The Influence of Elevated Intraocular Pressure on Vascular Pressures in the Cat Retina

Rajpaul Attariwala, Claudine P. Giebs, and Matthew R. Glucksberg

Purpose. Elevated intraocular pressure is known to reduce retinal blood flow, although the effect of intraocular pressure on retinal vascular pressures is unknown. Direct measurements of intravascular pressures were taken in the cat retina at various intraocular pressures.

Methods. Micropipettes of 2- to 3-μm tip diameter were used in conjunction with a servonull pressure-measuring system to determine retinal intravascular pressures in supine anesthetized cats. Pressures in large (80 to 120 μm diameter) vessels near the optic disc were measured over a wide range of intraocular pressures.

Results. Measurements show that retinal artery pressure depends on both intraocular pressure and mean systemic blood pressure, and that retinal vein pressure is determined by, but generally is different from, intraocular pressure, with no significant correlation to mean systemic blood pressure. Empirical equations are presented that predict statistically significant retinal artery, vein, and microvascular perfusion pressures.

Conclusions. Intraocular pressure is an important determinant of the microvascular perfusion pressure in the retina of the cat, particularly at low mean systemic blood pressure. It is also apparent that retinal vein pressure is always greater than intraocular pressure, which implies the existence of a high-resistance extraretinal segment of the retinal vein. The results suggest mechanisms for the loss of visual function in glaucoma and other retinal circulatory disorders.


Elevated intraocular pressure (IOP) impairs visual function1-3 in the eye, perhaps by reducing blood flow in the retinal circulation.4,5 The decreased blood flow has been attributed to a decreased microcirculatory perfusion pressure.6 The effects of elevated IOP on retinal vascular pressure, however, have not been directly measured.

The micropuncture servonull technique has long been an effective method for determining fluid pressures within small-diameter blood vessels in vivo7-11 preparations. In the eye, the servonull technique has been used to measure pressures in extraocular and choroidal vessels.12,13 Our laboratory has reported servonull pressure measurements in the vasculature of the cat retina14 that showed lower perfusion pressures through the retinal vascular bed than previously believed. We now present the direct measurements of intravascular pressures in the retina of the cat eye subjected to elevated IOP.

MATERIALS AND METHODS

Eight mixed-breed, conditioned cats were used for this study, and we adhered to the ARVO Resolution for the Use of Animals in Ophthalmic and Vision Research. We anesthetized (30 mg/kg ketamine hydrochloride mixed with 0.5 mg/kg acepromazine, intramuscularly) cats weighing 3 to 4.5 kg and cannulated the left saphenous vein with a catheter containing 5% sodium thiamylal, which was given as needed during surgery. We cannulated the right saphenous vein to administer a loading dose (300 mg/hr) followed by a maintenance dose (50 mg/kg/hr) of urethane for long-term anesthesia. We cannulated the right femoral artery with Teflon tubing, then used this line to sample blood gas, pH (128 blood gas monitor; Corning, Corning, NY), and to measure systemic arterial pressure with a physiologic pressure transducer (P23XL;
Spectraned, Oxnard, CA). We maintained the animal’s body temperature using a rectal probe connected to a continuous temperature monitor (73A; YSI, Yellow Springs, OH) that controlled the operation of a heated water blanket.

We performed a tracheotomy and inserted a breathing tube into the trachea, and all incisions were closed with surgical wound clips. The spontaneously breathing cat was placed in the supine position on a flat bed, and we immobilized the head in a stereotaxic-type head holder. After a lateral canthotomy, which involved removing some of the orbital bone, we stabilized the eye with a stainless steel eye ring fixed to the conjunctiva. We dilated the pupil with topical mydriatics (atropine and cyclopentolate hydrochloride; Alcon, Fort Worth, TX) and instilled flurbiprofen sodium 0.03% (Ocufen; Allergan, Irvine, CA) drops to maintain mydriasis at high IOP and to minimize the physiological effects of the surgical trauma. A 22-gauge needle connected to a height-adjustable saline reservoir was placed in the anterior chamber for IOP regulation, and the IOP was continuously monitored via a second 21-gauge needle placed in the anterior chamber and connected to a pressure transducer.

To prepare for vascular micropuncture, we positioned the tip of a 17-gauge, thin-walled needle with trocar at the center of rotation of our goniometric micromanipulator and aligned it with the superior retinal vessels. After a topical application of proparacaine hydrochloride (Alcaine; Alcon), we positioned the needle near the equator of the eye and pushed the needle through the sclera.

We filled a sharp, 2- to 3-μm tip diameter glass micropipette, which we manufactured in our own laboratory,15 with dyed (Fast Green; Sigma Chemical Co., St. Louis, MO) 2 M saline. We used concentrated saline to achieve a low electrical impedance through the tip of the micropipette in accordance with the principles of the servonull device (Model 5A; IPM, La Mesa, CA), and included the dye in the mixture to improve the micropipette visibility in the vitreous. We then removed the trocar from the needle and fed the pressure-measuring micropipette though the needle lumen and into the vitreal chamber before securing the micropipette to the micromanipulator. The micropipette was connected electrically and hydraulically to the servonull apparatus. The details of our micropuncture set-up and procedures have been previously described.15

We placed a plastic planoconcave lens on the cornea and viewed the tip of the micropipette and the retina through the dilated pupil with an operating microscope (Zeiss OPMI1; Carl Zeiss, Thornwood, NY). Using the positioning knobs on our goniometric micromanipulator, we maneuvered the micropipette tip above a retinal vessel at a location within 1.5 disc diameters of the edge of the optic disc. Just before micropuncturing the vessel, we checked that the servonull system was operating properly by increasing the gain until the pressure tracing showed that the fluid at the micropipette tip was oscillating. The eye is particularly well suited to the servonull system because a calibration check is built into the measurement: before each measurement we recorded the cardiac-induced pulse of the IOP that is transmitted throughout the aqueous and vitreous (Fig. 1). As a further check of the system, we momentarily increased the IOP by elevating the controlling reservoir and observing the simultaneous pressure increase measured by the anterior chamber needle and the servonull micropipette apparatus. We then set the reference pressure for the servonull equal to the IOP before directly measuring intravascular pressure. The details of our calibration procedures and testing criteria have been described previously in detail.14

With the micropipette tip above a retinal artery or vein, we punctured the vessel with a smooth forward movement of the microdrive (Narishige, Greensville, NY) mounted on the micromanipulator. We determined that the micropipette was within the lumen of the vessel by visual inspection and by observing a distinct pressure tracing on the chart recorder (RS3400; Gould, Cleveland, OH). This tracing was characterized by smooth, pulsatile oscillations at the same frequency as the systemic blood pressure (Fig. 1). Conversely, pressure measurements produced by the micropipette

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933180/)

**FIGURE 1.** Portion of a sample tracing showing BP, IOP, venous transmural pressure (Ptv), and retinal venous pressure (Prv). The time axis starts shortly after micropuncture, and the measured pressure can be seen to return to reference pressure after removal of the micropipette from the vein at 44 seconds. The IOP and venous pulse amplitudes measured by the servonull are nearly identical. BP is measured in the femoral artery, and therefore the phase offset is of little consequence.
resting on, or perhaps partially within, the wall of the vessel or surrounding connective tissue would typically produce flat traces with distinct spikes due to either cardiac or respiratory movements. As a further check to be certain that the micropipette was within the lumen of the vessel, we occasionally injected dye through the micropipette tip; if the dye was not visible or appeared as a plume proceeding downstream within the vessel, the micropipette tip was judged to be inside the vessel, whereas if the dye appeared as a stagnant cloud in the vitreous, the micropipette tip was judged to lie outside the lumen of the vessel. Dye injection was particularly useful for venous measurements at extremely elevated IOP (> 60 mm Hg), where a small difference between venous pressure and IOP was observed. In virtually every case where we injected dye after a recording, the characteristically stable intravascular pressure tracing was sufficient for confirming that the micropipette tip was located within the vessel lumen.

Systemic blood pressure, IOP, and servonull pressure were monitored continuously on a chart recorder, and digitized for further analysis by a PC-based data acquisition system (Keithley Dataq [Cleveland, OH] system in a Zenith [Franklin Park, IL] AT class computer). We normally left the micropipette within the vessel lumen for over a minute, which was sufficient time to change IOP; however, we were unable to change IOP with the micropipette tip in the vessel lumen because of the globe deformation and vessel movement that accompanies a change in IOP. To measure intravascular pressures at different values of IOP without damage to the vessel or micropipette tip, we withdrew the micropipette from the immediate vicinity of the vessel while we changed IOP. Each measurement therefore represents a separate micropuncture.

The servonull pressure recordings, with the pressure offset equal to IOP, measured transmural pressures directly. Therefore we analyzed all the data as average arterial (P\text{ta}) or venous (P\text{tv}) transmural pressure, whereas IOP was averaged and mean systemic blood pressure (BP) was calculated from the femoral diastolic plus one-third pulse pressure. Minitab (State College, PA) software was used for statistical regression analysis, giving Student's $t$ test for coefficients and the $F$-statistic for the relationships reported between transmural pressure, IOP, and BP.

RESULTS

Retinal Vein Pressure

We made 54 measurements of P\text{tv} at IOPs set between 14 and 80 mm Hg. A portion of a sample tracing from a 120-μm retinal vein measurement is shown in Figure 1. The transmission of the cardiac pulse pressure to the IOP and retinal vein pressure is apparent. The venous pressures were typically characterized by pulse pressures ranging from 1 to 3 mm Hg, with an average pulse (systolic–diastolic) of 2 mm Hg.

The P\text{tv} measurements at specific IOP values are plotted in Figure 2. These values were consistently greater than IOP until vascular collapse prevented any further micropuncture. The best-fit equation predicted by linear regression for 14 < IOP < 80 mm Hg is given by:

$$ P_{\text{tv}} = 9.12 - 0.0964 \text{ IOP} \quad (1) $$

The coefficients and the relation are significant ($t$ test $P < 0.001; F$-stat $P < 0.001), with an RMS error of 2.17 mm Hg. We used multiple linear and nonlinear regression to determine the influence of BP to be not significant ($P \geq 0.88$). We could not determine the influence of P\text{ta} on P\text{tv} because we lacked a sufficient number of paired data.

Retinal Artery Pressure

We made 49 measurements of P\text{ta} in 100-μm diameter arteries with IOP ranging from 18 to 75 mm Hg. P\text{ta} measurements typically had pulse pressures between 7 and 20 mm Hg, but clean pulse pressures sometimes were difficult to obtain because the motion of the arteries often caused the micropipette tip to strike the inside of the vessel wall, causing spikes in the tracings. A portion of a sample arterial trace is given in Figure 3.

The dependence of P\text{ta} on both BP and IOP made the data analysis more complex than the analysis for P\text{tv}. BP varied widely between cats, and ranged from 77 to 167 mm Hg. For each measurement, IOP was set at specified pressures between 18 and 75 mm Hg. Figure 4
FIGURE 3. Portion of a recording for a retinal artery, showing IOP, Pta, and retinal arterial pressure (Pra). The micropipette was removed from the artery at 46 seconds, where the measured pressure returned to the reference pressure. Systemic blood pressure for this measurement was 195/120 mm Hg.

shows the complete set of Pta measurements divided into arbitrarily assigned BP ranges for clarity. Thus, multiple regression was required to obtain a relation between Pta and both IOP and BP. We propose a statistically derived empirical equation for this relation based on both x (IOP, BP) and y (Pta) coordinate transformations, which results in the inverse logarithmic equation:

\[ P_{ta} = (10^{5/2+(BP/157)^{2}} - 60)^{1/2} \]  

(2)

The coefficients and the nonlinear relation are significant (t test P < 0.001; F-stat P < 0.001) with an r² of 0.771. Equation (2) fits the data well for 20 < IOP < 80 mm Hg and 80 < BP < 170 mm Hg. This relationship is also shown in Figure 4, where it was generated at the midpoint of each graph’s BP range. Figure 5 shows the family of curves generated by equation (2), plotted relative to atmospheric pressure as retinal arterial pressure (Pra, where Pra = Pta + IOP) versus IOP.

Retinal Microvascular Perfusion Pressure

We generated perfusion pressure curves based on equations (1) and (2); therefore, the perfusion pressure curves are valid under the same conditions. The perfusion pressure plotted against IOP, with BP isobars, is shown in Figure 6, and Figure 7 shows the perfusion pressure versus BP with IOP isobars.

DISCUSSION

The servonull micropuncture technique is an effective method for measuring pressures within retinal vessels.
The sharp, 2- to 3-μm tip diameter allowed repeated measurements without vascular hemorrhage or noticeable vasoconstriction.

The results of this study are consistent with the results of our previous work in cats with IOPs set at 10 and 20 mm Hg. Both studies obtained values for \( P_{tv} \) that were significantly above IOP, and values for \( P_a \) that were well below ophthalmodynamometric measurements, which are the only estimates of ophthalmic artery pressure available. In this series of experiments, we measured \( P_a \) and \( P_{tv} \) with IOP between 14 and 80 mm Hg and generated regression equations to fit this data.

We point out that the pressures we have recorded in cat retinal veins contradict some current thought, and preclude the possibility of the retinal veins being partially collapsed, leading to a "vascular waterfall" within the globe near the edge of the optic disc.

The regression equation for the \( P_{tv} \) data (equation [1]) has an RMS error of 2.17 mm Hg; therefore, adding and subtracting this value to equation (1) statistically envelopes at least two thirds of the data distributed around the regression line. This property allows us to approximate equation (1), with a reasonable degree of accuracy (\( P_{tv} \) error < 0.3 mm Hg for IOP < 40 mm Hg), by the following simpler equation:

\[
P_{tv} = 9 - \frac{IOP}{10}
\]

In the cat, \( P_{tv} \) above IOP for all pressures below vascular collapse indicates that a resistance segment must be present somewhere downstream. Bill has measured extraocular venous pressures by cannulation of the cat's anterior ciliary veins and found the pressure to be equal to 7 mm Hg for IOPs between 12 and 26 mm Hg. The anterior ciliary vein and the retinal veins drain directly into a venous plexus in the retrolaminar region of the optic nerve. Hence, the resistance segment must maintain the large pressure drop from intraocular venous pressure relative to atmospheric pressure (\( P_{tv} \), where \( P_{tv} = P_{tv} + IOP \)), which increases with increasing IOP, to extraocular venous pressure. Based on anatomy, the resistance segment responsible for this pressure drop undoubtedly lies anterior to the venous plexus. Although it is conceivable that the extraocular resistance is controlled by active mechanisms, the rapidity of the change in vascular pressure after a change in IOP (they are nearly simultaneous) leads us to the reasonable conclusion that the mechanism is purely a passive mechanical effect.

Taylor et al, by serial sectioning of the human lamina cribrosa, have shown the presence of a collagenous narrowing around the central retinal vein. They hypothesize that this narrowing is responsible for maintaining the intraocular venous pressure above IOP. This mechanism for venous regulation is consistent with our measurements in the cat, because the narrowing of the lamina cribrosa around the veins represents a possible anatomic site for passive control of
extraretinal venous resistance, and may represent the site of the so-called venous waterfall.

Our measurements of pressures in retinal arteries demonstrate that the \( P_a \) depends on both IOP and BP, and not just BP alone, as predicted by ophthalmodynamometric approximations. In ophthalmodynamometry, IOP is elevated until vascular collapse near the disc is observed, but Bill's \(^{18} \) has shown that elevating IOP causes a subsequent elevation in ciliary artery pressure. Therefore, this method cannot be used to determine accurately arterial pressures at any IOP below vascular collapse because it overestimates the arterial pressures that influence the retinal circulation, particularly at low BP, where a strong dependence on IOP is observed.

Statistical analysis of the data led to a nonlinear inverse logarithmic equation (\( P_a \)), which, although complex, reasonably predicts \( P_a \) at all IOPs and BPs measured. The general trend obtained from the multiple nonlinear regression shows a small plateau region for low IOPs, followed by a nearly linear drop in \( P_a \) with increasing IOP (refer to Figs. 5, 6). The \( P_a \) therefore increases with increasing IOP, consistent with Bill's \(^{18} \) speculation that the pressure increase in the ciliary artery would translate intraocularly to the retina at acutely elevated IOP.

The nonlinear response of \( P_a \) to BP at low IOPs is not surprising, given that the arterial system is not a passive conduit of blood. \(^{23} \) Within the retina, autoregulation \(^{24} \) and metabolic regulation \(^{25} \) have been well documented. Traditionally, autoregulation is defined as the maintenance of constant flow with changes in perfusion pressure by vascular caliber changes in the resistance vessels, and the retinal artery falls well within the range of diameters for resistance vessels. Our determination of perfusion pressures provides complementary data to blood flow measurements in the retina. \(^{4,5,26} \) Based on principles of fluid mechanics, without changes in vascular caliber, flow and pressure gradients are proportional. Figure 6 shows a near-linear drop in perfusion pressure at IOP's above 40 mm Hg, whereas below 40 mm Hg, there is an even greater drop in perfusion pressure, which results in a "semiplateau" region. This semiplateau region, determined by regression analysis of our pressure data, is consistent with measurements of flow in normal human subjects by the blue field entoptic phenomena technique \(^{4} \) and laser Doppler flowmetry studies. \(^{5} \) From these flow studies, the region below 30 mm Hg is interpreted as the range in which the retina's autoregulatory function is most effective. It is important to recognize that the term "perfusion pressure" has been widely used to refer to the difference between ophthalmic artery pressure and IOP, whereas we have defined it to be the difference between \( P_a \) and \( P_r \). Because our results show that \( P_r \) is not equal to IOP, and that \( P_r \) is not necessarily equal to the pressure in the ophthalmic artery, our use of the term differs from the traditional definition. The semiplateau region in Figure 6 is predominantly the result of the nonlinear response of \( P_a \) to changes in IOP. The fact that \( P_a \) changes nonlinearly with IOP necessarily implies that the site of artery micropuncture lies within a region of the circulation that participates in blood flow regulation.

Cats, compared to humans, are normally hypertensive, with widely varying BPs. \(^{27} \) This, along with the vascular differences in the retrolaminar optic nerve region, might lead to speculation that the response of perfusion pressure to BP changes could be different in cats and humans. We point out, however, that our results are consistent with noninvasive laser Doppler velocimetry studies in humans. \(^{26} \) This suggests that the nature of the retinal vascular pressure response to BP may be common to both cats and humans, and we may therefore speculate on the importance of our results in disease. Our measurements are particularly relevant to the glaucomas, which are characterized by elevated IOP or cardiovascular crises, and impaired retinal autoregulation with elevated IOP. \(^{28} \)

Drance \(^{29} \) speculated that patients with glaucoma probably have increased venous resistance, because he and other researchers \(^{30} \) have found that flame-shaped hemorrhages in the optic disc frequently precede the development of retinal nerve fiber bundle damage. Our measurements of high \( P_r \) even at elevated IOP, along with recent anatomic evidence of a venous constriction in the human lamina cribrosa, \(^{32} \) and the predisposition of patients with glaucoma to retinal vein occlusion, \(^{31} \) support this hypothesis. Our data clearly show that reduced BP, elevated IOP, or both, reduce perfusion pressure more than expected from previous assumptions, and suggest a direct link between the etiology of primary open-angle glaucoma and normal-tension glaucoma.

Beyond glaucoma, we speculate that our results may have profound implications for the understanding of the physiology of the retinal circulation in health and disease. Although we currently are unable to identify the mechanisms by which \( P_r \), is controlled, it is possible that disease alters these mechanisms and changes \( P_r \) as well as autoregulatory performance of the arterial side of the circulation. Our data indicate that perfusion pressure is normally lower than common estimates, and, if further lowered by disease, the resulting reduction in blood flow and consequently lower hydrodynamic shear may promote the formation of retinal vascular occlusions. Retinal vein pressure, if normally greater than IOP, may become further elevated, and prove to be important in the formation of microaneurysms and eventually the neo-
vascularization seen in diabetic and hypertensive retinopathies, and may contribute to increased transvascular fluid flux and the formation of retinal edema.

Key Words

micropuncture, intraocular pressure, retina, glaucoma, perfusion pressure

Acknowledgments

The authors thank R. Dunn, R. A Linsenmeier, and T. K. Goldstick for useful discussions, and G. S. Attariwala for supplies and clinical input.

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