Doppler measurement of vortex vein blood flow in animals

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The blood flow was measured continuously in the intact vortex vein of the anesthetized rabbit using an ultrasonic Doppler flowmeter with a frequency response of approximately 4 Hz. Blood flow correlated well with perfusion pressure (arterial pressure minus intraocular pressure). The intraocular pressure was changed stepwise by perfusion from a saline reservoir. Tonometry was performed with the eye connected to the reservoir and with the valve to the reservoir closed. Blood flow decreased with decreasing perfusion pressure in all cases.

Key words: ocular blood flow tests, vortex veins, intraocular pressure change, Schiotz tonometry, ocular blood circulation, rabbits.

Studies on the ocular pressure-volume relationship, homeostatic pressure mechanisms, tonometry, tonography, suction cup pressure decay curves, and retinal metabolism require an understanding of the changes in ocular blood flow and volume.

Monnick,1 in 1870, suggested that the ocular blood volume decreased during tonometry. Since that time, many investigators have studied the problem of ocular blood volume and flow and made some attempts to quantitate these effects. Even recent studies2-7 have provided little or no information on rapid changes in blood flow.

This series of experiments was an attempt to record the changes in outflow of blood in an intact vortex vein associated with changes in intraocular pressure. An ultrasonic Doppler flowmeter was used to obtain flow recordings with approximately 4 Hz. frequency response.

Methods

White New Zealand rabbits were anesthetized topically with proparacaine hydrochloride and intravenously with ethylcarbamate or sodium pentobarbital. The right femoral artery, the median ear artery, and the nose vein were cannulated. The vein cannula was directed towards the eye. The conjunctiva of the right eye was incised superiorly and medially at the limbus, and the upper lid and superior orbital margin were removed in most cases. The superior medial vortex vein was exposed and the ultrasonic transducer was either placed over the point of emergence of the vortex vein, glued on with Eastman 910, or sutured in place. In some animals, the superior rectus and superior oblique muscles were sectioned. The rabbit was heparinized with 2,000 to 4,000 U.S.P. units intravenously. We cannulated the anterior chamber with a 21 gauge needle connected to a supply reservoir of heparinized saline and used a separate 23 gauge needle for measurement of the intraocular pressure. If the surgical preparation on the right side was not perfect, the left side was used instead (two rabbits).

The intraocular pressure, saline reservoir supply pressure, nose vein pressure, median ear artery pressure, and femoral artery pressure were recorded continuously with 387BC Sanborn-Hewlett...
Packard transducers and 350-1,100 carrier pre-amplifiers. The flowmeter signal was obtained from a 10 mHz. Parks Electronic Transcutaneous Doppler Flowmeter* with the use of a specially constructed flow transducer applied to the superior nasal vortex vein (see Fig. 1). The output from this flowmeter was recorded through a modified Hewlett Packard frequency meter amplifier, 350-2,600. These six signals were recorded simultaneously on the 350-7,700 Sanborn direct-writing recording system and on a seven-track 3,955B Hewlett Packard magnetic tape system. One of the following procedures was followed:

1. **Stepwise changes in intraocular pressure (ten rabbits).** The saline reservoir connected to the anterior chamber was set at 20 mm. Hg, held for two to four minutes, and then raised in 10 mm. Hg steps, each held for the same length of time. When 90 mm. Hg was reached, the intraocular pressure was lowered in similar steps to zero mm. Hg. Then the intraocular pressure was raised stepwise to 90 mm. Hg and returned to zero again, one to three times. In three rabbits, the pressure steps were not sequential but were randomized (Fig. 2).

The voltages representing vortex vein flow, intraocular pressure, and median ear arterial pressure from the magnetic tape system were passed through identical filters to remove arterial pulsations. The intraocular signal was subtracted electronically from the arterial signal, producing a voltage proportional to perfusion pressure, which we applied to the X-axis of the X-Y recorder. The flow voltage was applied to the Y-axis (Fig. 3).

2. **Tonometry.** In two of the preceding rabbits, tonometry was performed after the stepwise changes in intraocular pressure, either with the eye at 20 mm. Hg not open to saline reservoir or with the eye open to the saline reservoir at 20 mm. Hg.

3. **Calibration.** In a different group of five rabbits, before the application of the ultrasonic transducer, the vortex vein was cannulated approximately 10 mm. from the eye with drawn-out PE-10 tubing. The cannula tip rested approximately 5 mm. from the point of emergence of the vortex vein. This tubing was attached to a horizontal 1 ml. pipette 5 cm. above the eye. The time required for a flow of 100 μL was determined for intraocular pressures of 20 and 40 mm. Hg with simultaneous recording of the Doppler flow signal.

**Results**

1. **Stepwise changes in intraocular pressure.** Stepwise changes in intraocular pressure produced rapid changes in vortex vein flow; changes in flow were also produced by spontaneous changes in arterial pressure (Fig. 2). A plot of derived perfusion pressure and flow is shown in Fig. 3. The heavy traces represent retracing of the flow-perfusion pressure curve for each fixed intraocular pressure.

2. **Tonometry.** The changes in pressure and flow produced by Schiötz indentation tonometry with open and closed manometry are shown in Fig. 4. The 25 cycles per minute wave shown to some degree in all traces represents the respiratory cycle.

3. **Calibration.** Our preliminary attempts at calibration resulted in considerable scatter, but the average flow was 325 μL per minute for the single vein, which corresponds to 1.3 ml. per minute for all vortex veins. The measured frequency shift was used to calculate the average velocity, approximately 50 mm. per second.

**Discussion**

The shift in frequency which occurs when ultrasound is scattered or reflected from moving, formed elements in the bloodstream is an example of the "Doppler" effect. The change in frequency which occurs is proportional to the velocity of the
formed elements in the blood. The signal which is returned is a spectrum of frequencies because the blood flow in a vessel consists of lamellae moving with different velocities, faster near the axis than the wall. The method for obtaining a voltage proportional to the mean velocity of the moving elements in the blood was given by Franklin and co-workers. 8

The Doppler output voltage is proportional to average velocity. Actual volume flow will depend on the cross-sectional area of the vessel being measured and the velocity profile across the vessel. In these experiments, the vortex vein diameters were not measured, but no gross changes were observed. In an attempt to determine whether the placement of the transducer was altering the cross-sectional area of the vortex vein, the transducer was fixed to the sclera in three different ways: laid on the surface, glued in place, and fixed with sutures. No differences were observed in the results obtained with these three methods of transducer application. The nose vein was used as an estimate of the distending pressure in the vortex vein. The changes recorded in the nose vein did not exceed 2 mm. Hg during a run, and the distension of the vortex vein probably did not change significantly. The finding that the flow signal was related to perfusion pressure with an extremely consistent curve in all our experiments, except at intraocular pressures lower than 20 mm. Hg, is an indication that our flow signal may represent volume flow.

When the intraocular pressure was reduced below the normal pressure in the rabbit, there was great variability in flow signal. This was probably due to two factors: (1) When the eye pressure was reduced below its normal value, the venous pressure probably should have been substituted for the intraocular pressure in the perfusion pressure equation: Perfusion pressure equals arterial pressure minus intraocular pressure, as defined in Fig. 3. (2) At this pressure level, the sclera is no longer under normal tension and there are probably changes in the exit resistance of the vein.
Fig. 3. The signals were taken from the tape system through the analogue manifold to an X-Y recorder. Blood flow was plotted continuously against perfusion pressure and the excellent correlation shown above was consistently obtained.

Fig. 4. The effect of repeated applications of a Schiotz tonometer on intraocular blood flow in the rabbit eye during open and closed manometry.
Although the scatter was large in our records of intraocular pressure below 20 mm. Hg, the changes in flow appeared to become smaller for a given change in pressure.

Bill\(^3\) indicated that ocular blood flow approaches zero at an intraocular pressure lower than the average femoral arterial pressure. Our results indicate that zero flow occurs close to or below the measured diastolic pressure of the median ear artery.

In this preliminary series of experiments (Fig. 4), we did not achieve completely open manometry and there were some changes in intraocular pressure upon application of the Schiötz tonometer. However, the results would appear to indicate that the ocular blood flow is primarily dependent on the intraocular pressure during tonometry. As the tonometer is removed, vortex vein flow decreases transiently, which probably indicates a filling of intraocular vascular channels. These results are consistent with Ytteborg\(^9\) work. A transient increase in blood outflow was observed almost invariably each time the intraocular pressure was elevated by the reservoir and was seen about one time in three when the tonometer was applied to the eye.

**REFERENCES**