The Correlation between Cerebrospinal Fluid Pressure and Retrolaminar Tissue Pressure

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Purpose. To measure the effects of cerebrospinal fluid pressure (CSFp) on retrolaminar tissue pressure (RLTp) and the translaminar pressure gradient (TLPG), particularly at low CSFp, which is the normal situation in erect posture.

Methods. Micropipettes coupled to a servonull pressure system were passed into eyes of anesthetized dogs to the optic disc and advanced in steps through the lamina cribrosa to the optic nerve subarachnoid space (ONSAS), while pressure measurements were taken. Cerebrospinal fluid pressure and intraocular pressure (IOP) were monitored and controlled. The TLPG was measured at varying IOPs and CSFps. The RLTp and ONSAS pressure (ONSASp) were measured at varying CSFps. In separate experiments, the optic nerve dura was incised, and pressure measurements were taken across the pia mater.

Results. The TLPG was strongly correlated to the difference between IOP and CSFp ($r = 0.93$; $n = 18$) when CSFp was more than zero. Mean RLTp was 3.7 ± 0.2 mm Hg (SEM; $n = 15$) when CSFp was 0 mm Hg. The ONSASp and RLTp were largely dependent on the presence of CSFp higher than break point pressures of −0.5 mm Hg and 1.33 mm Hg, respectively. However, below these break points, RLTp (slope 0.07) and ONSASp (slope 0.18) were little influenced by CSFp. Separate measurements across the pia mater revealed that 95% of the pressure drop occurred within 100 μm of the pial surface.

Conclusions. The TLPG and RLTp are dependent on CSFp when CSFp is more than −0.5 mm Hg. Below this level, there is no hydrostatic continuity between the intracranial and optic nerve subarachnoid space. In this range, RLTp is stable and is little influenced by CSFp changes. (*Invest Ophthalmol Vis Sci.* 1998;39:1419-1428)

The optic disc is the portion of the optic nerve that lies between two pressure compartments, the eye and the subarachnoid space. Pressure in the subarachnoid space largely determines retrolaminar tissue pressure (RLTp). The difference between the latter and the eye occurs across the lamina cribrosa.[1] The optic disc deforms posteriorly in glaucoma and anteriorly in papilledema; both diseases are associated with loss of ganglion cell axons. In glaucoma, which is the second most common cause of blindness in the developed world, elevated intraocular pressure (IOP) is the most potent known risk factor.[2] In this disease, evidence of rapid orthograde and retrograde axonal transport blockage[3] and axonal interruption[4] occur at the level of the lamina cribrosa. It is well known that elevated intracranial cerebrospinal fluid pressure (CSFp) causes optic disc swelling in papilledema. However, it is unclear to what extent CSFp influences the tissue pressure within regions of the anterior optic nerve.

Pressure gradients across axons are known to alter axonal transport. Peripheral nerves can tolerate absolute pressure increases to 3800 mm Hg,[5] whereas small pressure differences of 30 mm Hg, with pressure gradients of 4.5 mm Hg/100 μm along axons, can significantly reduce rapid orthograde axonal transport.[6] The dog translaminar pressure gradient (TLPG) is 3.08 mm Hg/100 μm when CSFp is 0 mm Hg and IOP is 20 mm Hg.[1] There is fluid continuity between the intracranial cerebral spinal fluid (CSF) and optic nerve subarachnoid space (ONSAS) when CSFp is more than 0 mm Hg, and retrolaminar tissue pressure (RLTp) is significantly higher than CSFp and is dependent on CSFp when CSFp is more than 0 mm Hg.[1] Thus, it is likely that the TLPG is determined by IOP and CSFp. The pia mater connective tissue separates the ONSAS from the optic nerve neural tissue and so probably supports the pressure difference between these two compartments.

It is known that 95% of humans in the erect posture have a CSFp at eye level of 0 mm Hg to −10 mm Hg.[7] However, whether CSF pressures less than 0 mm Hg influences optic nerve subarachnoid space pressure (ONSASp) or RLTp is unknown. Previous investigators measuring optic nerve tissue pressure have used passive transducers connected to 300-μm diameter needles[8,9] or 25-μm diameter micropipettes.[1] The problems with these techniques include slow response times and a tendency toward blockage of the needle or micropipette

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tip, which makes it impossible to measure an increase in tissue pressure.

Servonull counterpressure systems[10] use a sensing system and a pump to oppose the fluid pressure external to the micropipette tip while measuring pressure within the system. They have a rapid response time of 20 Hz and virtually zero compliance and fluid shifts across the tip of several nanoliters only.[11] They have been used to measure microvascular pressures in various vascular beds[10,12] and to measure neural tissue pressure.[13,14]

The desire to determine the RLTp and TLPG across a broad range of CSFps and the ability to take tissue pressure measurements with a servonull counter pressure system led us to perform the following experiments. We were particularly interested in determining the correlation between the TLPG and the pressure difference across the lamina cribrosa. We wanted to know whether the TLPG was accentuated at high pressure differences, which would suggest the occurrence of lamina cribrosa compression. Also, we wanted to determine the response of RLTp and ONSASp to varying CSFps, particularly at low CSFp, and we wanted to measure the pressure gradient across the pia mater.

Methods

Animal Preparation

Twenty-four mixed-breed dogs were used in the procedures in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The animal setup has been described previously,[1] with the following differences: The animals were anesthetized by halothane inhalation for induction only and then by a continuous intravenous infusion of a 50:50 mixture of 60 mg/ml pentobarbital and 50 mg/ml thiopental, administered while animals were ventilated with 80% nitrogen and 20% oxygen. The animal was suspended prone with its head held in a maxilla bite clamp and a second magnetic stand holding its head in an ear clamp. A second cannula, connected to a hydrostatic reservoir filled with Krebs solution, was inserted into the lateral ventricle. The CSF and anterior chamber were connected to fluid reservoirs in an open-stopcock manner, which allowed control of CSFp and IOP.

Aortic blood pressure was monitored at heart level, and blood gas sampling was performed by a cannula inserted in the femoral artery. Cerebrospinal fluid pressure and IOP were monitored by second cannulae in the lateral ventricle and anterior chamber connected to pressure transducers held at eye level. All pressure transducers were connected through conditioning modules (Analog Devices, Norwood, MA) to a chart recorder (LR8100; Yokogawa, Tokyo, Japan). The eye was sutured to a fixed eye ring, the superior and lateral rectus muscles were cut, and the sclerostomy was made just posterior to the insertion of the lateral rectus.

Micropipettes

Micropipettes (Industrial Science Associates, Richmond, NY) were pulled from borosilicate glass (outer diameter, 1 mm; inner diameter, 0.5 mm), bevelled on three facets to 25° with a tip size of 2.5 μm (inner diameter), and hydraulically connected to a servonulling counter pressure system (5A; Instrumentation for Physiology and Medicine, San Diego, CA) designed using principles described initially by Wiederhielm et al.[10] The pipettes were filled with 2 M sodium chloride, and the resultant impedance was 2 MΩ to 7 MΩ.

Translaminar Pressure Gradient

Before all experiments, excluding the transpial measurements, calibration of the transducer connected to the micropipette was checked with the micropipette tip located in the vitreous, using controlled IOP as the standard. Tissue pressure measurements were accepted according to the following criteria: high-frequency oscillations were visible on the servonulling machine's video monitor; measurement did not vary with changes in gain, indicating fluid continuity at the pipette tip; and vitreous pressures, measured with the micropipette before and after vessel puncture, were within 1 mm Hg of the IOP recorded from the anterior chamber cannula.

In these 17 animals, CSFp was first set at the minimum stable pressure higher than zero. Intraocular pressure was set at varying levels to determine the difference between IOP and CSFp. The micropipette tip was manipulated across the central third of the optic disc or superior to this region along the vertical meridian (superior), then slowly advanced (Fig. 1). Visual judgment of the micropipette tip position and the development of spike artifacts was used to determine the position of the inner limiting membrane. Spike artifacts are produced when tissue temporarily occludes the micropipette tip, inducing counter pressurization of the micropipette by the servonull system. All subsequent depth measurements were taken relative to this position. The pressure at zero depth was the IOP.

The micropipette was then advanced forward in steps of between 50 μm and 100 μm, and measurements were taken when the standard acceptance criteria were met and oscillation of pressure could be observed as it related to respiration. The pressure usually began to decrease within the first 600 μm of penetration. We assumed that the micropipette tip was in the retrolaminar tissue if the pressure reached a minimum and remained stable (within $\pm$1 mm Hg) when the micropipette was advanced a distance of 100 μm.

Cerebrospinal Fluid Pressure's Influence on Retrolaminar Tissue Pressure

In 11 animals, CSFp was reduced in steps of approximately 3 mm Hg by lowering the level of the Krebs solution reservoir. Measurements of RLTp were taken at each step. The CSFp was then elevated above baseline to between 10 mm Hg and 20 mm Hg in steps of 3 mm Hg, and measurements were taken.

Optic Nerve Subarachnoid Space Pressure Measurements

In seven animals, the micropipettes were advanced farther, in the manner previously described,[5] until there was a sudden reduction in tissue pressure at a depth consistent with the micropipette's insertion in the optic nerve subarachnoid space (ONSAS). We presumed that the ONSAS had been entered when the recording was uniform without spike artifacts and remained stable as the micropipette was advanced 100 μm. Additionally, in this situation, variations in bridge balance, forcing larger fluid movement across the micropipette tip, had no effect on the pressure measurement. Varying the bridge balance with the pipette in tissue causes large recording artifacts and a change in measurement baseline as a result of attempting fluid movement in the small extracellular space.

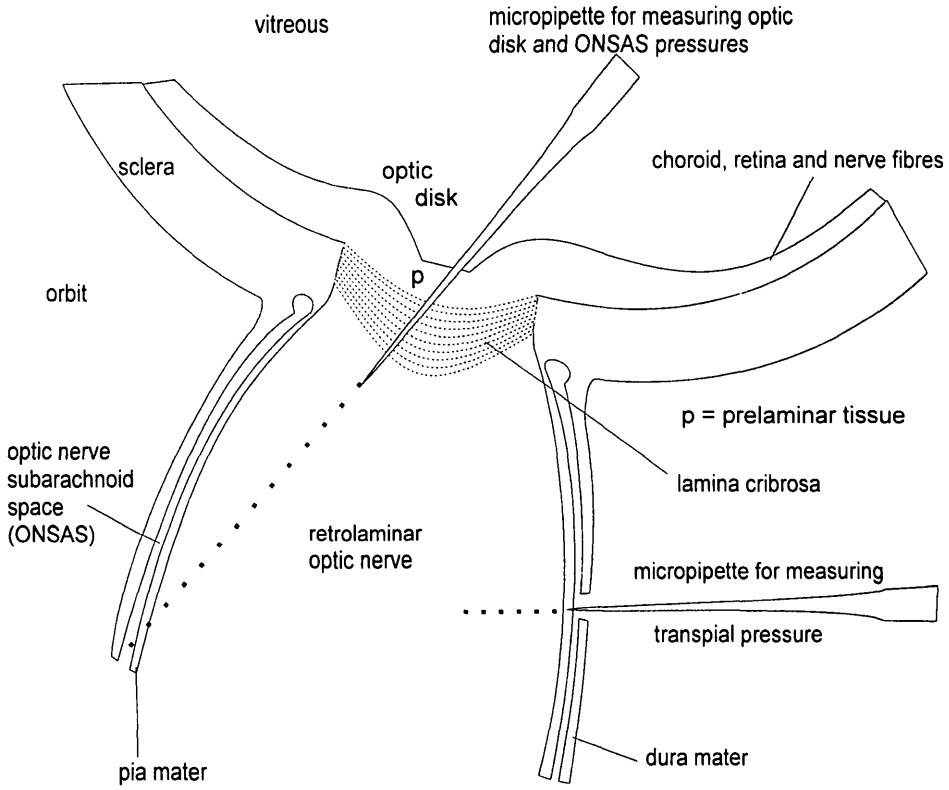

Figure 1. Schematic diagram showing optic disc and retrolaminar region. The typical paths of two micropipettes are shown. One measures the translaminar pressure gradient, retrolaminar tissue pressure, and optic nerve subarachnoid space pressure, and the other measures the pressure gradient across the pia mater.

Cerebrospinal fluid pressure was reduced by lowering the level of the Krebs solution reservoir to provide a minimum pressure. The CSFp was then increased stepwise to between 10 mm Hg and 20 mm Hg. Pressure measurements were taken at each step when the previously described criteria were met.

Transpial Pressure Measurement

Seven dogs were used in these experiments. The same animal positioning and anesthetic methods were used, with a single cannula in the lateral ventricle inserted to allow monitoring of CSFp. Similarly, the eye was sutured to an eye ring held in a magnetic clamp. All rectus muscles were cut, allowing the eye to be held more anteriorly. A lateral orbitotomy was performed and then blunt dissection of orbital fat down to the optic nerve. Extreme care was taken in dissecting down to the dura and incising it; however, small, short posterior ciliary artery branches in this region are numerous, and it was difficult to avoid minor hemorrhaging. To gain sufficient exposure to introduce the micropipette into the dural incision, the eye was held anteriorly under some tension. A 2-mm incision was made in the dura 4 mm posterior to the globe and in line with the optic nerve. Clear CSF was universally observed to appear in the incision, then the incision was coated in 2% methylcellulose fluid. The servonull pressure transducer was calibrated with the micropipette in a pressure chamber. The micropipette was then transferred to the arc manipulator and maneuvered to position its tip within the methylcellulose overlying the dural incision. This was exposed to atmosphere at 0 mm Hg pressure. Further measurements with the micropipette were conducted only if zero pressure output was recorded from the servonull pressure transducer and the acceptance criteria were met.

The micropipette was advanced until the surface of the pia mater was reached, after identification in the same manner as the optic disc surface. This was defined as zero depth, and the micropipette was advanced farther in 50- to 100-μm steps to a depth of 400 μm. Measurements were accepted only when the pressure measured when the micropipette was withdrawn back into the methylcellulose was 0.0 $\pm$ 1 mm Hg.

Histologic Analysis

All eyes from animals in which optic disc penetration had been performed were removed and immersion-fixed in 10% formalin, after a 1-ml formalin injection into the vitreous cavity to replace the vitreous. The eyes were sectioned along the anteroposterior axis that intersected the sclerostomy, avoiding the optic nerve. The angle of penetration was measured between lines from the sclerostomy to the optic disc center and the disc surface.

In 13 animals in which the translaminar pressure gradient had been measured, the optic disc with surrounding sclera was removed and embedded in paraffin. Sagittal serial sections were made at 100-μm intervals and stained with hematoxylin and eosin. The most central section, indicated by the widest lamina cribrosa, was used for measurements. The central axial thickness of the prelaminar tissue and lamina cribrosa was measured. The thickness of the pia mater was measured 4 mm from the termination of the ONSAS.

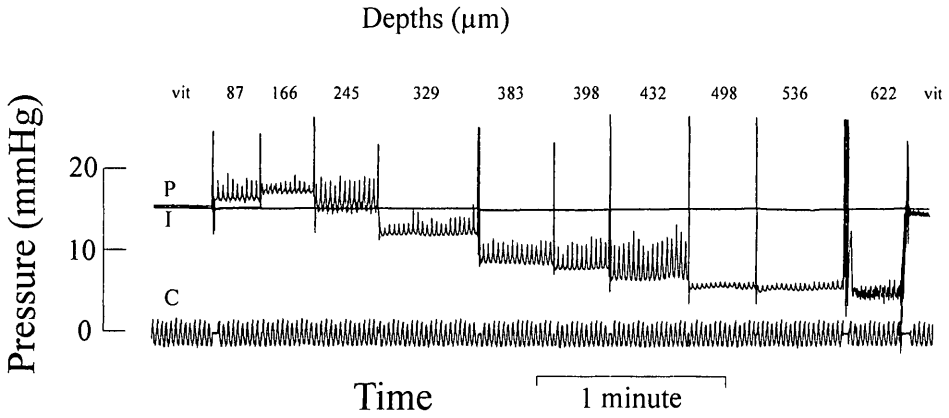

FIGURE 2. Typical recording of tissue pressure (P) at different depths into the dog optic nerve from the anterior surface of the optic disc. Intraocular pressure (I) was 15 mm Hg, cerebrospinal fluid pressure (C) was 0 mm Hg, and mean aortic blood pressure was 94 mm Hg throughout the recording. A pressure spike artifact was produced as the micropipette tip advanced from vitreous (vit) into the disc surface and as it advanced to successive depths. Throughout the recording, the pressure measurement varied in relation to respiration and the cerebrospinal fluid pressure pulse. There was a slight increase in tissue pressure at depths of 87 μm and 166 μm. A retrolaminar tissue pressure of 4 mm Hg was reached at 536 μm.

All results are expressed ± SE unless otherwise stated. Ninety-five percent confidence intervals (95% CI) were calculated using Student's two tailed *t*-test. Nonlinear curve fitting was done using commercial software (Sigmaplot version 4.1; Jandel scientific, San Rafael, CA) by determination of least mean squares. All depth measurements were corrected for angle of penetration.

RESULTS

Translaminar Pressures

A typical recording of tissue pressure at different depths in the canine optic nerve is shown, along with IOP and CSFp, in Figure 2. Note the pressure spike artifact as the micropipette tip was advanced into the most anterior tissue (inner limiting membrane) and as it was advanced farther posteriorly. Throughout the recording, the pressure measurement varied in relation to respiration and the CSFp pulse. There was a slight increase in tissue pressure at depths of 87 μm and 166 μm. A retrolaminar tissue pressure of 4 mm Hg was reached at 536 μm.

Figure 3 shows three typical pressure profiles across the optic disc from one animal experiment in which CSFp remained at 0 mm Hg and IOP was varied to 15 mm Hg, 26 mm Hg, and 35 mm Hg. Pressure measurements are plotted against depth. Pressure at 0 μm depth is IOP. It can be seen that in two penetrations, the pressure appeared to increase slightly in the first 200 μm below the optic disc surface. The pressure in each case decreased across a 400-μm range of depths to 5 mm Hg. Figure 4 shows six typical profiles from six animals at varying IOP and CSFp. The depth across which tissue pressure approximated IOP varied from 100 μm to 300 μm. Again, the tissue pressure appeared to increase initially in some animals, then to decrease through a depth range of 350 μm to 500 μm. The minimum tissue pressure ranged from 4 mm Hg when CSFp was 0 mm Hg to 15 mm Hg when CSFp was 10 mm Hg. This minimum pressure did not change appreciably with further advancement of the micropipette. The curves in these two figures resemble sigmoid patterns.

The mean of the maximum increase in tissue pressure compared with IOP in the first 400 μm in 17 animals was 1.2 ± 0.19 mm Hg. This maximum tissue pressure was significantly different from IOP (median IOP, 22 mm Hg; median tissue pressure, 24 mm Hg; $n = 38$; $P < 0.001$; Wilcoxon signed rank test).

Based on previous experience, we assumed that the mean distance between the last tissue pressure recording equal to IOP and the first pressure recording equal to RLTp corresponded to pressures in the lamina cribrosa.[1] Data in this range from each profile were used to calculate the translaminar pressure gradient (TLPG). Measurements were included for analysis only when there were at least five valid pressure recordings in this range. We assumed that the TLPG was equal to the slope for individual curves calculated by linear regression. Only the first valid TLPG measurement from each animal was included in the analysis. The mean thickness of the lamina cribrosa by these measurements was 480 ± 38 μm.

The TLPG versus the difference between IOP and CSFp measured in the first penetrations in each of 17 animals, in a pressure difference range of 14 mm Hg to 45 mm Hg, with an IOP range of 15 mm Hg to 52 mm Hg and mean CSFp of 4 ± 5 mm Hg (SD; range, 0–14 mm Hg) is plotted in Figure 5. Despite this variation in CSFp and IOP, there was a strong linear correlation ($r = 0.93$, $P < 0.01$). By least mean squares linear regression analysis, the slope was 0.23 ± 0.05 (95% CI) and the *y*-intercept was −1.2 ± 1.4 (95% CI). This linear correlation across a range of IOP-CSFp suggests that the thickness of the lamina cribrosa remained constant.

The data were further analyzed to determine whether there was other evidence for a change in lamina cribrosa thickness with increasing pressure differences. The first optic cup profile from 12 animals and the first superior disc profile

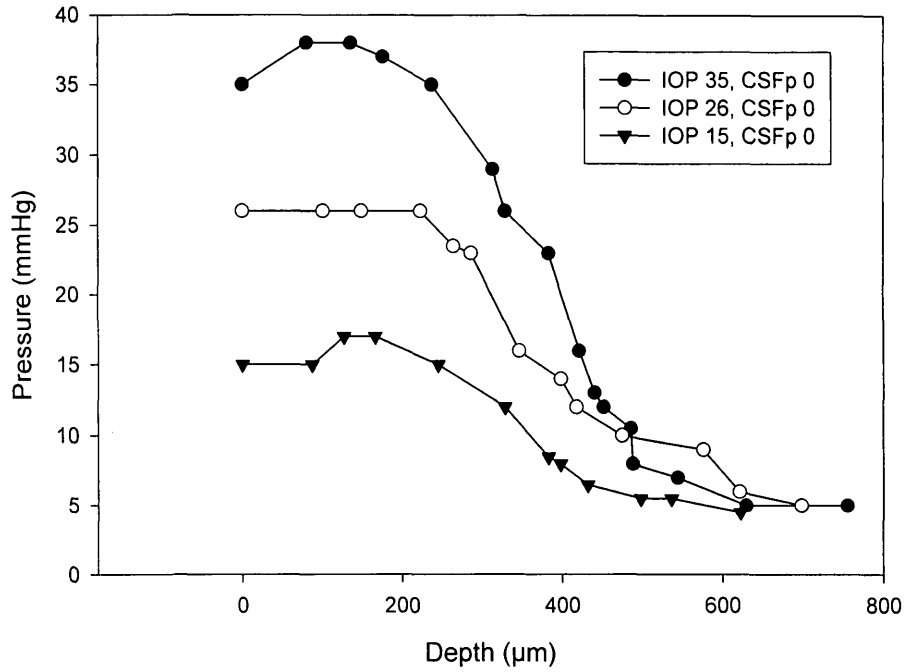

Figure 3. Three typical pressure profiles across the optic disc from one animal experiment in which cerebrospinal fluid pressure (CSFp) was 0 mm Hg and intraocular pressure (IOP) was 15 mm Hg, 26 mm Hg, and 35 mm Hg. These pressure-versus-depth profiles are sigmoid shaped. Pressure at 0 μm depth is IOP. In two penetrations, the pressure appeared to increase slightly in the first 200 μm of tissue below the optic disc surface. The pressure in each case decreased across a 400-μm range to 5 mm Hg. The curves are sigmoid shaped.

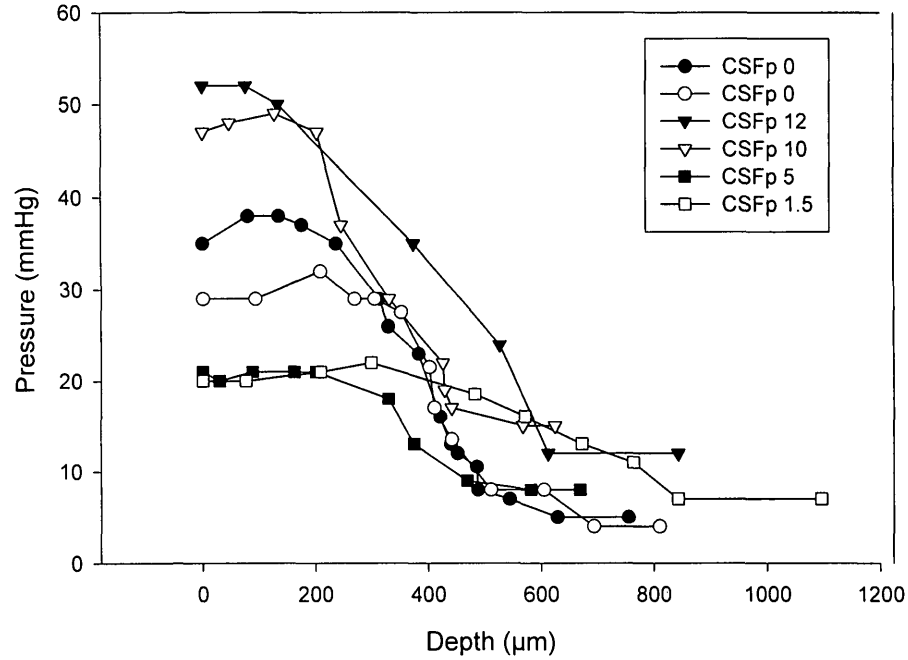

Figure 4. Six typical profiles from six animals, in which pressure is plotted against depth. The depth across which tissue pressure approximated intraocular pressure varied from 100 μm to 300 μm. The tissue pressure rose initially in some animals, then decreased in the next 350 μm to 500 μm. The minimum tissue pressure ranged from 4 mm Hg when cerebrospinal fluid pressure (CSFp) was 0 mm Hg, to 15 mm Hg when CSFp was 10 mm Hg. This minimum pressure did not change appreciably with further advancement of the micropipette.

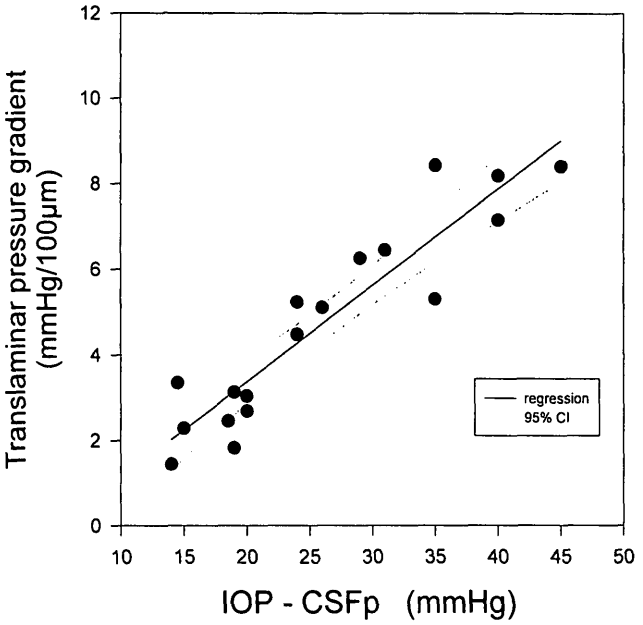

FIGURE 5. Translaminar pressure gradient versus the difference between intraocular pressure (IOP) and cerebrospinal fluid pressure (CSFp) from the first penetrations of 18 animals. The regression line and 95% confidence interval of the regression line are plotted.

from 5 animals were compared. We assumed that a rate of decrease in pressure would be inversely proportional to the involved tissue thickness. It was impossible to determine the exact anatomic position corresponding to particular depth values in any one pressure-depth profile. These profiles resemble sigmoid functions, which we used as our theoretical model for normalizing and comparing the profiles. We did not attempt to model the small increase in prelaminar tissue pressure, but we assumed that pressure decreased from a maximum asymptote (IOP) to a minimum asymptote (RLTp). Normalized pressure (NP) was defined as the the percentage between these two parameters.

$$NP = \{(P - RLT_p)/(IOP - RLT_p)\} \times 100 \quad (1)$$

Best fit for each set of normalized pressure versus depth (D) data were sought for the following two-parameter sigmoid curve function

$$NP = 100/\{1 + e\text{^}(s(D - d_{50}))\}, \text{ where } e \text{ is natural } e \quad (2)$$

where e is natural e and parameter d_{50} is the depth corresponding to 50% normalized pressure. We subtracted d_{50} from each depth value, so that normalized depth was correlated with the 50% normalized pressure position. Normalized pressure and normalized depth data from central disc penetration were grouped together and compared with data from superior disc penetrations (Fig. 6A). A two-parameter sigmoid curve fit was again sought for the grouped data. The slope parameter s was 0.018 ± 0.0014 (SEM; n = 58) for the superior disc, not significantly different ($0.1 < P < 0.2$) from that of the disc (0.0157 ± 0.00082; n = 131). The disc penetration results were divided into two groups (Fig. 6B): one in which the difference between IOP and RLTp was 20 mm Hg or less (mean, 14.5 mm Hg in six animals) and another in which the difference was more than 20 mm Hg (mean, 30.3 mm Hg in six animals). There was no significant difference ($P > 0.05$) in parameter s was when comparing the group with a high difference in pressures (s = 0.0162 ± 0.001; n = 73) with the group with a low difference in pressures (s = 0.01496 ± 0.0013; n = 58).

A typical recording of RLTp as CSFp is altered is shown in Figure 7. The RLTp (5 mm Hg) does not vary when CSFp is lowered from 0 mm Hg to −5 mm Hg, and then increased to +2 mm Hg. However, it moves with CSFp when CSFp is increased above 5 mm Hg.

One hundred five measurements of RLTp versus CSFp were taken from 10 animals that had a range of CSFp from −12 mm Hg to 22 mm Hg. Additionally, 83 measurements of ONSASp versus CSFp were taken from seven animals that had a range of −12 mm Hg to 29 mm Hg. The values of ONSASp and RLTp were grouped at 1- to 3-mm Hg CSFp intervals and the mean and SE were calculated (Fig. 8). There appeared to be two linear correlations for each data set describing RLTp or ONSASp versus CSFp. One had a shallow slope (m_1) below a certain break point (c), and the other had a higher slope (m_2) above the break point. The data were fitted to the following function, which describes a line with two separate linear slopes

$$\text{when CSFp} \leq c,\ y = m_1 \times \text{CSFp} + b \quad (3)$$

$$\text{when CSFp} > c,\ y = m_2 \times \text{CSFp} + (m_1 - m_2) \times c + b \quad (4)$$

The parameters b, c, m_1, and m_2 were not fixed during the curve fit. Table 1 is a display of these calculated parameters, which were compared using Student's t-test. Slope m_1 was significantly different from slope m_2 ($P < 0.001$) for RLTp and ONSASp. Slope m_1 for RLTp was not significantly different from zero or from ONSASp slope m_1 ($P > 0.2$); but for ONSASp, slope m_1 was significantly different from zero ($P < 0.05$). Slope m_2 for RLTp was significantly different from that of ONSASp and 1 ($P < 0.001$). The CSFp break point for ONSASp (−0.52 mm Hg) was significantly less than that for RLTp (1.33 mm Hg; $P < 0.025$).

Measurements of RLTp in 15 animals when CSFp was close to the ONSASp break point demonstrated a mean RLTp of 3.7 ± 0.2 mm Hg at mean CSFp of 0.6 ± 0.3 mm Hg and IOP of 23 ± 2.4 mm Hg. The mean ONSASp when CSFp was −4 mm Hg in five animals was −1 ± 1 mm Hg (range, +1 to −4 mm Hg).

The pressure results at varying depths from the pia mater surface in the seven animals in which the transpial pressure was measured are shown in Figure 9. The mean CSFp before lateral canthotomy was 8.9 ± 2.8 mm Hg. The mean tissue pressure at a depth of 300 μm was 7.7 ± 0.7 mm Hg. Intracranial CSFp at the time of the recording is included in the legend. It appears that most of the pressure change from atmospheric to optic nerve tissue occurs in the first 50 μm to 100 μm of tissue. The mean ratio of pressure at a depth of 100 μm to pressure at 300 μm was 0.95 ± 0.2 (n = 7) with 95% confidence interval from 0.74 to 1.16, calculated using Student's two tailed t-test. The pia mater width measured at 4 mm from the sclera from eight fixed eyes examined histologically was 25 ± 2 μm.

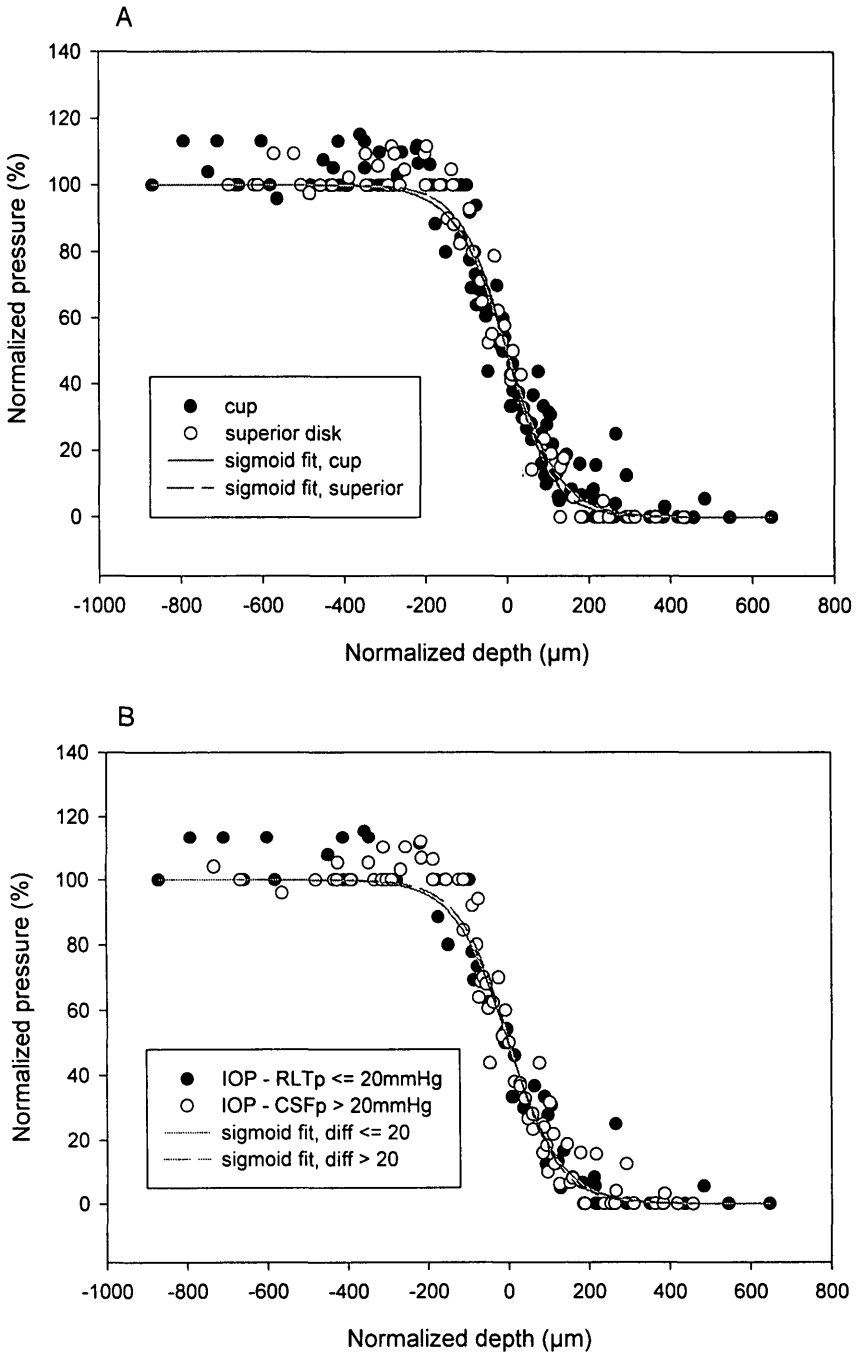

Figure 6. Normalized pressure versus normalized depth. The line of best fit to a two-parameter sigmoid function is shown for each group. Measurements (**A**) from the central disc in 12 animals and the superior optic disc in 6 animals are compared. The cup penetration results were divided into two groups (**B**): one in which the difference between intraocular pressure (IOP) and retrolaminar tissue pressure (RLTp) was 20 mm Hg or less (mean, 14.5 mm Hg in six animals) and another in which the difference was more than 20 mm Hg (mean, 30.3 mm Hg in six animals). CSFp, cerebrospinal fluid pressure.

Histologic examination of the eye that underwent surgery revealed an accumulation of leukocytes within the prelaminar tissue in some eyes. No other evidence of optic nerve trauma was seen in the histologic sections. Measurement of the laminar thickness was difficult, because there is no definite boundary between the posterior laminar beams and the retrolaminar tissue. The laminar beams merge into the pial septa, and myelination of the axons continues through the lamina region. The posterior limit was defined as where the connective tissue orientation changed from mainly transverse to longitudinal. Our measurements from these stained sections revealed a mean central prelaminar thickness of 245 ± 19 μm, and a mean laminar thickness of 531 ± 29 μm (n = 13). The mean distance from disc surface to the midpoint of the lamina cribrosa was 483 ± 24 μm, which was not significantly different from the mean d_{50} of 382 ± 75 μm ($P > 0.2$).

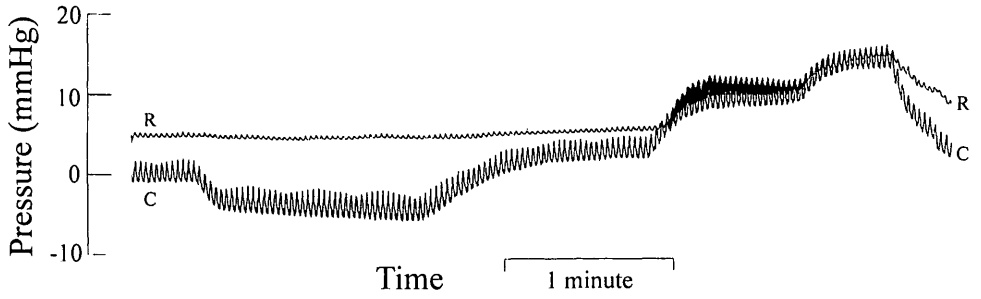

FIGURE 7. A typical recording of retrolaminar tissue pressure (R) as cerebrospinal fluid pressure (C) is altered. Retrolaminar tissue pressure (5 mm Hg) did not vary when cerebrospinal fluid pressure was reduced from 0 mm Hg to −5 mm Hg and then increased to 2 mm Hg. However, it moved with cerebrospinal fluid pressure when cerebrospinal fluid pressure was increased above 5 mm Hg. Intraocular pressure was 25 mm Hg, and mean aortic blood pressure was 104 mm Hg throughout the recording.

DISCUSSION

These results demonstrate that the pressure distribution across the anterior optic nerve is sigmoidal and that the pressure decrease occurs across the lamina cribrosa. The magnitude of this pressure decrease, or TLPG, is influenced by CSFp when CSFp is higher than 0 mm Hg. Additionally, the intracranial CSF space is connected hydraulically to the ONSAS and exerts a major influence on RLTp when CSFp is higher than −0.5 ± 0.33 mm Hg. However, when CSFp is less than −0.5 mm Hg, fluid continuity between the ONSAS and intracranial CSF is lost.

At variance from the sigmoidal pressure distribution pattern was the observation that the optic nerve tissue pressure rose an average 1.2 ± 0.19 mm Hg higher than IOP within the prelaminar region. There is evidence of physiologic obstruction of orthograde axonal transport at the level of the lamina cribrosa in a variety of mammalian species.[15] This axonal congestion may increase cytoplasmic pressure toward the cell body in the prelaminar region.

These results demonstrate that there is a strong linear correlation between TLPG and the difference between IOP and CSFp, when CSFp is 0 mm Hg or higher (Fig. 5). The TLPG in a normal situation with IOP of 15 mm Hg and CSFp of 0 mm Hg was 2.3 mm Hg/100 μm. The presence of the TLPG causes the pressure to decrease across the lamina cribrosa in the orthograde direction. Paradoxically, there is some orthograde axonal transport obstruction within the lamina cribrosa in the normal situation,[15] which is exacerbated when IOP is elevated.[3] The reason for this apparent paradoxical effect is unknown. Mechanical forces applied to nerves are known to cause ischemia[16] and cytoskeletal disruption[17] in association with axonal transport inhibition. Within the lamina cribrosa, the axonal cytoskeleton is subject to the pressure gradient, which may be increased in regions adjacent to the lamina cribrosa connective tissue beams. This may explain why maximum axonal transport block is seen at these sites.

There was no significant difference in the sigmoid functions fitted to the normalized pressure and depth data (Fig. 6B) when the pressure difference across the lamina cribrosa (IOP − RLTp) was high or low. Therefore, the pressure decreased, and there were similar tissue thicknesses in both situations, which implies that no significant lamina compression occurs at high pressure differences. There would have to be tissue compression to 82% of original thickness for this method of analysis, with the same SEs and number of data points, to demonstrate significant compression ($P < 0.05$). If there had been significant compression of the lamina cribrosa at high pressure differences, the TLPG may have been skewed upward at high pressure differences (Fig. 5), which was not observed. Our results are in agreement with those of Yan et al.,[18] whose measurements also suggest that there is no significant acute compression of the lamina cribrosa at elevated IOPs.

The mean retrolaminar tissue pressure of 3.7 ± 0.2 mm Hg that we measured when CSFp was 0 mm Hg was less than the previously measured RLTp of 7 mm Hg in the dog[1] and cat.[8,9] These results may be more representative of the the RLTp in the undisturbed situation because our micropipettes had smaller tips than any used previously and did not produce

TABLE 1. Mean Parameter Results from Curve Fits of RLTp and ONSASp versus CSFp

	Above Break Point			*Below Break Point*						
	m_1	*SEM*$_1$	r_1	m_2	*SEM*$_2$	r_2	b	*SEM*	c	*SEM*
RLTp	0.07	0.06	0.44	0.82	0.02	0.998	2.92	0.32	1.33	0.59
ONSASp	0.18	0.07	0.93	0.99	0.01	0.998	−0.21	0.26	−0.52	0.33

Assumes different slopes below (m_1) and above (m_2) break point (c). Y-intercept is b. Correlation coefficients r_1 and r_2 are calculated for first and second portions of the curves, respectively.

CSFp, cerebrospinal fluid pressure; ONSASp, optic nerve subarachnoid space pressure; RLTp, retrolaminar tissue pressure; SEM, standard error of the mean.

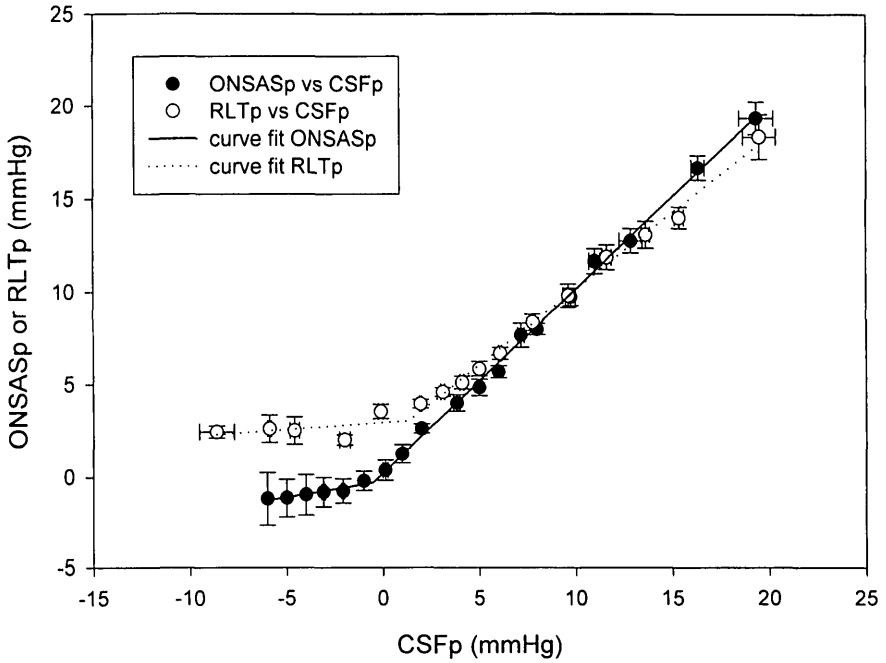

FIGURE 8. Averaged measurements of optic nerve subarachnoid space pressure (ONSASp) versus cerebrospinal fluid pressure (CSFp) and retrolaminar tissue pressure (RLTp) versus CSFp; error bars are equivalent to the SE. At low CSFp, a linear correlation exists, and there is a small slope between CSFp and the two parameters. As CSFp increases above a certain point, a separate linear correlation exists between the two parameters, and there is a larger slope. A line of best fit with two slopes and a break point junction was plotted for each parameter.

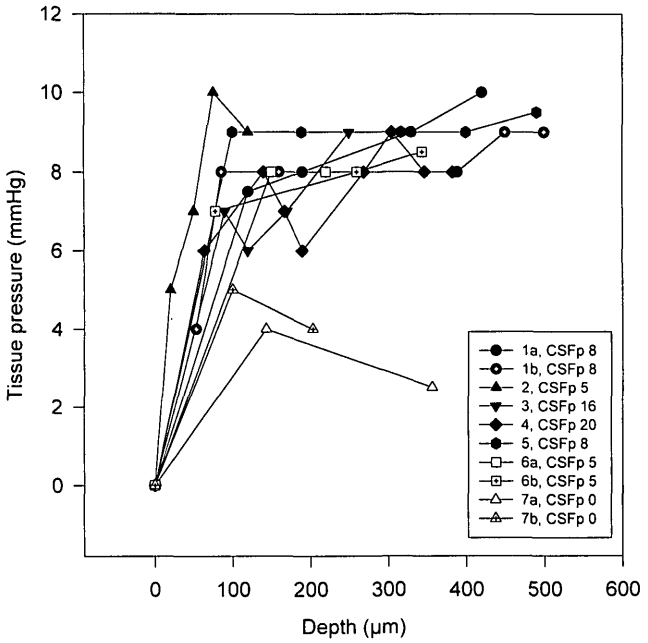

FIGURE 9. The pressure measurements at varying depths from the pia mater surface as the micropipette was advanced into the optic nerve 4 mm behind the globe in seven animals. Intracranial cerebrospinal fluid pressure at the time of the recording is included in the legend. CSFp, cerebrospinal fluid pressure.

observable tissue disruption, unlike previous experiments that included histologic examination.[1,8]

We have clearly demonstrated that RLTp is largely determined by CSFp when CSFp is greater than 1.3 ± 0.6 mm Hg (Fig. 8). Also, we have confirmed our earlier observations that when CSFp is greater than −0.5 ± 0.3 mm Hg, optic nerve subarachnoid space pressure is equivalent to CSFp.

However, these correlations change dramatically when the CSFp is low. When CSFp was lower than 1.3 mm Hg, RLTp appeared to reach a stable level so that when CSFp decreased farther, the change in RLTp was much less than the change in CSFp. The mean slope of RLTp versus that of CSFp in this range was not significantly different from zero; RLTp may therefore reach a floor level below which it will not decrease. Similarly, when CSFp was less than −0.3 mm Hg, the ONSASp varied little within the same animal. This implies that hydrostatic continuity between the ONSAS and intracranial CSF is lost when CSFp decreases below −0.5 mm Hg in the dog.

We postulate that when CSFp is higher than orbital pressure, CSF will fill the ONSAS and exert a dominant pressure influence on the retrolaminar optic nerve. However, when the CSFp is less than orbital pressure, orbital pressure will compress the ONSAS and exert a dominant pressure influence on the retrolaminar optic nerve. This is analogous to the situation in the spinal cord of the dog, where spinal cord CSFp cannot be lower than the surrounding epidural space pressure.[19] It is likely that orbital pressure will determine the minimum pressure acting on the pia mater and retrolaminar optic nerve.

Orbital pressure in supine humans averages 3 mm Hg[20] when measured by 800-μm diameter needles connected to a passive transducer. No direct measurements of orbital pressure

in the erect posture have been taken. In other regions of the body such as skin[21] and subcutaneous tissue,[22] interstitial pressures are subatmospheric. If ONSASp is equivalent to orbital pressure at low CSFp, then dog orbital pressures may range between 1 and −4 mm Hg.

The retrobulbar optic nerve pressure measurements demonstrate that the pressure gradient between CSF and the optic nerve lies in the first 50 μm to 100 μm tissue. Our histologic measurements indicate that the pia mater is at least 25 μm wide where the pressure measurements were taken. The pia mater comprises mainly collagen, which is capable of bearing stress and therefore a pressure gradient. Additionally, no significant pressure gradient existed deeper within the nerve. So it is likely that the pressure gradient lies largely across the pia mater. The optic nerve tissue pressure was probably elevated artifactually by the tension on the optic nerve exerted when holding the eye anteriorly, by hemorrhages, and by the elevated CSFp before dural incision. This may explain the difference between mean tissue pressure of 7.7 mm Hg in the retrobulbar approach compared with the mean 3.7 mm Hg when measured across the lamina cribrosa.

When CSFp was higher than 1.3 mm Hg, the gradient between RLTp and CSFp was 0.82, significantly less than the gradient between ONSASp and CSFp. Therefore, the difference between RLTp and ONSASp declined as ONSASp increased (Fig. 8). If the optic nerve is incompressible, the pressure difference would be expected to remain constant. We postulate that the optic nerve is compressible or that optic disc movement out of the subarachnoid compartment into the ocular compartment occurs at high CSFp. This effective volume reduction would reduce the tension across the pia mater and thus reduce the transpial pressure difference.

Measurements from rat cortex suggest that there is no transpial pressure difference in the brain.[14] Monkey pia mater surrounding the optic nerve contains more collagen than that surrounding the cortex.[23] If we assume that these observations hold in all mammalian species, then it is possible that optic nerve pia mater may play a role in increasing RLTp above low ONSASp and orbital pressures. Thus, the pia mater may act to damp the effect of reductions in these pressures.

In the human, the same anatomic compartments and correlations exist as in the dog. We can therefore expect similar stability of RLTp at low CSFp. Because RLTp is a key determinant of the TLPG, this would act to keep the TLPG stable in the face of postural changes. A stable, low TLPG may facilitate optimal axonal transport along the optic nerve.

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References

1. Morgan WH, Yu DY, Cooper RL, Alder VA, Cringle SJ, Constable IJ. The influence of cerebrospinal fluid pressure on the lamina cribrosa tissue pressure gradient. *Invest Ophthalmol Vis Sci.* 1995; 36:1163-1172.
2. Sommer A. Intraocular pressure and glaucoma. *Am J Ophthalmol.* 1989;107:186-188.
3. Minckler DS, Bunt AH, Johanson GW. Orthograde and retrograde axoplasmic transport during acute ocular hypertension in the monkey. *Invest Ophthalmol Vis Sci.* 1977;16:426-440.
4. Vrabec F. Glaucomatous cupping of the human optic disk. *Graefes Arch Clin Exp Ophthalmol.* 1976;198:223-234.
5. Ochs S. Energy metabolism and supply of −P to the fast axoplasmic transport mechanism in nerve. *Fed Proc.* 1974;33: 1049-1058.
6. Hahnenberger RW. Inhibition of fast anterograde axoplasmic transport by a pressure barrier. The effect of pressure gradient and maximum pressure. *Acta Physiol Scand.* 1980;109:117-121.
7. Magnaes B. Body position and cerebrospinal fluid pressure, II: clinical studies on orthostatic pressure and the hydrostatic indifferent point. *J Neurosurg.* 1976;44:698-705.
8. Ernest JT, Potts AM. Pathophysiology of the distal portion of the optic nerve 1: tissue pressure relationships. *Am J Ophthalmol.* 1968;66:373-380.
9. Hedges TR, Zaren HA. The relationship of optic nerve tissue pressure to intracranial and systemic arterial pressure. *Am J Ophthalmol.* 1973;75:90-98.
10. Wiederhielm CA, Woodbury JW, Kirk S, Rushemer RF. Pulsatile pressures in the microcirculation of frog's mesentery. *Am J Physiol.* 1964;207:173-176.
11. Intaglietta M. Pressure measurements in the microcirculation with active and passive transducers. *Microvasc Res.* 1973;5:317-323.
12. Morgan WH, Yu DY, Cooper RL, Alder VA, Cringle SJ, Constable IJ. Retinal artery and vein pressures in the dog and their relationship to aortic, intraocular, and cerebrospinal fluid pressure. *Microvasc Res.* 1997;53:211-221.
13. Myers RR, Powell HC, Costello ML, Lampert PW, Zweifach BW. Endoneural fluid pressure: direct measurement with micropipettes. *Brain Res.* 1978;148:510-515.
14. Wiig H, Reed RK. Rat brain interstitial fluid pressure measured with micropipettes. *Am J Physiol.* 1983;244:H239-H246.
15. Hollander H, Makarov F, Stefani FH, Stone J. Evidence of constriction of optic nerve axons at the lamina cribrosa in the normotensive eye in humans and other mammals. *Ophthalmic Res.* 1995; 27:296-309.
16. Dahlin LB, Rydevik B, Lundborg GL. Pathophysiology of nerve entrapments and nerve compression injuries. In: Hargens AR, ed. *Tissue Nutrition and Viability.* New York: Springer Verlag; 1986: 135-160.
17. Povlishock JT, Christman CW. The pathobiology of traumatically induced axonal injury in animals and humans: a review of current thoughts. *J Neurotrauma.* 1995;12:555-564.
18. Yan DB, Coloma FM, Metheetrarut A, Trope GE, Heathcote JG, Ethier CR. Deformation of the lamina cribrosa by elevated intraocular pressure. *Br J Ophthalmol.* 1994;78:643-648.
19. Bengis RG, Guyton AC. Some pressure and fluid dynamic characteristics of the canine epidural space. *Am J Physiol.* 1977;1:H255-H259.
20. Moller PM. The pressure in the orbit. *Acta Ophthalmol Suppl.* 1955;43:1-100.
21. Wiig H, Noddeland H. Interstitial fluid pressure in human skin measured by micropuncture and wick in needle. *Scand J Clin Lab Invest.* 1983;43:255-260.
22. Guyton AC. A concept of negative interstitial pressure based on pressures in implanted perforated capsules. *Circ Res.* 1963;12: 399-414.
23. Anderson DR. Ultrastructure of meningeal sheaths: normal human and monkey optic nerves. *Arch Ophthalmol.* 1969;82:659-674.