Local Choroidal Blood Flow in the Cat by Laser Doppler Flowmetry

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Purpose. To develop a procedure using a noninvasive technique that will allow the investigation of choroidal blood flow (ChBF) regulation in discrete regions of the cat eye. Validation of this procedure will provide a method to study intrinsic, neural, and pharmacologic factors that regulate regional ChBF.

Methods. The technique to measure ChBF is based on laser Doppler flowmetry. However, in contrast to conventional laser Doppler flowmetry, which uses fiber-optic probes in direct contact with the tissue to deliver the laser beam and detect the scattered light, with this technique the beam is delivered through a fundus camera and the scattered light is detected in the retinal image plane of the camera. Measurements were made in 34 anesthetized cats under conditions that would ensure that the flow measured represented ChBF in the choriocapillaris: the laser beam was aimed at retinal intervascular sites in the tapetal region of the fundus; the Doppler shift power spectrum of the light scattered by the red blood cells had the shape and frequency range typical for a microvascular bed; and the recorded flow did not decrease by more than 5% when the cat was given 100% O2 to breathe for 4 minutes. The responses to various physiologic and pharmacologic stimuli were tested and compared with those obtained from retinal vessels.

Results. Intravenous infusions of acetylcholine increased ChBF in a dose-response fashion, whereas sympathetic nerve stimulation at various frequencies decreased ChBF as predicted by previous studies. By comparison, retinal blood flow was negligibly affected by these two stimuli. In contrast to retinal blood flow, ChBF was unaffected by diffuse luminance flicker. ChBF was found to be pulsatile. The mean of the pulsatile component of ChBF represented approximately 34% of mean ChBF, a value similar to those derived from ChBF measurements in minipigs and retinal blood flow in the cat.

Conclusions. This study demonstrates that laser Doppler flowmetry is a valid technique for obtaining local, noninvasive, and continuous recordings of relative ChBF. Tested under steady-state conditions for blood pressure, heart rate, and acid-base balance, ChBF is stable for long periods of time, allowing the investigation of the effect of various physiologic stimuli and pharmacologic agents on this flow.

Since the early attempt by Bornshein and Zwiauer to obtain quantitative information on choroidal blood flow (ChBF), a variety of techniques have been developed for this purpose. These include calorimetry, direct measurements from choroidal veins, radioactive krypton desaturation, labeled microspheres, hydrogen clearance, and more recently laser Doppler flowmetry (LDF). Except for the hydrogen clearance and LDF as applied by Kiel and Shepherd, all these techniques measure ChBF to large regions of the choroidal vasculature. However, recent studies suggest that more local measurements of ChBF may be needed to better understand the physiology of the choroidal circulation. Thus, Yancey and Linsenmeier have demonstrated that the choroidal pO2 in a region just nasal to the optic disk in cats was unaffected by increases in intraocular pressure (IOP) over a large range of IOPs. To explain this finding, they hypothesized that in this area of the fundus, the "choroidal arteries...may be..."
physiologically more similar to the retinal circulation and therefore capable of autoregulating.” Furthermore, Yamamoto et al.,\textsuperscript{19} observing in cats that the pH in the subretinal space increased in response to a step elevation of IOP in the area centralis and in the adja-
cerning near discal region, but not in the superior tem-
poral periphery, hypothesized that this alkalinization could be due to “a regional increase in choroidal
blood flow.”

The purpose of this work was to develop a method to determine local ChBF in the cat eye that will enable the investigation of regional differences. Our method uses the principle of LDF, but in contrast to previous applications of this technique,\textsuperscript{7,8} it is noninvasive because it does not require surgery or intraocular penetration of a fiber optic probe.

**MATERIALS AND METHODS**

**Animal Preparation**

Thirty-four adult cats, weighing 2 to 3.5 kg were used and prepared as described previously.\textsuperscript{11} Each cat was premedicated with atropine (0.04 mg/kg, subcutane-
ously) and anesthetized with intramuscular ketamine hydrochloride (22 mg/kg) and acepromazine maleate (2 mg/kg). Catheters were placed in a femoral artery and vein, and a tracheostomy was performed. A loading dose of pancuronium bromide (0.2 mg/kg) was given intravenously and the animal was ventilated with 21% O\textsubscript{2} and 79% N\textsubscript{2}. Arterial blood pressure, end tidal CO\textsubscript{2}, and heart rate were monitored continuously. Arterial pH, pCO\textsubscript{2}, and pO\textsubscript{2} were monitored intermittently using a blood gas analyzer and adjustments of the inspired gas mixture, end tidal volume, and respiration rate were made to keep pH \(\approx 7.4\), pCO\textsubscript{2} 31 mm Hg, pO\textsubscript{2} \(\geq 90\) mm Hg, and mean arterial blood pressure (MAP) between 85 and 110 mm Hg. Rectal temperature was maintained at \(\approx 38^\circ\text{C}\). A dose of enflurane (1.7 to 2.5%) was administered and pancuronium bromide (0.10 mg/kg/hr) infused continuously. The pupils were dilated with 1% tropicamide and 10% phenylephrine and the cat was placed prone on a table with the head secured in a stereotactic head holder. A ring was sutured to the eye with three stitches at the limbus and held in a fixed position to prevent eye motion. A zero diopter contact lens was placed on the cornea protected with Healon (Pharmacia Ophthalmics, Monrovia, CA). All experimental procedures conformed to the ARVO Resolution on the Use of Animals in Research and The Presbyterian Medical Center of Philadelphia Guidelines on Animal Research.

**ChBF Measurements**

ChBF was determined using the technique of LDF, which is based on the Doppler effect.\textsuperscript{12} Laser light scattered by a moving particle is shifted in frequency by an amount \(\Delta f = (2\pi/\lambda) (K_i - K_s) \cdot V\). \(K_i\) and \(K_s\) are the wave vectors of the incident and scattered light, respectively, \(V\) the velocity vector of the particle and the wavelength of the incident light. When \(|V| \ll \epsilon\), \(|K_i| \approx |K_s| = 2\pi n/\lambda\), where \(n\) is the refractive index of the medium, \(\lambda\) is the wavelength \textit{in vacuo} of the laser light, and \(\epsilon\) the speed of light. When the laser beam impinges on red blood cells (RBCs) moving at different velocity vectors, the spectrum of the scattered light, the so-called Doppler shift power spectrum (DSPS), has a width that is due not only to the multiplicity of \(V_s\), but also to the effect of scattering of the laser light by the tissue itself. As a result, the RBCs receive light from numerous random directions rather than from a single direction of incidence. Furthermore, light scattered by an RBC can reach the detector along various directions due to subsequent scattering by the tissue or other RBCs. These processes give rise to a multiplicity of \(K_s\) and \(K_i\)-vectors and, consequently, to a broader DSPS than would otherwise result solely from the various \(V_s\).

While observing the fundus through a modified laser Doppler Topcon (Tokyo, Japan) fundus camera (model TRC),\textsuperscript{15} a diode laser beam (670 or 810 nm) was aimed at a site in the tapetal region of the fundus away from visible retinal vessels (intersceral sites) and between recognizable “black dots.” These dots are the cross-sections of vessels that radially pierce the tapetum and branch into the capillaries that compose the choriocapillaris.\textsuperscript{14} The beam was defocused and its diameter at the fundus was about 500 \(\mu\text{m}\). The optical fiber aperture (nominal diameter 450 \(\mu\text{m}\)) used to detect the scattered light was placed at the center of the illuminated region. The location of the image of this aperture at the fundus, as well as the site illuminated by the laser beam, was observed on the monitor of a television camera mounted on the Topcon camera.\textsuperscript{15} The photocurrent generated by the scattered light was analyzed by the electronic system of a blood perfusion monitor (BPM 403A, Vasamedics, Minneapolis, MN; or PeriFlux PF3, Perimed, Inc., Stockholm, Sweden), which provided relative flow, \(F\), defined as the product of the velocity and volume of the RBCs within the illuminated volume of this tissue.\textsuperscript{18,16} A spectrum analyzer also displayed the DSPS on an oscilloscope screen.

In addition to aiming the laser beam at intersceral regions (inclusion criterion #1), we included in our studies only those sites where: a) the DSPS decayed exponentially with increasing frequency and the frequency at which the power of the DSPS was 10% of the power at the low frequency end was below 800 Hz (inclusion criterion #2); and b) \(F\) did not decrease by more than 5% when the cat was given 100% O\textsubscript{2} to breathe for 4 minutes (inclusion criterion #3). At these sites the measured \(F\) parameter was identified with ChBF and we tested that ChBF responded to
various maneuvers as predicted by previous studies. We also compared the responses of ChBF with those of relative blood velocity (Vel) recorded from adjacent retinal vessels.

**Relationship Between Measured ChBF and Actual ChBF**

In 14 cats, the relationship was tested between the perfusion pressure, PP, defined as MAP minus IOP, and ChBF by simultaneously recording MAP and ChBF after pentobarbital was lethally injected. In six cats the IOP was kept at a constant value between 10 and 15 mm Hg by inserting a needle into the anterior chamber and connecting this needle to a reservoir of physiologic saline. The IOP was measured using another needle connected to a pressure transducer (Harvard Apparatus, Dover, MA). In the other cats IOP was not measured and a value of 15 mm Hg was used to calculate PP.

**Pulsatile Versus Mean ChBF**

In 13 cats ChBF was recorded using a time constant of 0.1 sec. By analogy to calculations of mean arterial blood pressure, the mean ChBF was calculated as ChBF_{mean} = (ChBF_{syst} - ChBF_{diast})/(ChBF_{syst} + ChBF_{diast}) where ChBF_{p} = 1/3 (ChBF_{syst} - ChBF_{diast}) is an approximation of the mean of the pulsatile component of ChBF. Tested on a number of recordings in two cats, ChBF_{p} was found to underestimate the rigorously defined ChBF_{p} = 1/T \int (ChBF - ChBF_{diast})dt by less than 15%. Because the frequency response of the flowmeters decreased above 1 Hz and because the heart rate ranged from 2.5 to 3.5 Hz, we determined this response and corrected the ChBF_{p} values accordingly.

**Retinal Vessel Blood Velocity and Diameter**

Vel, in a retinal arteriole (second or third branch) near the site of measurements was measured by focusing the probing laser beam on the vessel and analyzing the scattered light with the BPM 403A flowmeter. In vitro measurements in capillary tubes (Appendix) confirmed a highly linear relationship between Vel and the actual mean velocity, provided that the DSPS was scattered light with the BPM 403A flowmeter. In vitro measurements in capillary tubes (Appendix) confirmed a highly linear relationship between Vel and the actual mean velocity, provided that the DSPS was below approximately 4000 Hz, a condition that was fulfilled in this study. Retinal vessel diameter was measured from red-free fundus photographs taken with a Topcon TRC 50FT fundus camera.

**Multiple Flash Stimulation**

In eight cats, multiple diffuse luminance flashes of 20 \( \mu \)sec duration were administered to a region of the fundus (30° in diameter) that was roughly centered at the site of LDF measurements. The flashes were generated by a Xenon arc flash bulb Visual Stimulator (Model PS22, Grass Instruments, Quincy, MA) and delivered to the eye through the illumination system of the fundus camera by means of a fiber optics cable. The frequency of the flashes was 10 Hz and the radiant energy \( \approx 50 \mu J/\text{flash/cm}^2 \) at the retina. After positioning the fundus camera, the fundus illumination was turned off and ChBF was then recorded continuously. The flashes were administered after at least 10 minutes of dark adaptation. The beam was then moved to a nearby retinal vessel. After a few minutes of baseline recording of Vel, the flicker stimulus was administered again. Changes in ChBF and retinal Vel from baseline values were determined at 1 minute of stimulation.

**Infusion of Acetylcholine**

In 13 cats, 1.35 mg acetylcholine (ACh) were diluted in 50 ml of physiologic saline and this solution was infused into a femoral vein at rates of 10, 20, and 40 \( \mu \)g/min, with an interval of about 10 minutes between each rate. The changes in ChBF were calculated based on the baseline (preinfusion) value and ChBF at 4 minutes of infusion. In seven of these cats, Vel was also recorded from a retinal vessel. Location of the beam on the vessel was checked at various times during the period in which the MAP changes were important and appropriate repositioning was done. In three cats, red-free fundus photographs were taken to measure the diameter of retinal vessels at baseline and at 4 minutes of infusions of 20 and 40 \( \mu \)g/min ACh.

**Sympathetic Stimulation**

In five cats, the left cervical sympathetic nerve and superior cervical ganglion were exposed using an anterior midline approach. The nerve and ganglion were dissected from the accompanying vagus nerve and no-dose ganglion, taking care to preserve these as well as the major blood vessels in this region. Silver electrodes were placed on the sympathetic nerve immediately proximal to the superior cervical ganglion and the nerve was ligated proximal to the electrodes using 3-0 silk ties to prevent retrograde stimulation. The nerve was moistened with mineral oil and insulated from the surrounding tissues with latex material. Electrodes were connected to the terminals of an electric stimulator (model SD 5, Grass Instruments, Quincy, MA) with the positive electrode placed orthograde on the nerve with respect to the negative electrode. Changes in ChBF were obtained from the average value of ChBF before stimulation and the minimum value (maximum change) during the stimulation (4 to 10 V, frequencies 1 to 64 Hz, 4 ms pulse duration). When the stimulation was observed to move the fundus (motion estimated to be < 100 \( \mu \)m), and consequently to shift the incident laser beam away from its original position, the beam was then repositioned at the desired site.

**Statistical Analysis**

Percentage changes in the measured quantities were expressed as mean ± SEM. Paired Student’s \( t \) tests were used to calculate PP.
TABLE 1. Summary of Choroidal Blood Flow and Retinal Blood Velocity in the Various Experiments

<table>
<thead>
<tr>
<th>Type of Experiment</th>
<th>Number of Cats</th>
<th>% ChBF-Changes From Baseline</th>
<th>% Vel Changes From Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% O₂, 4 min</td>
<td>23</td>
<td>-1 ± 1</td>
<td>-30 ± 2†</td>
</tr>
<tr>
<td>Multiple flashes 10 Hz, measured at 1 min</td>
<td>8</td>
<td>3 ± 2</td>
<td>20 ± 3‡</td>
</tr>
<tr>
<td>Infusion of ACh, measured at 4 min</td>
<td>11/7</td>
<td>28 ± 7†</td>
<td>1 ± 2</td>
</tr>
<tr>
<td>10 μg/min</td>
<td>11/4</td>
<td>55 ± 8‡</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Sympathetic stimulation 16 or 32 Hz, measured at maximum change</td>
<td>5</td>
<td>-39 ± 5†</td>
<td>-1 ± 3</td>
</tr>
</tbody>
</table>

ChBF, choroidal blood flow; Vel, mean velocity of blood in retinal vessels. Results are given as mean ± SEM. Paired Student’s t-tests were applied to evaluate the statistical significance of the results.

*11 cats for ChBF and 7 (4) for retinal Vel measurements.
†P < 0.01.
‡P < 0.001.

were applied to evaluate statistical significance. The changes were considered as statistically significant when the P values were ≤ 0.05.

RESULTS

In approximately 90% of the cats, no more than three sites needed to be probed to identify a site that satisfied the three inclusion criteria mentioned earlier. Approximately half of them were within two disk diameters from the rim of the disk, the others were farther out. Table 1 summarizes the ChBF and retinal Vel changes observed in the various experiments. The changes in ChBF, retinal Vel, and MAP induced by 4 minutes of 100% O₂ breathing obtained in one of the cats are shown in Figure 1. Similar responses were observed in the other animals. While retinal Vel decreased by about 60% in this cat, ChBF increased slightly during the hyperoxia. For the sites that met the inclusion criteria, the changes in ChBF at 4 minutes of 100% O₂ ranged from -5 to 33% with a mean value that was not significantly different from baseline. In contrast, retinal Vel decreased significantly (Table 1). Vessel diameter also decreased significantly by 14 ± 2% (P < 0.01).

The stability of ChBF is demonstrated by a typical recording of ChBF in Figure 2A. Time constant of the flowmeter was 5 seconds, a value too large to permit the recording of the variations induced by the pressure pulse. Slow variations (a period of many minutes) as well as oscillations with a period of one third to one half a minute were often present, as can be seen at various places of this recording. Figure 2B shows a recording of ChBF and retinal Vel that were obtained in another cat, using a flowmeter time constant of one tenth of a second. In this example, ChBF values range from approximately 33% of mean ChBF. For the various cats, the ChBF values ranged from 16 to 66% of mean ChBF (mean 34 ± 2%, n = 13). By comparison Vel, the mean of the pulsatile component of retinal Vel, de-

FIGURE 1. Effect of 4 minutes of 100% O₂ breathing (hatched bar). Top: ChBF measured at an intervascular site; middle: relative mean velocity, Vel, in a retinal arteriole (diameter approximately 40 μm); and bottom: mean systemic arterial blood pressure, MAP.
fined as $1/3(V_{el_{pre}} - V_{el_{stim}})$, represented approximately 30% of mean Vel ($= V_{el_{stim}} + V_{el_{p}}$).

The effect of multiple flash stimulation is shown by the representative recordings in Figure 3. In the retinal vessels, the mean increase in Vel at 1 minute of stimulation was significant, whereas the change in ChBF was not significant (Table 1).

Figure 4 demonstrates the effect of ACh on ChBF. In spite of the rapid decrease in MAP, ChBF often did not decrease, as seen in this example, although in some cases a transient decrease was observed. More importantly, however, even in these cases, ChBF was back at baseline while the MAP was still below its baseline and increased above baseline to reach a constant level, which depended on the amount of ACh infused (Table 1). In contrast (Fig. 5), Vel in retinal vessels first decreased transiently in parallel with MAP. It returned to a value not significantly different from the preinfusion value (1 ± 5%) in less than 30 seconds. Except during the rapid decrease in MAP, there was no significant change in retinal arteriolar diameter and therefore no significant change in retinal blood flow during the infusion of ACh.

Figure 6A shows the effect of sympathetic stimulation on the time course of ChBF. A decrease in ChBF was observed in all cats, even when there was an increase in MAP. This decrease depended on the frequency of the stimulation (Fig. 6B). Because of the small number of animals used in this experiment, the peak in the curve of Fig. 6B observed at 10 Hz is not significant. In the various cats, the maximum decreases in ChBF in response to approximately 30-second stimulations at 16 or 32 Hz ranged from 21 to 38% of baseline (Table 1). The effects are shown in Figure 6A for representative animals.

Figure 2. (A) Seventy-minute recording of ChBF obtained with a 5-second time constant of the flowmeter. (B) Relative ChBF recording obtained with a 0.1-second time constant of the flowmeter. The Doppler signal was recorded on magnetic tape (4 channel Racal, FM channel) and played back at 1/4 the recording speed. No attenuation of the amplitude of ChBF occurred under these conditions. Heart rate of the cat was 248 beats/min. Also shown for purpose of comparison is a recording of Vel taken approximately 1 hour later. No attempt was made to synchronize the phases. The vertical axes are linear scales.

Figure 3. Typical ChBF and Vel responses induced by multiple diffuse luminance flashes at 10 Hz (hatched bar). The illuminated area (30° in diameter) was temporal to the optic disk, with the disk at its edge. Vel was measured in a retinal vessel (diameter approximately 50 μm) within this area. These measurements were performed after approximately 15 minutes of dark adaptation. Time constant of the flowmeter was 5 seconds.

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FIGURE 4. Typical ChBF and MAP responses induced by an infusion of 20 μg/min acetylcholine (ACh). The period of infusion is indicated by the hatched bar. The second response was recorded approximately 15 minutes after the first one.

FIGURE 5. Typical Vel response obtained from small retinal vessels coursing the area where ChBF responses in Figure 4 were recorded. The lack of increase in Vel after the transient decrease associated with the rapid drop in MAP was not due to saturation of the flowmeter since increases in Vel of more than 20% above baseline (not shown here) could be recorded in these vessels in response to multiple flashes. Time constant of the flowmeter was 0.2 seconds.

FIGURE 6. (A) Typical time course of ChBF during sympathetic stimulation (hatched rectangle). The frequency of stimulation was 16 Hz, 6 V. The mean ChBF during baseline was determined from the data during the 1-minute interval preceding the stimulation. (B) Dependence of ChBF on sympathetic stimulation frequency obtained from two other cats.
59% and was significant (Table 1), whereas the mean change in retinal Vel (range, −8% to 12%) was not significant. The mean changes in MAP was 7 ± 6%, also not significant.

Recordings of MAP, ChBF, and IOP after a lethal injection of pentobarbital are shown in Figure 7A. The linear fit to the corresponding plot of ChBF as a function of MAP (inset) shows a highly significant correlation between ChBF and MAP. Correlation coefficients obtained in the 14 cats ranged from 0.953 to 0.998 (P < 0.01). In Fig. 7B the results obtained from all the cats were combined, after the average preinjection values of PP and ChBF had been normalized to 1. The linear fit is given by ChBF = 0.997 × PP(mm Hg) − 1.45 mm Hg and has a highly significant correlation (r = 0.896, P < 0.001). The fit to the data measured in the six cats with constant IOPs was not significantly different from the one obtained from the other cats (ChBF = 1.01 × PP + 1.4, R = 0.98, P < 0.001).

DISCUSSION

An important question in this study pertains to the contribution of the retinal circulation to the LDF signal. Although the beam was aimed at intervascular sites, some light scattered by RBCs moving in retinal capillaries must be present in the Doppler signal. This contribution can be minimized relative to the choroidal signal by probing the tapetal rather than the pigmented region of the fundus because in the tapetal region the incident light reaching the choriocapillaris and returning to the detector after reflection by the tapetum is not attenuated by pigment. The relative contributions of the RBCs in the retinal capillaries and the choriocapillaris can be estimated as follows. Because the RBCs scatter predominantly in a small angle in the forward direction,17 the light reaching the detector will have been scattered forward by the RBCs in the retinal capillaries and choriocapillaris, reflected by the tapetum and again forward scattered by those RBCs. Assuming the RBCs (number N) in the sampled volume to be randomly distributed, the intensity of the scattered light will be N times the intensity of the light scattered by a single RBC.18 Therefore the relative contributions of the retinal and choriocapillaris RBCs will be proportional to the number of RBCs in the respective layers. Histologic findings show that the choriocapillaris is equivalent to a uniform layer of blood with a thickness of approximately 20 μm.19 In contrast, there are less than ten retinal capillaries in a 100 μm line segment of a retinal cross-section (Fig. 5 of Snodderly et al20). Assuming each capillary to be 5 μm in diameter, the thickness of an equivalent uniform layer of blood would be less than 2.5 μm. Thus the contribution of the retinal RBCs should be about 1/8
Choroidal Blood Flow in Cats

previous studies have demonstrated that ChBF is insensitive to increased blood O_{2} concentration, in contrast to retinal blood flow (RBF). This criterion ensures that the contribution of the retinal circulation to the measured flow is less than 10%. This can be seen as follows. Previous results, as well as those of the current study, demonstrate that, during 4 to 5 minutes of 100% O_{2} breathing, blood velocity in cat retinal vessels decreases by 30 to 40%. This study and previous ones in other species show that vessel diameters decreases by more than 10%. Using a value of 10%, the resulting decrease in RBF amounts to 43 to 51%. Using a value of 50%, we can estimate the relative contribution of RBF and ChBF in the detected signal at sites where the decrease in ChBF at 4 minutes of hyperoxia was ≤ 5%. Let us call F = ChBF + RBF = 100 units during air breathing. At 4 minutes of 100% O_{2}, ChBF + 0.5 RBF ≥ 95. Combining both equations, RBF ≤ 10%.

To obtain valid relative measurements of ChBF with LDF, the measured ChBF must vary linearly with actual choroidal flow. If changes in PP occur faster than any regulatory change in vascular resistance, R, then the change in ChBF will be proportional to that of PP, because ChBF = PP/R. Studies in cats by Alm and Bill have failed to show an effect of a myogenic stimulus on ChBF. Responsiveness to metabolic stimuli, however, could not be ruled out. These stimuli are, however, much slower than the 6 seconds (Fig. 7) it takes for the arterial blood pressure to markedly drop after the injection of pentobarbital. Therefore, the measurements in Figures 7A and 7B provide a valid test of the linearity between actual and measured flow and the results clearly confirm such a relationship.

Chandra and Friedman found that high doses (10 μg) of ACh injected into the car femoral artery caused ChBF to increase by about 30%, a value somewhat lower but still in the range of our results (Table 1), if one takes into account that the MAP in their study decreased by 42% compared to about 10% in our animals (Fig. 4). As mentioned by these investigators, the larger decrease in MAP they observed may have attenuated the local increase in ChBF. In contrast, except for a transient decrease in response to the rapid drop in MAP, retinal Vel and vessel diameter did not change significantly, indicating no detectable effect of ACh on retinal blood flow.

Sympathetic stimulation reduced ChBF by 40% (Table 1), a result consistent with previous findings of Gherezghiher, who found that 16 Hz stimulation produced a 45% decrease in ChBF, and of Weiter et al., and Alm and Bill who reported that stimulation at 10 Hz decreased ChBF by 45% and 60%, respectively. As expected from the results reported by Alm and Bill, our study demonstrates that retinal blood velocity is insensitive to sympathetic stimulation. The lack of significant change in ChBF (3 ± 4%) in response to multiple flash stimulation, while retinal Vel increased by an average of 20% during 1 minute of stimulation, strongly supports our premise that the retinal circulation contributes little to the Doppler signal when the measurements are done as described in this study.

ChBF was found to be pulsatile, in phase with the heartbeat. The pulsatile component amounts to 34% of the mean ChBF. By comparison, a value of 25% was calculated for ChBF in a vessel of the minipig (Fig. 19b of reference 12) and values of 36% and 17% were obtained for retinal arteries in cats (Fig. 2a and 2b, ref. 32). The knowledge of the contribution of the pulsatile flow to the total choroidal flow is of major importance in the application of the ocular blood flow technique of Langham, a technique that attempts to determine pulsatile ocular blood flow from continuous IOP measurements.

With the continuous recording of ChBF, it is now possible to better characterize the time course of various regulatory processes in discrete regions of the choroidal vascular system. This may help the physiologist formulate hypotheses about the mechanisms involved in these processes and how these mechanisms can be affected by pharmacologic agents. Because of the local nature of these recordings, it will be possible to investigate potential regional differences in ChBF regulation. Furthermore, measurements over long periods of time may provide new information on slow fluctuations of ChBF and thus help identify some of the factors that affect the choroidal circulation.

APPENDIX

Linearity of the Relationship Between Actual Mean Flow Velocity and Vel

This relationship was investigated for flows with parabolic velocity profile (Poiseuille flow) with the TSI BPM 403A flowmeter. Two cases were considered. One in which the scattering particles were smaller and
the other in which they were larger than the wavelength of the laser light. In the first case, the Doppler shift power spectrum (DSPS) has a rectangular shape with a sharp cutoff, in the second, the DSPS decays exponentially with increasing frequency. The rectangular shape is exhibited by spectra obtained from retinal vessels in front of the heavily pigmented region of the cat fundus. It can be simulated by having polystyrene spheres (diameter < 0.2 \mu m) flowing in capillary tubes, 200 \mu m in diameter. The exponential shape is obtained when Doppler measurements are made from red blood cells flowing in a microvascular bed or from retinal vessels in front of the tapetum in the cat eye. This shape can be simulated using large polystyrene spheres (5 \mu m in diameter) or milk flowing through a capillary tube.

**DSPS with Rectangular Shape**

Polystyrene spheres (0.1 \mu m in diameter) were diluted in water and injected through a glass capillary tube (200 \mu m internal diameter) at known flow rates. The optical setup to obtain the DSPS has been described in detail elsewhere. In brief, the capillary tube was placed in the retinal plane of a Topcon model eye. The DSPS were obtained by Fourier analysis of the photocurrent produced by the laser light (670 nm) scattered by the spheres using a digital spectrum analyzer (Model 4520, Multigon, Mt. Vernon, NY). They were observed on the screen of an oscilloscope. The cutoff frequency of the spectra (corresponding to the centerline velocity of the particles) was determined by visual inspection. The photocurrent was also fed into a TSI BPM 403A (Vasamedics, Minneapolis, MN) blood perfusion monitor to obtain the mean velocity of the flowing spheres, Vel.

Figure 8A shows the cutoff frequency, \( f_c \), versus the pump flow rate of the spheres and the TSI Vel versus \( f_c \). The linear fit between Vel and \( f_c \) obtained with velocities below \( f_c \approx 3.6 \) kHz provides a correlation coefficient \( R = 0.994 \) (\( P < 0.01 \)).

**FIGURE 8.** (A) Linearity of relationship between actual mean flow velocity and relative flow velocity, Vel, as measured in kHz with the BPM 403A flowmeter, for polystyrene spheres with a diameter of 0.1 \mu m flowing through a 200 \mu m glass capillary tube. Bottom: Cutoff frequency, \( f_c \), versus the pump flow rate of the spheres. Top: Vel versus \( f_c \). The linear fit between Vel and \( f_c \) obtained with velocities below \( f_c \approx 3.6 \) kHz provides a correlation coefficient \( R = 0.994 \) (\( P < 0.01 \)). (B) Linearity of relationship between actual mean flow velocity and relative flow velocity, Vel (kHz) for whole milk (1/3 diluted in water) flowing through the same tube as in (A). Bottom: \( f_{1/10} \) versus pump flow rate. \( f_{1/10} \) is the frequency at which the spectral power of the DSPS (which decays exponentially with increasing frequency) was 1/10 the power at a frequency of zero kHz. Top: Vel versus \( f_{1/10} \). A linear relationship provides a highly significant fit with \( R = 0.99 \) (\( P < 0.001 \)) for \( f_{1/10} \) up to about 8 kHz.
DSPS with Exponential Shape

When the polystyrene spheres were replaced by whole milk (1/3 diluted in water), the DSPS decayed exponentially with increasing frequency. A frequency $f_{1/10}$ was arbitrarily defined as the frequency where the spectral power was 1/10 the power at 0 kHz. Plots of $f_{1/10}$ versus pump flow rate and TSI Vel versus $f_{1/10}$ are shown in Figure 8B. A linear relationship provides a highly significant fit with $R = 0.99$ ($P < 0.001$) for $f_{1/10}$ up to about 8 kHz.

Key Words
choroidal blood flow, laser Doppler flowmetry, acetylcholine, sympathetic stimulation, choroidal blood flow regulation

Acknowledgments
The authors thank Dr. J. Koelle for helpful suggestions regarding all aspects of this work and Dr. B.L. Petrig for his technical expertise.

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