt;/sub&gt; (mm Hg)</td>
<td>90.5 ± 2.1</td>
<td>86.6 ± 5.0</td>
<td>81.1 ± 4.3</td>
<td>80.6 ± 3.9</td>
</tr>
<tr>
<td>Pulse rate (per minute)</td>
<td>326 ± 9</td>
<td>335 ± 11</td>
<td>361 ± 14</td>
<td>333 ± 13</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>38.5 ± 0.2</td>
<td>38.3 ± 0.2</td>
<td>38.0 ± 0.1</td>
<td>37.7 ± 0.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.40 ± 0.03</td>
<td>7.42 ± 0.02</td>
<td>7.39 ± 0.01</td>
<td>7.41 ± 0.02</td>
</tr>
<tr>
<td>Pco2 (mm Hg)</td>
<td>29.1 ± 2.3</td>
<td>26.6 ± 2.4</td>
<td>27.5 ± 4.0</td>
<td>26.5 ± 1.7</td>
</tr>
<tr>
<td>Po2 (mm Hg)</td>
<td>79.9 ± 1.9</td>
<td>84.5 ± 2.9</td>
<td>82.5 ± 2.0</td>
<td>83.0 ± 7.4</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 8, respectively). Doses: Unoprostone, eyes were administered 0.12% unoprostone; control, eyes were administered normal saline.

FABP<sub>mu</sub>, mean femoral arterial blood pressure.
Topical Unoprostone. There was no significant change in pupil diameter during the unoprostone experiment. Comparison of NBiris and microsphere data on iridial blood flow rate is shown in Table 3. In controls, both NBiris and iridial blood flow rate changed little after the instillation of saline. After the instillation of unoprostone, the NBiris was significantly increased by 146.6 ± 11.7% (mean ± SEM; \( P = 0.0277 \), Wilcoxon rank sum test, \( n = 8 \)), and the blood flow determined by the microsphere technique was significantly increased by 145.2 ± 10.2% (\( P = 0.0273 \), \( n = 8 \)). The correlation coefficient of changes measured by the two techniques was 0.93 (\( P = 0.0068 \), \( n = 8 \)).

Effect of Ocular Perfusion Pressure Change

There was no significant change in pupil diameter during this experiment. The cornea became cloudy at IOP levels of 60 mm Hg, but there was little effect on normalized blur measurement because the speckle pattern produced by a cloudy, immobile cornea does not vary during the measurement period. Calculation of normalized blur depends on the variation in speckle intensity, not on the speckle intensity itself. In addition, the optical system was adjusted so that light from the iris surface, not from the cornea, was reflected onto the image sensor. The relationship between the OPP and the NBiris is shown in Figure 4. The NBiris at IOP levels of 30, 40, 50, and 60 mm Hg was expressed as a percentage of the NBiris at 20 mm Hg. The OPPs at each IOP level were 45.3 ± 5.3, 39.2 ± 5.1, 28.0 ± 4.9, 25.0 ± 6.8, and 16.8 ± 5.5 mm Hg, respectively (mean ± SEM, \( n = 9 \)). The NBiris decreased as OPP decreased: there were significant decreases at IOP levels of 30, 40, 50, and 60 mm Hg (9.1%, 19.9%, 33.8%, and 48.9%) in the NBiris (\( P < 0.016-0.0059 \); \( n = 9 \); Wilcoxon rank sum test). The correlation coefficient obtained by the linear regression passing thorough the origin of the coordinates between the NBiris and the OPP was 0.99 (\( P = 0.00006 \)).

Excision of Extraocular Muscles

There was no significant change in pupil diameter during the experiment. NBiris changes are shown in Figure 5. After excision of superior rectus or inferior rectus muscles, the mean NBiris varied significantly in the time course (\( P = 0.0344 \) for superior rectus muscle; \( P = 0.0114 \) for inferior rectus muscle; ANOVA), with a decrease from the baseline at 5, 30, and 60 seconds for superior rectus muscle (−35.3%, −15.3%, and −10.7%; \( P = 0.0343 \), 0.0277, and 0.0464; Wilcoxon rank sum test) or at 5, 30, 60, and 120 seconds for inferior rectus muscle (−25.4%, −28.9%, −17.86%, and −20.8%; \( P = 0.0496 \), 0.0277, 0.0357, and 0.0173; Wilcoxon rank sum test).

Table 3. Iridial Blood Flow and NBiris, before and after Topical Instillation of Unoprostone or Saline

<table>
<thead>
<tr>
<th>Blood Flow Rate (mg/min)</th>
<th>NBiris (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After 2 hours</td>
</tr>
<tr>
<td>Unoprostone</td>
<td>149.9 ± 15.2</td>
</tr>
<tr>
<td>Control</td>
<td>143.8 ± 7.9</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (\( n = 8 \)). Unoprostone and Control indicate eyes instilled with 0.12% unoprostone and those instilled with physiological saline, respectively.

Figure 3. Comparison of blood flow rates simultaneously determined by the microsphere technique (abscissa) and NBiris (ordinate). Each plotted point represents the mean blood flow rate and NBiris at each intraocular pressure, with error bars (± SEM). \( n = 10, 12, 10, \) and \( 8 \) in the 20-, 30-, 40-, and 50-mm Hg experiments, respectively.

Figure 4. Relationship of ocular perfusion pressure (OPP; abscissa) and %NBiris (at 20 mm Hg of intraocular pressure [IOP]). Each plot represents the mean OPP and %NBiris in nine eyes of nine rabbits at each IOP, with SEM (error bars). *, \( P < 0.01 \); †, \( P < 0.05 \); by the Wilcoxon rank-sum test for difference from NBiris at IOP of 20 mm Hg.
FIGURE 5. The NB_iris changes after excision of various recti. Each plot represents the mean NB_iris with SEM (error bars). Eyes numbered 6, 9, 4, and 5 for the superior, inferior, lateral, and medial recti experiments. SRM, IRM, LRM, and MRM indicate superior, inferior, lateral, and medial rectus muscles. *, P < 0.05, by Wilcoxon rank sum test for difference from pre-excision NB_iris.

Wilcoxon rank sum test). There was no significant change in NB_iris after excision of either lateral rectus or medial rectus muscles (P = 0.8999 and 0.8953, respectively; ANOVA).

Effects of Topical Timolol and Betaxolol

There was no significant difference in baseline NB_iris, IOP, or pupil diameter in any of the groups, and no significant change in pupil diameter was seen during the experiment. Figures 6 and 7 show the patterns of changes of NB_iris and IOP after the instillation of timolol, betaxolol, or normal saline. IOP and NB_iris were expressed as the discrepancy from the preinstillation measurement. The changes in IOP were significantly different between timolol-treated and control eyes (P = 0.000015; ANOVA), with marked decreases at 1, 1.5, 2, and 2.5 hours after instillation (P ≤ 0.0274; Mann–Whitney test). The differences found by NB_iris, however, were not significant (P = 0.0531; ANOVA).

FIGURE 6. The NB_iris changes and intraocular pressure (IOP), after instillation of 0.5% timolol (■) or normal saline (▲). Delta IOP and Delta NB_iris indicate differences from baseline. Each plot represents mean NB_iris or IOP with SEM (error bars) in eight rabbits. *, P < 0.01; †, P < 0.05; by the Mann-Whitney test for difference between timolol and control groups.

FIGURE 7. The NB_iris changes and intraocular pressure (IOP) after instillation of 0.5% betaxolol (■) or normal saline (▲). Delta IOP and Delta NB_iris indicate differences from baseline. Each plot represents mean NB_iris or IOP with SEM (error bars) in eight rabbits. *, P < 0.01; †, P < 0.05; by the Mann-Whitney test for difference between betaxolol and control groups.
In betaxolol-treated eyes, IOP was significantly lower than in control eyes \( (P = 0.000015; \text{ANOVA}) \), and there were marked differences at 1.5, 2, and 2.5 hours \( (P = 0.0181; \text{Mann–Whitney test}) \). \( NB_{\text{iris}} \) changes in betaxolol-treated eyes were significantly different from those in control eyes \( (P = 0.0149; \text{ANOVA}) \), and there was a marked difference seen at 2.5 hours after instillation \( (P = 0.0117; \text{Mann–Whitney test}) \).

**DISCUSSION**

This study found that the average \( NB_{\text{iris}} \) at the periphery was significantly larger than at the midpoint or pupil margin; the least variation in \( NB_{\text{iris}} \) was found at the midpoint of the iris. In the rabbit, iris processes extend from the ciliary process, with the major iridial arterial circle lying near the limbus.\(^{35}\) The \( NB_{\text{iris}} \) of the periphery may be affected by blood flow through this arterial circle, causing both the larger average and the larger coefficient of variation than those found at the other sites. The lowest coefficient of variation was found at the midpoint, probably because the vascular distribution was relatively uniform and without large vessels. No significant differences were found in \( NB_{\text{iris}} \) obtained from the eight radials (Fig. 1; A–H). With these data, and the fact that there was occasional interference with measurement in the nasal direction, we selected the \( NB_{\text{iris}} \) at the midpoint of the temporal radial (Fig. 1; striped box in C) as the representative site.

The coefficients of reproducibility for the 5-minute- and 24-hour-interval \( NB_{\text{iris}} \) measurements were 8.8% and 14.1%, respectively, which confirms the usefulness of this technique for monitoring changes in \( NB_{\text{iris}} \) during long time intervals. The normal pulse rate of a rabbit is approximately 300/minute. The \( NB_{\text{iris}} \) level was measured consecutively for 0.5 second; therefore, any rhythmic change in tissue blood flow resulting from pulse would have been averaged, contributing to the relatively good reproducibility.

The iris blood flow rate found by the colored-microsphere technique at the baseline IOP, before instillation of unoprostone, coincides with the rate determined by the radioactive microsphere technique in a normal rabbit eye.\(^{1,5}\) In the experiment with manometrically controlled IOP, the \( NB_{\text{iris}} \) was significantly correlated with the blood flow rate found with the microsphere technique (correlation coefficient, 0.61). When the average blood flow rates and \( NB_{\text{iris}} \) at each IOP level were calculated,\(^{3,4\text{a}}\) the correlation was higher (correlation coefficient, 0.99). In the unoprostone experiment, topical instillation significantly increased \( NB_{\text{iris}} \) and blood flow rate determined by the microsphere technique. Results obtained by these two methods also correlated well (correlation coefficient, 0.93) when averages were compared for increased rates. Topically applied unoprostone disrupts the blood-aqueous barrier in rabbit eyes\(^{30}\); therefore, topical application probably affected the blood flow in the anterior uvea of eyes in this study. These results suggest that \( NB_{\text{iris}} \), introduced as a quantitative index of iridial blood flow velocity, applies to an approximate of iridial circulation in a broad sense.

Previous studies have demonstrated that blood flow through the anterior uvea in rabbits\(^{3}\) does not possess autoregulation protection from OPP change, whereas the vascular beds of the anterior uvea in cats\(^{1}\) and monkeys\(^{5}\) do. The \( NB_{\text{iris}} \) found in the present study decreased with reduction of the OPP and showed a significant correlation. These results suggest that there is no apparent regulation between the \( NB_{\text{iris}} \) and the OPP. Because the \( NB_{\text{iris}} \) significantly correlated with the blood flow rate determined with the microsphere technique, the present result is not incompatible with the previous results.\(^{3}\)

The blood flow in the anterior uvea of rabbits has two major pathways: the muscular and the long posterior ciliary arteries.\(^{35}\) The muscular arteries follow the vertical recti and merge into major arterial rings, after confluence with the perforating branches of the long posterior arteries. The horizontal rectus muscles, however, are not accompanied by a large artery. The present results showed that excision of the vertical rectus muscles reduced iridial circulation temporally, but horizontal rectus muscle excision brought no significant change in \( NB_{\text{iris}} \). This may be explained by the anatomic features peculiar to rabbits. In addition, the quick recovery of the iridial circulation after excision of vertical muscles suggested rapid compensation from the posterior ciliary artery circulation.

In the timolol experiment, \( NB_{\text{iris}} \) tended to decrease after a single instillation of timolol, although there was no significant difference from controls. \( (P = 0.0531, \text{ANOVA}) \); the analysis powers were 46%, 80%, and 96%, corresponding to effect size changes of 1X, 1.5X, and 2X SD, respectively.) The vascular, \( \beta_{2}\)-receptor-blocking effect is thought to cause vasoconstriction.\(^{36,37}\) Previous reports on the effects of topical timolol on iridial circulation have differed, however. Van Buskirk et al.\(^{38}\) documented constriction of the ciliary body artery after long-term topical instillation of timolol, and Watanabe et al.\(^{7}\) reported a reduction of blood flow rate, using the microsphere method, after a single instillation of 0.25% timolol. Although the \( NB_{\text{iris}} \) as a parameter of the blood velocity, does not specify the blood flow rate, our present results suggest that topical timolol has a suppressive effect on iridial circulation, under the conditions of this experiment.

In the betaxolol experiment, \( NB_{\text{iris}} \) increased significantly after instillation in the betaxolol-treated eyes. Betaxolol has little \( \beta_{2}\)-blocking effect on vasoconstriction in peripheral tissues, compared with other beta blockers such as timolol.\(^{39}\) In addition, it shows direct vascular-relaxing properties, similar to Ca\(^{2+}\) channel blockers, in the porcine long posterior ciliary artery,\(^{40}\) bovine retinal artery,\(^{41}\) and rat arteries from various tissues,\(^{42}\) at concentrations ranging from 0.3 \( \mu \text{M} \) to 100 \( \mu \text{M} \). After one instillation of 0.5% betaxolol, the aqueous humor and choroid concentrations in Dutch rabbits reached 6.54 \( \mu \text{M} \) and 6.90 \( \mu \text{M} \).\(^{43}\) Because topically applied drugs are absorbed into the internal ocular structures chiefly through the cornea and the aqueous humor, the iris concentration after one instillation of betaxolol should be equivalent or greater than that in choroid and sufficient to cause the Ca\(^{2+}\)-blocking-like effect.\(^{43}\)

In this study, we used urethane, which is an anesthetic that provides the minimum effect on peripheral circulation.\(^{44}\) However, all commonly used general anesthetics, including urethane, affect results in physiological or pharmacologic studies.\(^{44,45}\) Therefore, we compared nearly all present results with those of similarly prepared control rabbits.

To use the present apparatus for measuring human iris circulation, the safe light level should be estimated. Assuming that backscattering of laser beam light is not so different between the pigmented rabbit iris and the colored human iris, a 3-mW exposure on the iris surface should be necessary to measure \( NB_{\text{iris}} \) in the human iris. At a usual iris measurement, approximately all laser light is thought to be absorbed into the iridial or ciliary tissues of pigmented rabbit or colored human irises. Even in the case of the whole laser beam (3 mW on the iris surface for an approximate 1-second exposure for iridial measurement, includ-
ing alignment) reaching the retina through the pupil or the lucent iris of a white person, it would be well below the maximum permissible exposure of the retina, which is 460 mW/cm² for a 10-second exposure (estimated according to the American National Standards Institute standard).

In summary, use of the laser speckle phenomenon permits noninvasive, in vivo, serial monitoring of iridial circulation in a variety of conditions, with reasonable reproducibility. Therefore, this method offers advantages not only for laboratory animal studies but for clinical application in humans.

**References**


