A Rabbit Model to Study Orbital Venous Pressure, Intraocular Pressure, and Ocular Hemodynamics Simultaneously

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PURPOSE. To measure orbital venous pressure (OVP) and determine the effects of changes in mean arterial pressure (MAP) on OVP, intraocular pressure (IOP), episcleral venous pressure (EVP), and ciliary and choroidal blood flows.

METHODS. The experiments were performed in anesthetized rabbits. In all animals, MAP, IOP, and OVP were measured by direct cannulation of the central ear artery, the vitreous, and the orbital venous sinus, respectively. Laser Doppler flowmetry was used to measure choroidal blood flow in one group, and ciliary blood flow in a second group. A servonull micropressure system was used to measure EVP in a third group. The protocol for all three groups entailed varying MAP mechanically with occluders on the aorta and vena cava.

RESULTS. The OVP and IOP relationship correlated linearly (r = 0.99) during mechanical manipulation of MAP. EVP also correlated well with OVP (r = 0.9). Resistance calculations based on choroidal and ciliary blood flows and the pressure gradients indicate active adjustment of arterial resistance and passive changes in venous resistance in response to changing MAP in both circulations.

CONCLUSIONS. The rabbit orbital venous sinus permits continuous measurements of OVP. The present findings show that OVP is not static and suggest that OVP may play an important role in IOP homeostasis and ocular hemodynamics. (Invest Ophthalmol Vis Sci. 2002;43:3728–3734)

Because the veins draining the eye are small or difficult to reach without disturbing the eye and orbit, measurements of venous pressure outside the eye are not performed routinely. However, as the downstream recipient of conventional aqueous outflow and the efflux of the ocular circulations, the orbital venous system's physiology is intertwined with aqueous dynamics and ocular hemodynamics. In the case of the episcleral veins, the episcleral venous pressure (EVP) is the pressure head that must be overcome for aqueous passage through the trabecular pathway, and so the EVP is acknowledged as a key determinant of steady state intraocular pressure (IOP). However, although EVP is often measured in studies of drug effects on aqueous dynamics, it has rarely been manipulated experimentally, aside from studies of pseudofacility. In contrast to EVP and aqueous dynamics, the effects of orbital venous pressure (OVP) on ocular hemodynamics are less clear. To the best of the authors' knowledge, the only information in the literature comes from the study by Bill in which the blood flow from a cannulated vortex vein was measured as the cannula pressure was varied while holding IOP constant at different levels. Otherwise, we are aware of no studies in which blood flow in an ocular circulation was measured while OVP was varied or measured.

Given the paucity of information about OVP, we sought a method to measure it. We found that the rabbit's skull offers a unique opportunity to measure OVP by direct cannulation of the orbital venous sinus through the posterior supraorbital foramen. This article presents the results of continuous measurements of OVP, EVP, IOP, mean arterial pressure (MAP), and choroidal and ciliary blood flows. The relationships between these parameters obtained during mechanical manipulation of MAP over a wide range show that OVP is not static and that it may play an important role in ocular hydrodynamics.

METHODS

All animal procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. At the end of the experiment, all animals were killed with an overdose of anesthetic without regaining consciousness.

Animal Preparation

New Zealand albino rabbits (2–3 kg, n = 25) of both sexes were housed for 1 to 3 days in the vivarium with access to food and water ad libitum before the experiments. The animals were anesthetized with pentobarbital sodium (30 mg/kg, intravenously, supplemented as needed) and paralyzed with gallamine triethiodide (1 mg/kg) to eliminate eye movement. The animals were intubated through a tracheotomy and ventilated with room air. Expired PCO2 was monitored (Nor-mocap 200; Datex, Tewksbury, MA) and maintained at 40 to 45 mm Hg. A heating pad was used to maintain normal body temperature (38–39°C). All intravenous injections were given through cannulas placed in the marginal ear veins. The right eye and the right orbital venous sinus were used in all experiments.

To estimate the ocular arterial pressure (AP) and ensure the adequacy of anesthesia, a catheter was inserted into the right ear artery and connected to a pressure transducer positioned at the same height above the heart as the eye. After the initial surgical preparation, the animals were mounted in a stereotaxic head holder, and the right eye was cannulated with a 23-gauge needle inserted into the vitreous cavity through the pars plana to measure the IOP with a pressure transducer. To avoid the rabbit ocular trauma response and release of prostaglan-
dins, the right eye was anesthetized topicaly with lidocaine before cannulation and care was taken not to disturb the cornea and anterior chamber.

Measurement of OVP

The orbital venous sinus covers most of the backside of the rabbit eye. To measure OVP (n = 25), a 23-gauge needle was connected to a...
pressure transducer, inserted into the posterior supraorbital foramen (PSF), and advanced into the sinus, as shown in Figure 1. A moderate vacuum was applied to the catheter during insertion of the needle and the resultant visible blood reflux into the catheter used to indicate successful penetration. Figure 2A shows a typical unedited recording of OVP in relation to AP, IOP, and EVP. Figure 2B shows unedited tracings from an experiment in which IOP was raised above MAP to stop ocular blood flow and assess its contribution to OVP.

Measurement of Choroidal and Ciliary Blood Flow

Laser Doppler flowmetry (LDF) was used to measure choroidal ($n = 10$) and ciliary ($n = 10$) blood flow. LDF provides three indices of perfusion derived from the frequency spectra collected from tissue illuminated with laser light—that is, the number of moving blood cells, their mean velocity, and the flux, which is the product of the velocity and number of moving blood cells. The flux has been shown to correlate linearly with independent measures of blood flow in a variety of tissues. A detailed description of LDF and its validation is published elsewhere.9

The laser Doppler flowmeter (PF4000; Perimed, Stockholm, Sweden) used in this study has an infrared laser diode (780 nm, 1 mW) coupled to a fiber-optic probe (PF403; 0.25 mm fiber separation; Perimed). The flowmeter was calibrated so that the flux registered 250 perfusion units (PU) when the probe was placed in a suspension of latex particles at 22°C and 0 PU when placed against a plastic disk. This calibration procedure insures that comparable flux readings are obtained with the same flowmeter model (e.g., PF4000; Perimed) in the same tissue preparation (e.g., posterior choroid in albino rabbit) under similar conditions. However, because the flux is dependent on the light-scattering properties and vascular organization of the target tissue, the flux readings from different tissues are not comparable quantitatively. In the present study, this caveat means that the flux values and associated vascular resistances reported for the choroid and ciliary body cannot be compared directly (e.g., it would be incorrect to conclude that choroidal blood flow is 12.75 times higher than ciliary blood flow, based on the baseline data; see Table 1). In other words, quantitative flux comparisons in the same tissue between subjects are appropriate; quantitative flux comparisons in different tissues within or between subjects are not.

To measure choroidal perfusion, the probe was advanced through the pars plana of the right eye with a micromanipulator. The probe and the wavelength used in this study provide a sampling volume (1 mm$^3$) large enough to measure blood flow in both the retina and the choroid.9 The tip of the probe was positioned over the visual streak where the retina is avascular and the flux signal is caused only by choroidal blood flow.

To measure ciliary perfusion, the probe was attached to a modified cartridge holder of a record player tonearm. The tonearm counterweight was set so that the probe tip was held against the sclera with a force of approximately 0.5 g. The tip was placed on the right eye at a site overlying the ciliary body from which the conjunctiva had been removed. The tonearm allowed the probe tip to move with the eye during the large changes in MAP, thereby insuring that the measurements were not influenced by changes in the force of the probe against the tissue and that the measurements were made at the same site throughout the experiments. The measurement site was 1 mm posterior to the limbus and was identified as the peak flow between the vessels at the limbus and the pars plana. We confirmed previously that the LDF measurement depth is sufficient to measure through the sclera to the underlying ciliary body.9

EVP Measurements

A micropipette-based servonull pressure system (Model 900A; World Precision Instruments, Sarasota, FL) was used to measure episcleral venous pressure ($n = 5$).11–13 Micropipettes were pulled (P87 pipette puller; Sutter Instrument Co., Novato, CA), and their tips were beveled in two planes to inner diameters between 2 and 3 μm on a micropipette beveler (BV-10; Sutter Instrument Co.) using a grinding plate (104-F; Sutter Instrument Co.). The pipettes were filled with 2 M sodium-chloride solution and connected to the servonull pressure system.

The conjunctiva was cut near the superior limbus, and the conjunctival flaps were used to form a small (3 $\times$ 3 mm) calibration well that was filled with 0.9% sodium-chloride solution. The eye was stabilized with conjunctival sutures that were gently attached to a fixed ring, and the upper lid was slightly retracted. Otherwise, the eye was kept in its normal position. The pipette tip was first advanced into the well where it was calibrated in saline solution at zero pressure, and then it was further advanced into an episcleral vein. The episcleral veins were punctured near their exit point from the sclera under visual control. The double beveled pipettes were sharp and slid easily through the vessel walls. A small puff of saline emerging from the pipette was observed when the tip passed the vessel wall and reached the lumen of the vein. The pipette was then advanced further into the lumen while the pressure signal was watched carefully to detect the sharp pressure increase that occurs when the tip reaches the opposite vessel wall. The measurements were performed between these two points.

Experimental Protocols

Two protocols were performed. In both, the goal was to vary MAP over a wide range, by placing hydraulic occluders around the thoracic aorta and the inferior vena cava through a right thoracotomy. The aortic occluder was used to redirect the cardiac output to the upper body,
thus increasing the MAP in the eye. The caval occluder was used to impede venous return, thus lowering cardiac output and reducing MAP throughout the circulation. In the ciliary and choroidal blood flow experiments, the protocol entailed ramp increases and decreases in MAP. In the EVP experiments, stepwise pressure changes achieved with the occluders were usually necessary to optimize the positioning of the micropipette. To illustrate the protocols, Figure 3 shows representative unedited traces from a ciliary blood flow experiment (Fig. 3A) and an episcleral venous pressure experiment (Fig. 3B).

**Data Analysis**

All variables were recorded with a data acquisition system (PowerLab; ADInstruments, Grand Junction, CO) at a sampling rate of 20 or 100 Hz. The data were later reduced off-line by averaging the measured variables in 5-mm Hg bins of MAP (i.e., all values temporally associated with MAP between 80 and 75 mm Hg were averaged, then those between 75 and 70 mm Hg, and so forth). All results are expressed as the mean ± SE. An unpaired t-test was used.
to compare the baselines in the ciliary and choroidal blood flow experiments. Because there were no significant differences between baseline values, the MAP versus OVP and OVP versus IOP data were pooled.

Three different resistance indices were calculated for ciliary and choroidal blood flows: $R_1 = (\text{MAP} - \text{IOP})/\text{flux}$; $R_2 = (\text{MAP} - \text{OVP})/\text{flux}$; and $R_3 = (\text{IOP} - \text{OVP})/\text{flux}$.

$R_1$ is the traditional resistance calculation for the ocular circulations and reflects the Starling resistor behavior of the intraocular veins (i.e., the pressure in the intraocular veins as they near their exit through the sclera is slightly greater than IOP). R2 and R3 are novel resistance calculations, because OVP has not been measured previously. R2 is the summed resistance of all the intraocular vascular segments, whereas R3 is the venous resistance from inside to outside the eye.

**FIGURE 3.** Experimental protocols. (A) Representative traces from a ciliary blood flow experiment. (B) Representative traces from an EVP experiment with repositioned micropipette measurements stable for 25 seconds at each pressure step. MAP was changed by aortic and caval occlusion.
FIGURE 4. (A) Effect of MAP on IOP (r = 0.99, n = 20) and OVP (r = 0.98, n = 20) and (B) the corresponding linear relationship between IOP and OVP (r = 0.99, n = 20).

Table 1. Baseline Data

<table>
<thead>
<tr>
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<th>EVP Experiments (n = 5)</th>
<th>Blood Flow Experiments (n = 10)</th>
<th>Ciliary Blood Flow Experiments (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>64.7 ± 0.9</td>
<td>69.0 ± 1.2</td>
<td>66.1 ± 0.8</td>
</tr>
<tr>
<td>IOP (mm Hg)</td>
<td>16.4 ± 1.1</td>
<td>17.2 ± 1.2</td>
<td>15.8 ± 0.8</td>
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<tr>
<td>EVP (mm Hg)</td>
<td>9.6 ± 0.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>OVP (mm Hg)</td>
<td>3.1 ± 0.6</td>
<td>3.1 ± 0.4</td>
<td>2.9 ± 0.4</td>
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</table>

* Flux data from different tissues are not comparable quantitatively.

RESULTS

Table 1 shows the baseline data for the measured variables. MAP, IOP, and OVP were similar in the three sets of experiments with pooled (n = 25) means of 66.96 ± 0.67 mm Hg, 16.48 ± 0.61 mm Hg, and 3.01 ± 0.24 mm Hg, respectively.

Figure 4A shows the changes in IOP and OVP in response to changes of MAP (n = 20). The relationship of MAP with IOP is consistent with previous reports on this relationship in the rabbit eye. The mean IOP data were well fit by an exponential regression (IOP = 6.5 · 10^{0.0060·MAP}, r = 0.99) over the MAP pressure range of 18.6 ± 0.2 to 91.7 ± 0.2 mm Hg. The second curve shows the less steep relationship of MAP with OVP. OVP varied between −1 and +5 mm Hg. The mean OVP data were well fit by linear regression (OVP = 0.067 · MAP − 1.3; r = 0.98). Figure 4B shows IOP plotted as a function of OVP, which was also well fit by linear regression (IOP = 2.86 · OVP + 7.47; r = 0.99).

Figure 5A shows the pooled IOP, EVP, and OVP data plotted as a function of MAP (n = 5). As with the mean data in Figure 4A, the pooled IOP data were well fit by an exponential regression (IOP = 6.4 · 10^{0.0045·MAP}, r = 0.94) and the OVP data well fit by linear regression (OVP = 0.053 · MAP − 1.0; r = 0.90). By contrast, the pooled EVP data were less well fit by linear regression (EVP = 0.133 · MAP + 0.14; r = 0.78). However, when the experiments were analyzed individually, the regression coefficients ranged from 0.8 to 0.97 (mean, 0.92), and the average regression equation changed slightly (EVP = 0.157 · MAP − 1.08). A similar improvement in the regression coefficients occurred when the OVP versus EVP data were analyzed by experiment (mean r = 0.9 versus pooled r = 0.72), and thus Figure 5B shows the data from each experiment represented by different symbols with their associated regression lines and correlation coefficients. The mean regression equation for the individual experiments was EVP = 3.25 · OVP − 0.8.

FIGURE 5. (A) Effect of MAP on IOP, EVP, and OVP (n = 5). The regression lines are shown for the pooled data (● r = 0.94, □ r = 0.78, △ r = 0.90). (B) EVP plotted as a function of OVP (n = 5). The regression lines are shown for each experiment (● r = 0.95, △ r = 0.92, □ r = 0.90, □ r = 0.99, ○ r = 0.74).

The choroidal (n = 10) and ciliary (n = 10) pressure-flow relations are shown in Figures 6A and 6B; both are in keeping with previous reports. The resistance calculations based on the measured pressure gradients are shown in Figures 6C and 6D. At MAPs less than 30 mm Hg, all three resistance indices are inversely related to MAP. At MAPs from 30 to 45 mm Hg, all three resistance indices are relatively stable. At MAPs greater than 45 mm Hg, R1 and R2 are positively related to MAP, but R3 is constant.

DISCUSSION

The impetus for seeking a method to measure OVP arose from a desire to assess the potential role of EVP in dynamic IOP responses and a comment by Alm that “the pressure in the vortex veins just outside the eye may be expected to be similar to that in the episcleral veins.” Given Alm’s comment, and given the large caliber of the rabbit vortex veins and their short length before joining the orbital venous sinus, it seemed pos-
sible to obtain continuous measurements of OVP as an index of EVP, if the sinus could be cannulated. Postmortem dissection of the skin surrounding the orbit revealed the PSF as a likely point of access to the sinus, and subsequent in vivo attempts at cannulation were successful.

**OVP Measurement**

The cannulation of the orbital venous sinus through the PSF is performed without visual guidance, which raises the concern that the needle tip could be anywhere in the orbit and measuring something other than OVP. However, several points argue against this concern. First, aside from an inadvertent puncture of the globe, the orbital venous sinus is the only large vascular structure that can provide the blood reflux used as the indicator of successful cannulation. In the event the globe is punctured, the pressure equals the IOP and so is easily distinguished from the lower OVP readings. Second, in addition to the blood reflux, successful sinus cannulation is also indicated typically by respiratory synchronous fluctuations in the pressure signal (Fig. 2A) that are not seen when the needle is in the orbit but not in the sinus. Third, raising the IOP above systolic blood pressure causes a small decrease in OVP that parallels the decrease and cessation of choroidal blood flow (Fig. 2B). This occurs because the orbital venous sinus receives blood from the ocular and orbital circulations and has low resistance connections to the rest of the venous circulation of the head. Consequently, selective elimination of ocular blood flow by raising the IOP removes the ocular contribution to sinus blood volume and so lowers OVP. Such a decrease in pressure would not be expected from a needle tip in the orbit outside the sinus; instead, either no change or a slight increase in pressure due to increased volume of the orbit would be more likely.

**Baseline Data**

The baseline data in Table 1 for MAP and IOP are similar to those previously reported for this preparation. The baseline EVP of 9.6 ± 0.9 mm Hg is similar to the mean EVPs in humans compiled by Zeitmer for pressure chamber venanometers (9.8 ± 1.8 mm Hg) and applanation venanometers (10.0 ± 1.5 mm Hg). Maepea and Bill obtained a similar baseline EVP of 10.4 ± 0.7 mm Hg in monkeys by using a micropipette servonull pressure system. To our knowledge, there are no other baseline OVPs reported in the literature, and the pooled (n = 25) mean pressure of 3.01 ± 0.24 mm Hg therefore needs further corroboration. However, the present data indicate that the rabbit has an EVP-to-OVP gradient of 6 to 7 mm Hg and that measurements of OVP cannot be used as an index of EVP, as we had originally hoped.

**MAP, OVP, and IOP**

Although the response of IOP to acute, mechanically induced changes in MAP shown in Figure 4A is comparable to that reported previously for this preparation, the corresponding response of OVP is novel and particularly intriguing because of the relation between OVP and IOP (Fig. 4B). Perturbations such as head-down tilt and the Valsalva maneuver are thought to increase IOP by engorgement of the ocular circulations due in part to increased OVP, and so some correlation between OVP and IOP was expected. Nonetheless, the strength of the correlation (r = 0.99) is noteworthy. Given the evident sigmoid shape of the relationship, the correlation for a third-order polynomial fit is slightly better (IOP = (-0.09 · MAP^3) + (0.7 · MAP^2) + (1.56 · MAP) + 7.91; r = 0.999), but this provides little additional insight into the relationship at present.

Because MAP was the manipulated variable, the interpretation of the OVP-IOP relation is complicated. The small response of OVP to the elimination of ocular blood flow to the sinus (Fig. 2B) indicates that OVP’s response to MAP is not due primarily to changes in ocular blood flow, but rather to the more global effect of MAP on blood flow into the venous circulation of the head. Thus, the response of IOP to MAP is due in part to the OVP’s acting to impede ocular venous outflow and in part to the MAP’s driving blood flow into the eye. Resolving their relative contributions to the response of IOP necessitates selective manipulation of MAP and OVP, which was beyond the scope of the present study but will be attempted in a future study. The effects of vasoactive drugs and different anesthetics will also have to be assessed, because the OVP-IOP relation is probably affected by changes in ocular and cranial vascular tone. Still, it is clear from Figure 4 that OVP is not static and that OVP may have a significant effect on IOP.

These acute results must be distinguished from more long-term pressure perturbations. In the rabbit (which has a negligible retinal circulation), OVP and IOP are coupled anatomically through two routes. The direct coupling is established between the uvea and the sinus by the vortex veins, whereas the indirect coupling results from the pressure gradient between the episcleral veins and the anterior chamber. OVP-dependent changes in uveal blood volume occur rapidly with simultaneous effects on IOP, but subsequent changes in IOP would be expected because of OVP-dependent changes in EVP and consequent changes in aqueous outflow (e.g., see Fig. 8 in Reference 18).

**OVP and EVP**

The pressure gradient from EVP to OVP is one of the significant findings of this study. The source of the resistance responsible for this pressure gradient is unknown, although the relatively long length of the small-caliber episcleral veins and their course through the extraocular muscles en route to the orbital sinus are likely contributors. It is also the unique circumstances of each episcleral vein tested that presumably gave rise to the variations in slopes when EVP was plotted against the more global measures of MAP and OVP in Figure 5. Nonetheless, it is clear from Figure 5B that EVP varies with OVP, although the slope for an individual episcleral vein probably differs from the mean slope of the OVP-EVP relation, indicating a 3.25-mm Hg increase in EVP for every 1-mm Hg increase in OVP.

As with the OVP-IOP relationship (Fig. 4), the relative contributions of MAP and OVP to the EVP response cannot be discerned with this experimental design. However, given the large resistance between the ocular arterial supply and the episcleral veins (a pressure decrease of 55 mm Hg) and the low resistance between the episcleral veins and the orbital sinus (a pressure decrease of 6.5 mm Hg), the influence of OVP should be relatively large. If so, variations in OVP have implications for aqueous dynamics, because the EVP is part of the pressure gradient that forces aqueous through the trabecular meshwork and so contributes to IOP homeostasis.

**OVP and Blood Flow**

The OVP is also the pressure that impedes blood efflux from the eye. However, because the eye acts as a Starling resistor and the intraocular venous pressure slightly exceeds the IOP when the IOP is varied over a wide range, the IOP has traditionally served as the effective venous pressure in ocular blood flow studies. The lack of a convenient method to measure OVP also made equating IOP with OVP the only option for estimating the ocular perfusion pressure gradient.

The ciliary and choroidal pressure-flow relations in Figures 6A and 6B are shown, with only the MAP used as the perfusion pressure, so that the corresponding vascular resistances could be plotted on the same scale (Figs. 6C, 6D). When plotted
using the traditional MAP minus IOP perfusion pressure gradient (data not shown), both pressure-flow relationships are similar to previous studies of this preparation.\textsuperscript{10,15} The analysis of the resistance responses to MAP, using the three possible perfusion pressure gradients, indicates qualitatively similar conclusions for MAP — IOP (R1) and MAP — OVP (R2)—that is, both R1 and R2 show resistance changes that promote preservation of blood flow, despite the change in MAP, except at low MAP levels when the vessels presumably start collapsing. However, the greater magnitude of the MAP — OVP gradient makes R2 exceed R1 over the entire range of MAP, which could be an important quantitative difference for some experimental designs (e.g., pressure-flow studies with vasoactive agents in which the slope or shape of the OVP-IOP relationship is altered).

In contrast to the responses of R1 and R2 to MAP, R3 is unaffected by MAP, except at low MAP levels. The IOP — OVP pressure gradient for R3 is the pressure decline from the intraocular to the extraocular veins, and the absence of R3 response over the physiological range of MAP in both circulations is consistent with their expected passive behavior.

**CONCLUSIONS**

The present study demonstrates that the orbital venous sinus of the rabbit can be cannulated through the PSF to obtain continuous measurements of OVP. The results also show that OVP is not static, and that OVP may play a significant role in IOP homeostasis and ocular hemodynamics.

**Acknowledgments**

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**References**


