No flow through the vitreous humor: How strong is the evidence?

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\begin{abstract}
When analyzing vitreous drug delivery, or the pharmacological effects of drugs on intraocular pressure, or when interpreting outflow facility measurements, it is generally accepted that the fluid in the vitreous humor is stagnant. It is accepted that for all practical purposes, the aqueous fluid exits the eye via anterior pathways only, and so there is negligible if any posteriorly directed flow of aqueous through the vitreous humor. This assumption is largely based on the interpretation of experimental data from key sources including Maurice (1957), Moseley (1984), Gaul and Brubaker (1986), Maurice (1987) and Araie et al. (1991). However, there is strong independent evidence suggesting there is a substantial fluid flow across the retinal pigment epithelium from key sources including Cantrill and Pederson (1984), Chihara and Nao-i, Tsuboi (1985), Dahrouj et al. (2014), Smith and Gardiner (2017) and Smith et al. (2019). The conflicting evidence creates a conundrum—how can both interpretations be true? This leads us to re-evaluate the evidence. We demonstrate that the data believed to be supporting no aqueous flow through the vitreous are in fact compatible with a significant normal aqueous flow. We identify strong and independent lines of evidence supporting fluid flow across the RPE, including our new outflow model for the eye. On balance it appears the current evidence favors the view that there is normally a significant posteriorly directed aqueous flow. This as-
\end{abstract}

1. Introduction

With few exceptions (Cantrill and Pederson, 1984; Moses, 1987), it is widely believed that the vitreous humor normally has little or no fluid flow through it (Maurice, 1957; Maurice, 1987; Araie and Maurice, 1991; Maurice, 1992; Missel, 2002; Tabibian et al., 2015; del Amo et al., 2017; Soubrane-Daguet and Coscas, 2017), so effectively all the aqueous produced by the ciliary body exits through the anterior pathways of the eye, via the uveoscleral and the trabecular meshwork routes (Goel et al., 2010; Johnson et al., 2017). Consequently, this means most models of solute transport within the vitreous humor are dominated by diffusive transport, and advective transport is assumed to be negligible (Balachandran and Barocas, 2008; Missel, 2012; Lamminsalo et al., 2018; Zhang et al., 2018). This also means that any change in (whole eye) outflow facility and intraocular pressure (IOP) arising from changes in fluid flow across the retinal pigment epithelium (RPE), or potentially a redirection of aqueous production from anterior outflow pathways to flow across the RPE, are not considered in interpretations of drug actions. Consequently, the presence of a significant posteriorly directed flow will have important implications for interpreting many types of experiments, including predicting drug distributions within the vitreous, and drug effects on aqueous production, outflow facility and intraocular pressure. In addition, it may have important implications for understanding the origins of pathological processes underlying glaucoma (Smith and Gardiner, 2017). For example, is a type of glaucoma arising from changes in outflow across the RPE possible? Therefore, the question of the presence or absence of a significant posteriorly directed aqueous flow is worth evaluating carefully and thoroughly.

The assumption that there is no significant posteriorly directed aqueous flow is supported by some seemingly strong experimental evidence (Tabibian et al., 2015). For example the clearance of tritiated water and sodium ions from the vitreous humor can be explained by diffusive transport acting alone (Maurice, 1957; Moseley et al., 1984). Diffusive transport is also sufficient to explain the loss of dextran from the vitreal chamber, prior to needle holes being made in the posterior sclera, which induce a posteriorly directed flow of aqueous (Maurice, 1987). However, in this paper we repeatedly demonstrate that this evidence, used to justify the assumption of negligible vitreal flow...
(Missel, 2002; Laude et al., 2010; del Amo et al., 2017; Tabibian et al., 2015), is very weak evidence. In fact, we show that the same data admits similar conclusions when one assumes there is normally a significant posteriorly directed flow of aqueous through the vitreous humor!

We begin by considering some of the key evidence supporting the conclusion there is no flow across the vitreous humor. Then we review some of the key evidence supporting the opposite view—that there is normally a significant vitreous fluid flow that exits across the RPE. This contradictory evidence presents a conundrum, so we then discuss how past researchers have previously attempted to resolve it. We demonstrate their explanations are inconsistent with the experimental evidence. Consequently, we present our research to resolve this question, first by using qualitative reasoning to resolve the conundrum, and then by employing state-of-the-art quantitative reasoning based on computational analysis, including the development of a three-dimensional (3D) computational fluid flow model coupled with a 3D diffusive-advective transport model for the rabbit eye, to reanalyze key data.

We conclude that the evidence for no flow through the vitreous humor is currently very weak. We conclude the balance of evidence currently supports that there is normally a significant physiological flow of aqueous through the vitreous humor and across the RPE, which exits the eye via the choriocapillaris and vortex veins. Our research suggests that for humans, this posteriorly directed flow is about equal to the anteriorly directed flow, while in the rabbit the posteriorly directed flow is probably about one half of the anteriorly directed flow. By accounting for this flow across the RPE in a new outflow model for the eye, we can proceed to reinterpret a wide range of experimental data pertaining to outflow facility and changes in intraocular pressure, drug effects on aqueous production and the transport of drugs within the eye.

2. Evidence for and against the absence of significant vitreous fluid flow

When modeling transport through the vitreous humor, the usual assumption is that fluid in the vitreous is ‘stagnant’ (Balachandran and Barocas, 2008; Missel, 2012; del Amo et al., 2017). This means there is either no advective transport, or at least the fluid flow in the vitreous is so small that advective transport is effectively zero and so may be neglected. In this scenario, diffusive transport completely dominates transport through the vitreous (Missel, 2002). We now proceed to present the key evidence given to support this hypothesis of diffusion-dominated transport through vitreous humor.

2.1. Diffusion of Na\textsuperscript{24} and tritiated water in the vitreous humor

We first consider a report on the injection of radioactive Na\textsuperscript{24} into the mid-vitreous of rabbits (Maurice, 1957). Sodium loss from the whole eye was measured with an external Geiger counter to have a mean transit time (t_{1/2}) of 7.5 h. The ratio of mean anterior chamber concentration to mean vitreal concentration was recorded to be 0.2. Using electrical conductivity measurements on the vitreous humor, Maurice (1957) estimated the diffusion coefficient for Na in the vitreous humor to be 90% of that for isotonic water at the same temperature, and so the diffusion coefficient for Na\textsuperscript{24} could be estimated as 1.7 \times 10^{-9} \text{m}^2/\text{s}. Employing these measurements, and using some vitreous transport parameters found in a previous analysis by Friedenwald and Becker (Friedenwald and Becker, 1955), Maurice concludes that 60% of the Na\textsuperscript{24} leaves the vitreous humor by entering the anterior chamber of the eye, with the balance of Na\textsuperscript{24} exiting the vitreous humor via sodium exchange with the choroid. The main conclusions of this analysis were (Maurice, 1957):

(a) “All exchanges between the aqueous humor and the vitreous body can be explained on the basis of free diffusion across their surface of separation, and of almost free diffusion in the vitreous body itself;

(b) The conductance of the intact vitreous body is about 90% of that of the aqueous humor. This suggests that the Na ion can diffuse almost freely within it;

(c) The direct loss of Na to the blood is not limited by the rate of diffusion in the vitreous body, but by a membrane of low permeability on its surface; this is probably the external limiting membrane of the retina.”

Because the data obtained in Maurice’s experiment could be successfully explained by diffusive transport alone, on first impression this implies that advective transport of fluid through the vitreous humor is negligible.

As a second piece of evidence, Moseley et al. reports on tritiated water injected into the mid-vitreous of rabbits (Moseley et al., 1984). For this experiment, tritiated water is estimated to have a vitreal self-diffusion coefficient of 1.73 \times 10^{-9} \text{m}^2/\text{s} and a mean transit time (i.e. the average time required for labelled water molecules to reach the vortex veins from the site of injection) is only 32 min (Moseley et al., 1984). Most of the labelled water is removed from the eye within about 80 min, with approximately 97% of the labelled water molecules existing via the vortex veins, and only about 3% exiting the eye via aqueous flow through anterior pathways (Moseley et al., 1984). Similar data is reported in further papers (Foulds et al., 1985; Foulds, 1987). This indicates most exchange of water molecules occurs between the vitreous humor and the surrounding choroid. This removal of labelled water molecules is, within experimental error, completely explained by diffusive transport alone (Moseley et al., 1984). Because the data is successfully explained by diffusive transport alone, Moseley’s experiment also suggests that advective transport of water through the vitreous is negligible.

2.2. Leaks through needle holes in the sclera

Johnson and Maurice discovered that large molecular weight fluorinated dextran (e.g. 70 kDa dextran FITC) injected into the mid-vitreous could be employed to estimate changes in aqueous flow rates through the anterior chamber of the eye (Johnson and Maurice, 1984). This ‘vitreal deposit’ method for measuring aqueous flow depends on a difference in time scales for two processes within the eye—the large molecular weight dextran FITC leaves the vitreous chamber on a time scale measured in weeks, while changes in aqueous production by the ciliary body occur on a time scale measured in minutes to hours. So, for practical purposes the rate at which large molecular weight dextran FITC leaves the vitreous humor and enters the posterior chamber of the eye may be regarded as constant on the time scale for changes in aqueous production. This means that assuming steady-state loss of dextran from the vitreous, changes in fluorescent intensity within the anterior chamber depends only on the inverse of the aqueous flow rate through the anterior chamber, thereby providing a means for tracking changes in the aqueous flow rate through the anterior chamber.

In a separate experiment, Gaul and Brubaker (1986) utilized two independent methods to fluorescently measure the flow rate through the anterior chamber of one eye of a rabbit (Jones and Maurice’s ‘corneal deposit’ method (Jones and Maurice, 1966), denoted here method A), while simultaneously employing Johnson and Maurice’s vitreal deposit method (Johnson and Maurice, 1984) in the fellow eye (now denoted method B). Specifically, Gaul and Brubaker report the effects of intravenous injection of acetazolamide, the intravenous injection of mannitol, and water ingestion, have on these two independent measurements of aqueous flow rates through the anterior chamber (Gaul and Brubaker, 1986). As their data shows (Gaul and Brubaker, 1986), the two methods have very similar estimates of aqueous production under normal conditions, but the estimates of aqueous production diverge quite considerably during treatments. While method A shows a large (or 50%) decrease in aqueous production when acetazolamide and mannitol were administered, method B shows only a 10% decrease.
in aqueous production. Why these two estimates by independent methods diverge is not immediately apparent.

As proposed by Maurice (1987), a possible explanation for this discrepancy is an increase in the posteriorly directed flow of aqueous through the vitreous. For then an increase in the posteriorly directed advective transport of dextran would oppose the anteriorly directed diffusive transport of dextran, so any increase in posteriorly directed flow would reduce the dextran FITC entering the posterior chamber of the eye in method B.

By extension we assume that the data presented by Gaul and Brubaker could be explained similarly, along the same lines as expressed in Arai et al. (1991). A treatment causing a simultaneous reduction in aqueous production and an increase in posterior flow rate has the effect of reducing the estimated change in the aqueous flow rate anteriorly, when measured using method B. And further, if one accepts the actual anterior flow rate is given by correctly by method A, then one could infer the dextran FITC flux ratio, say before and after treatment, and then calculate the change in posterior aqueous flow.

Maurice set out to test his theory by creating one or more needle holes through the posterior sclera into the vitreous (Maurice, 1987). Each needle hole creates an additional abnormal fluid flow pathway from the eye, and so a step increment in the posteriorly directed aqueous flow. Loading both eyes in pigmented rabbits with vitreal deposits of dextran 70 FITC, Maurice waited until quasi-steady-state conditions were achieved. Then he introduced the needle holes through the sclera into the posterior vitreal chamber of one eye of the pair. By comparing fluorescence measurements in both eyes, he was able to infer the ratio of flux of dextran from the vitreous into the anterior chambers. Maurice then presented this data as the ratio of dextran fluxes measured for each eye pair, versus increasing numbers of needle holes in the posterior sclera. We have taken the measured data and fitted a linear curve on a semi-log graph (see Fig. 1).

Maurice (1987) explained that it has been previously shown by Maurice (1957) that:

... the interchange of Na\(^{2+}\) between the vitreous and aqueous of the rabbit can, to a first approximation, be treated as if the main body of the vitreous humor was well stirred, but that its anterior 0.4 cm was stagnant, and served as a diffusional barrier in which the ion diffused freely. This ‘diffusional barrier’ could be analyzed using a ‘slab’ model (Maurice, 1987). Maurice then analyzed the data in Fig. 1 using an analytic solution to a one-dimensional (1D) steady-state diffusive-advective transport model, and from this analysis proposed to infer the posterior fluid flow rate through the vitreous (e.g. for one needle hole in the posterior sclera).

The solution for this 1D steady-state transport problem of length dimension \(L\) (m) (where \(L\) is the thickness of the ‘diffusional barrier’ slab within the vitreous humor adjacent to the posterior chamber (Maurice, 1987)), assumes the flow velocity \((v, \text{ m/s})\) is in a direction opposite to the anterior direction of solute diffusive transport. The diffusion coefficient is denoted \(D\) (m\(^2\)/s). The concentration boundary conditions (mol/m\(^3\)) are zero at the upstream (flow) face (i.e. zero at the junction of vitreous and posterior chambers i.e. \(c(0) = 0\)), and there is a constant concentration at the downstream face (i.e. \(c(L) = c_2\)). The solution for the concentration profile to this problem is given by,

\[
c(x) = c_2 \times \left(\frac{e^{xL/D} - 1}{e^{-xL/D} - 1}\right)
\]

where the origin of the \(x\) coordinate is taken to be at the junction of the vitreous humor and posterior chamber. The solute flux (mol/m\(^2\)/s) at any location in the domain for this problem is given by,

\[
f = \frac{D \cdot W_c}{D(e^{xL/D} - 1)}
\]

For otherwise identical conditions, the ratio of fluxes at two different velocities (i.e. say \(v_1\) and \(v_2\)) based on equation (2), is given by,

\[
\frac{f_2}{f_1} = \frac{v_2}{v_1} \left(\frac{e^{xL/D} - 1}{e^{-xL/D} - 1}\right)
\]

Finally, in the limit, when \(v_1 \rightarrow 0\), initially—meaning prior to any needle hole being made—and transport occurs by diffusion alone, equation (3) reduces to,

\[
\frac{f_2}{f_1} = \frac{L}{D} \left(\frac{v_2}{v_1} \left(\frac{e^{xL/D} - 1}{e^{-xL/D} - 1}\right)}
\]

We note that this analysis assumes that the upstream concentration is zero. If the concentration is not zero, there is an additional advective flux equal to the non-zero concentration multiplied by the advective velocity. Estimating the non-zero concentration in the posterior chamber for the actual problem in the in vivo eye is not at all straightforward, as it depends on the diffusive flux magnitude and the aqueous flow rate. In other words, the solution to the actual problem becomes non-linear and requires a full 3D diffusive-advective transport analysis (or at least a two-dimensional (2D) axisymmetric transport analysis). However to follow Maurice’s argument here, we assume that...
the concentration is zero, even though we acknowledge this assumption brings into serious doubt the validity of applying this 1D transport solution to analyze the actual eye problem being considered.

Maurice first assumed that initially (i.e. prior to any needle holes being made in the posterior sclera) there is no posterior flow. This means vitreal transport is initially by diffusion alone, and so Maurice employed equation (4) to analyze his data (Maurice, 1987). Following a single needle puncture, he found the experimentally measured flux ratio was approximately three, and that this flux ratio corresponds to a ‘Peclet number’ of 1.75 in equation (4). The ‘Peclet number’ is a nondimensional number defined to be $vL/D$, where $v$ is the posterior flow velocity, $L$ is a characteristic length and $D$ is the diffusion coefficient. For this particular problem, the diffusion coefficient is that for dextran 70 FITC in vitreous humor. Maurice noted the IOP did not change appreciably following needle punctures, supporting the idea that increments in flow caused by each needle puncture were small, and could be assumed equal.

Based on estimate of the Peclet number being equal to 1.75, (and with $L$ equals 4 mm, $D$ equals $5 \times 10^{-11}$ m$^2$/s and flow area of 1 cm$^2$), Maurice estimated the posterior flow rate following one needle puncture to be 8 μl/h (Maurice, 1987). He observed this is only 5% of the total aqueous flow rate by the rabbit (assuming an aqueous production rate of about 2.7 μl/min). We also note that employing Maurice’s method of analysis and using Maurice’s parameters, Arria et al. (1991) estimated for his experimental data obtained following administration of acetazolamide, that the posterior flow was only 2.6 μl/h, even smaller than that estimated by Maurice (1987).

We will re-analyze Maurice’s (1987) data later, but here we simply point out his conclusions depend on the validity of the assumptions made when interpreting the data using a 1D steady-state diffusive-advective transport analysis.

### 2.3. In vitro experimental measurements of fluid transport across the RPE

There is strong independent experimental evidence that demonstrates a significant fluid flow across the RPE in the normal eye (Tsueboi, 1987; Tsueboi and Pederson, 1987b, 1988; Kawano and Marmor, 1988). Gallemore et al. state in their review (Gallemore et al., 1997): **The fluid transport rates measured in the in-vitro preparations are remarkably similar to those measured in vivo, even though the in vitro preparations are undoubtedly missing some of the paracrine and hormonal input signals from the retina and blood that would normally help regulate fluid transport out of the sub-retinal space.**

This assessment confirms the consistency of a large body of research supporting a significant rate of resorption across the RPE in many types of animals gathered over many years. In 2004, Quintyn and Brasseur also make the same key observation—that in vitro measurements made on RPE from animals are largely supported by in vivo measurements on the rates of sub-retinal fluid absorption (Quintyn and Brasseur, 2004). We briefly consider the evidence for resorption of fluid across the RPE in the next section, highlighting key experiments that have employed independent methods for estimating the rate of fluid resorption across the RPE but nevertheless arriving at a similar conclusion.

### 2.4. In vivo experimental measurement of fluid transport from the subretinal space

In a variety of research papers there is evidence of significant absorption of fluid across normal RPE, be they experiments on epithelial sheets in cell cultures (Shi et al., 2008; Li et al., 2009; Adijanto et al., 2009; Baert et al., 2012) or tests on animal tissue, both in vitro and in vivo (Marmor et al., 1980; Miller et al., 1982; Hughes et al., 1984; Negi and Marmor, 1986; Tsueboi, 1987; Tsueboi and Pederson, 1988; Kawano and Marmor, 1988; Marmor, 1990; Dahrouj et al., 2014; Edelman and Miller, 1991). We note that experiments on the rate of resorption of subretinal fluid in animals often involve blebs of small dimensions, generally with diameters less than about 1.3 mm (Negi and Marmor, 1986), thereby involving comparatively little trauma to the in vivo tissue.

There is also compelling data for subretinal resorption for the human eye in vivo (Quintyn and Brasseur, 2004). Chihara and Nao-i report that clinically measured subretinal sorption rates applied to the whole retina are equivalent to resorbing about one half of the total vitreal chamber volume per day (Chihara and Nao-i, 1985). Interestingly, Adijanto et al. note the in vivo rate of fluid transport reported by Chihara and Nao-i is comparable to those reported by Adijanto et al. for cultured sheets of human RPE (Adijanto et al., 2009). For the normal human eye in vivo, Quintyn and Brasseur estimate a flow rate through the RPE to be about 2.5 ± 1.25 μl/min (Quintyn and Brasseur, 2004). This estimate is somewhat smaller than the mean estimate of 3.0 μl/min made using an independent method reported by Smith and Gardiner (2017), but well within the margin of experimental uncertainty.

Using a mathematical model for fluid flow in human eye with pressure-dependent outflow facility, and fitting this model to both in vivo animal and human data (e.g. in vivo human data on pressure-volume and pressure-time data obtained from intracameral manometric measurements obtained prior to cataract surgery (Dastiridou et al., 2013; Karyotakis et al., 2015)), Smith and Gardiner estimated the rate of fluid absorption across the human RPE at about 3.0 μl/min (Smith and Gardiner, 2017), which is approximately the same as the fluid as exits anteriorly through the trabecular and uveoscleral outflow pathways (we note that Table 1 in Brubaker suggests anterior pathway outflow rates at about 2.5 ± 0.7 μl/min (Brubaker, 1991) or measuring outflow in 51 younger adults between 20 and 30 years of age, Toris et al. reports 2.8 ± 0.8 μl/min (Toris et al., 1999)).

In a later paper, Smith et al. developed a 3D axisymmetric model of fluid flow in human eye, employing the same pressure dependent outflow facility model as developed earlier by Smith and Gardiner. The 3D porous media flow model predicts a fluid flow rate across the human RPE of 2.8 μl/min (Smith et al., 2019b). The magnitude and direction of the Darcy flow velocity through the vitreal chamber of the human eye predicted by the model is shown in Fig. 2. Smith et al. then employed the 3D model of the human eye and showed that if the silicon oil tamponade in contact with the retina completely blocks fluid transport across the RPE, then model predictions (see Fig. 3) are in accord with clinically measured rise and fall in mean IOP reported following silicon oil tamponade insertion and removal (Smith et al., 2019b). Importantly we note that provided the anterior outflow pathways are not compromised by the presence of excessive silicon oil, the ‘standard model’ for fluid flow through the eye (i.e. with fluid flow through anterior pathways alone, and stagnant fluid in the vitreous humor), predicts no change in IOP upon introduction of a silicone oil tamponade, provided the anterior outflow pathways are not compromised by the presence of excessive silicon oil. But this prediction is clearly at odds with the measured rise in median IOP for a group of 198 patients (Jonas et al., 2001).

Employing the same fluid flow model, Smith et al. show they could also predict IOP changes in the eye associated with Schwartz-Matsuo syndrome (Matsuo, 1994; Smith et al., 2019b). Together, these findings build confidence in the pressure dependent outflow model proposed by Smith et al. (Smith and Gardiner, 2017; Smith et al., 2019b). Interestingly, we observe that the final IOP rise predicted by our model applied to Schwartz-Matsuo syndrome (Matsuo, 1994) (i.e. 225% that of normal IOP) is of similar magnitude to the pressure rise when silicone oil is introduced into the anterior chamber of mice to completely block anterior outflow pathways (250% that of normal IOP) (Zhang et al., 2019).

The research by Cantrill and Pederson is also of particular interest here because it uses yet another independent method to estimate the rate of fluid transport across primate RPE (Cantrill and Pederson, 1984). Cantrill and Pederson performed a detailed set of experiments...
Table 1

<table>
<thead>
<tr>
<th>Method Based on these estimates or measurements</th>
<th>Modified by:</th>
<th>Retinal metabolic water production μl/min Fraction of 2.8 μl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Estimated whole body metabolic water production and blood flows for adults</td>
<td>Whole retinal fraction of aerobic and glycolytic metabolism pig</td>
<td>0.057 to 0.079</td>
</tr>
<tr>
<td>2 Measurements on oxygen consumption brain gray matter for adults</td>
<td>Pig</td>
<td>0.020</td>
</tr>
<tr>
<td>3 Measurements of oxygen consumption at inner retina for adults</td>
<td>Pig</td>
<td>0.072</td>
</tr>
</tbody>
</table>

on cynomolgus monkeys with sodium fluorescein as a tracer, specifically both intravitreal and intraperitoneal injections of fluorescein, with and without probenecid treatment (which inhibits or blocks organic anion transporters (OAT) and so active transport of fluorescein across the RPE). Using exemplary deductive logic, they calculated the in vivo flow rate across RPE to be 2.89 μl/min for normal cynomolgus monkey eyes (i.e. with retina and RPE intact). This is close to the estimates made by Quintyn and Brasseur and Smith et al. for the human eye.

However this particular analysis depends upon several assumptions, one of the more important being that fluorescein is passively advected with fluid flow across the RPE. Fluid flow across the epithelium depends mainly on net sodium ion transport (as well as chloride ion and bicarbonate ion transport) from the vitreal side to the choroidal side of the RPE, with water osmotically following the net movement of ions.

Miller and Edelman explain (Edelman and Miller, 1991):

\[ \ldots \text{net Na transport across the RPE is determined by the balance between:} \]

(1) Na secretion through the Na/K pump; and (2) Na absorption, which is driven by the transepithelial potential (TEP) through the paracellular pathway. Since ion and fluid transport are tightly coupled, one would expect that net ion flux also is modulated by changes in TEP. This notion has been verified in frog and bovine RPE. Short-circuiting the tissues (TEP = 0) reverses the direction of net Na flux from absorption to secretion, and this secretory flux is mediated by the Na/K pump.

This implies that any molecules passively transferred with fluid flow through the ‘paracellular route’ mostly pass through the tight junctions between the RPE cells (Fields et al., 2019). And so probably the most important question for the Cantrill and Pederson study is: can fluorescein be advected with the fluid through the tight junctions between epithelial cells as assumed in (Cantrill and Pederson (1984))? One observes that if the OAT in the cell membranes of RPE is blocked by probenecid, then the paracellular route is the only transport pathway available. As fluorescein is still transported across the RPE, the logical deduction is that fluorescein can traverse the tight junctions. But it would be reassuring to have some confirmatory anatomical insight into tight junction permeability to fluorescein.

While emphasizing the tight junctions are dynamic structures, Rizzolo et al. reports that organic molecules less than 4 Å in size can pass through the tight junctions unimpeded (see Fig. 1 in Rizzolo et al. (2011)). Liang and Webber report that there are at least two pathways through tight junctions: (i) ‘a high capacity pore pathway regulates paracellular flux of small ions and molecules, but does not pass macromolecules and (ii) a low capacity leak pathway that passes ions and macromolecules in a charge and relatively size non-selective manner’ (Liang and Webber, 2014). Liang and Webber report that mannitol (size 3.6 Å, MW = 182) can pass easily through the high capacity leak pathway, but inulin (at 11.5 Å, MW = 5200 (Czernicka et al., 2000)) cannot. Fluorescein has been reported to be 5 Å in diameter (Malmgren and Olsson, 1980). However, based on the average bond length in a benzene ring being 1.4 Å, we estimate from space filling models of fluorescein (MW 322), that it is a compact molecule, approximately ellipsoidal in shape, with a minor axis of about 4 Å, an intermediate axis of about 6.0 Å and a major axis of about 8.4 Å. We observe that the average of the minor and intermediate axes is about 5 Å, similar to that reported by Malmgren and Olsson. Consequently it seems likely that fluorescein can be advected through the RPE tight junctions relatively unimpeded, as assumed by Cantrill and Pederson. This conclusion is supported by a later study on the transport of carboxyfluorescein across the RPE (Tsui and Pederson, 1987a). Given the other assumptions employed by Cantrill and Pederson are reasonable, this means the calculated flow across the in vivo RPE of 2.89 μl/min in control eyes of cynomolgus monkeys is also reasonable. It is noteworthy that both Cantrill and Pederson’s method (Cantrill and Pederson, 1984) and Smith et al.’s method (Smith and Gardiner, 2017; Smith et al., 2019b) for estimating the rate of fluid transport across the RPE do not involve any experimental disruption to the in vivo retinal tissues (i.e. the retinal and RPE...
Table 2

Estimation of mutual diffusion coefficients at 37 °C for dextran FITC of various molecular weights in distilled water, saline solution and vitreous humor. Gajraj* denotes steady-state diffusion estimates made by the authors using Gajraj’s data (Gajraj, 2012), rather than based on breakthrough time, a method employed by Gajraj. Author* estimate for Dextran 70 kDa FITC is based on interpolation on Gajraj* data. Dias (Dias and Mitra, 2000) and Laurent (Laurent et al., 1976). PBS represents phosphate buffer solution.

<table>
<thead>
<tr>
<th>Solute FITC dextran molecular weight (kDa)</th>
<th>References</th>
<th>$D_m$ (× 10^{-6} cm²/s) in distilled water</th>
<th>$D_m$ (× 10^{-6} cm²/s) in PBS</th>
<th>$D_{m-PBS}$ (× 10^{-6} cm²/s) Percine Vitreous</th>
<th>$D_{m-PBS}$ (× 10^{-6} cm²/s) Rabbit Vitreous</th>
<th>$D_{m-PBS}$ Best Est Vitreous</th>
<th>Ratio Best estimate $D_{m-PBS}/D_{m-PBS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Gajraj</td>
<td>na</td>
<td>8.4</td>
<td>3.1</td>
<td>3.8</td>
<td>3.4</td>
<td>0.40</td>
</tr>
<tr>
<td>4</td>
<td>Dias*</td>
<td>na</td>
<td>7.0</td>
<td>2.0</td>
<td>2.4</td>
<td>2.2</td>
<td>0.31</td>
</tr>
<tr>
<td>4</td>
<td>Dias</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>7.6</td>
<td>7.6</td>
<td>na</td>
</tr>
<tr>
<td>9.3</td>
<td>Dias</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>6.2</td>
<td>6.2</td>
<td>na</td>
</tr>
<tr>
<td>20</td>
<td>Laurent</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>1.2</td>
<td>1.2</td>
<td>na</td>
</tr>
<tr>
<td>40</td>
<td>Gajraj</td>
<td>na</td>
<td>8.3</td>
<td>2.2</td>
<td>4.1</td>
<td>2.7</td>
<td>0.32</td>
</tr>
<tr>
<td>40</td>
<td>Author*</td>
<td>na</td>
<td>2.7</td>
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<td>40</td>
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<td>150</td>
<td>Gajraj</td>
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<td>Gajraj*</td>
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3. Explaining the conundrum

In summary we now have two sets of experimental data, one data set apparently suggesting little or no posterior directed flow of aqueous through the vitreous, while the other data set suggesting significant posteriorly directed flow of aqueous. This presents a conundrum.

3.1. Earlier attempts to resolve the conundrum

This conundrum was identified by Maurice and Marmor—colleagues at Stanford University—many years ago. Both researchers separately attempted to resolve this conundrum (Maurice, 1992).

3.1.1. Maurice’s attempt

Maurice relates to us in his paper titled: ‘Physiology of the Vitreous: a personal view’ (Maurice, 1992):

Another conclusion that can be drawn from the experiments on the leakage of fluid out of a scleral needle hole [see discussion above] is that there is normally very little seepage of aqueous humor backward across the vitreous interface. This is interesting because subretinal fluid is rapidly absorbed across the pigment epithelium by an active transport system. It is difficult to believe that this fluid volume could be replaced in the vitreous other than by the ciliary body, so that if the active system is transporting fluid at the same rate, under normal circumstances a marked anterior seepage should be detectable. It is piquant that one of the active investigators of the pigment epithelial transport system is Dr. Marmor of the Ophthalmology Department of Stanford; we have not yet devised an experiment that will resolve the apparent contradiction. Currently I am examining particular markers that may be able to reveal small drifts within the vitreous body … I am convinced that the vitreous still has mysteries to reveal.

This extract suggests that while Maurice had concluded there was very little posterior fluid migration on the basis of his ‘scleral needle’ experiments. At the same time he harbors suspicions there may normally be some posteriorly directed movement of aqueous fluid, as evidenced by his belief that the ‘vitreous still has mysteries to reveal’ and his ongoing research program ‘examining particular markers’ of vitreal flow. This investigation of particulate markers may have been influenced by the findings for rabbits reported by Fowlks (1963).

When nitro BT was injected 2 mm or closer to the retina or pars plana, it was swept posteriorly in a meridional flow pattern. Blue formazan was found to stain the retina immediately posterior to an injection at or near the corona ciliaris and as far back as the posterior pole and in as little as 15 minutes after injection.‘

These intriguing findings are clearly summarized by Fig. 4 of Fowlks (1963). Maurice may have also known of the research by Hayreh (1966) and the analysis by Fatt (1975). Following an advective-diffusive transport analysis employing plausible model parameters Fatt concludes (Fatt, 1975):

Furthermore, these [large] particles will simply travel with the convective flow because the diffusion process is too slow to diffuse the front. Under these conditions, the results summarized by Fowlks (1963) and Hayreh (1966) are not surprising. Large particle deposited retrogradely in the vitreous body of the living eye tend to move posteriorly and be deposited on the retina.

3.1.2. Marmor’s attempt

Marmor appears to agree with Maurice’s conclusion that there is normally little posteriorly directed flow through the vitreous, for Negi and Marmor say in their discussion (Negi and Marmor, 1986):

These figures apply to experimental accumulations of a balanced salt solution in the subretinal space, but may not be applicable under normal...
conditions when the subretinal space has minimal dimensions, and the only fluid available must come from the vitreous across the flow resistance of the retina.

In a later paper Marmor did a similar calculation to Quintyn and Brasseur’s on fluid transport across the RPE based on the rate of removal of subretinal fluid, and arrived at the same conclusion as Quintyn and Brasseur (Marmor, 1990). In an attempt to resolve this conundrum, Marmor again invokes ‘retinal resistance’ to fluid flow as the explanation that resolves the ‘paradox’, making the following comments (Marmor, 1990):

Some readers may discern a paradox in this last observation. If the RPE transports fluid at a rate comparable to the secretion of aqueous, how then is intraocular pressure maintained and what is the role of the trabecular meshwork? If the RPE routinely transported fluid at its maximal rate, the inconsistencies would hold, but my guess is that ongoing fluid transport across the normal RPE is actually very small: the rates quoted above represent RPE transport with an unlimited reservoir of fluid in the subretinal space. The retina provides substantial resistance to fluid flow (reference to Fatt and Shantinath (1971)) and only a small amount of fluid percolates through in response to intraocular pressure. In other words, the rate-limiting step is ordinarily the passage of fluid through the retina rather than the RPE. When there is a retinal detachment, however, the RPE can transport at its maximal rate (e.g. we know from clinical experience that large amounts of subretinal fluid can absorb in 24 hours after placement of a scleral buckle).

But unfortunately the reference employed by Marmor in support of his contention that the retina provides ‘substantial resistance’ to fluid flow, actually reports that retinal resistance to fluid flow is exceedingly small, resulting in an estimated pressure drop across of the retina of only 0.52 x 10^{-3} mmHg (Fatt and Shantinath, 1971). While we previously reported a somewhat larger pressure drop of 6.0 x 10^{-3} mmHg, as calculated in Smith et al. (2019b), this pressure drop is also negligible, indicating little flow resistance. In the same paper, Fatt and Shantinath also noted that their measured negligible resistance to fluid flow in the retina explains why Maurice could not measure any significant pressure difference between IOP and the subretinal pressure (Fatt and Shantinath, 1971).

Specifically Maurice et al. (1971) reports:

On connecting the subretinal cannula to the manometer a positive pressure was immediately recorded, the level of which showed little or no change with time. Its value was 15.5 (12–19) mmHg, similar to the intraocular pressure of the anaesthetised rabbit eye. A small pulse was generally displayed on the trace, and an immediate pressure rise.

There was no significant pressure difference between IOP and subretinal pressure, despite there being a significant pressure drop of 3.7 mmHg - 4.7 mmHg between the vitreous fluid pressure and the posterior suprachoroidal fluid pressure in the normal eye of cynomolgus monkeys (Emi et al., 1989), and a probable osmotic pressure drop between vitreous and choroid of about 2.5 mmHg in normal eyes of the cynomolgus monkey (i.e. about 10% of 25 mm Hg (Toris et al., 1990)). Indeed Maurice’s paper also demonstrates that Marmor’s contention that retinal resistance is so large it blocks posteriorly directed flow is not supported by the experimental evidence, as no significant pressure difference is found between the anterior chamber and the subretinal space in vivo for rabbits (Maurice et al., 1971).

A later paper by Antcliff et al. reports a retinal hydraulic conductivity for human retina, that is 200 times smaller than that observed by Fatt and Shantinath for rabbit retina (Antcliff et al., 2001), even though both human and rabbit retina contain inner and outer plexiform layers, which are the sites of most resistance to fluid flow within the retina (Antcliff et al., 2001). Using Antcliff et al.’s data the pressure drop across the retina is found to be about 1 mmHg in our human eye model (Smith et al., 2019b), but this still represents only about 20% or less of the total pressure drop between vitreous and the choroidal interstitial space.

This refutation of Marmor’s supposition is important because like Marmor, many now suppose that the fluid flow rate as estimated by the rate of resolution of subretinal blebs is an artefact of the subretinal fluid accumulation itself, and that fluid flow across the RPE somehow ceases (or becomes negligible) when the retina contacts the RPE (Tabibian et al., 2015; Soubrane-Daguet and Coscas, 2017). For example Soubrane-Daguet and Coscas explain: ‘RPE fluid transport is normally limited by the retina, which resists water flow from the vitreous.’ (Soubrane-Daguet and Coscas, 2017), and in support of this contention they cite Orr et al.’s paper. But Orr et al.’s paper is actually about the diffusion permeability of Tritiated water leaving the vitreous chamber, not hydraulic conductivity of the retina. Orr et al. remarks in their discussion (Orr et al., 1986):

It should be emphasized that both these experiments with tritiated water relate to diffusional molecular movement rather than non-diffusional flow across the sensory retina and the RPE. However, it is probable that the different permeabilities to diffusion movement reflect similar differences in permeability to water flow. It is unlikely, for example, that the retina is much more impermeable than the RPE to diffusion but more permeable to water flow.

But the diffusional permeability (solute transport) and hydraulic permeability/conductivity (solvent/fluid transport) are two quite different material properties, and while this much is recognized by Orr et al., it is unfortunately not possible to make any certain conclusion as how the two are correlated without measurement. This explains the paucity of correlations in the literature between these two material properties, and when a correlation is found, it is material specific and often complicated (for example, see Fig. 4 in Offeddu et al. (2018)). To give a simple and extreme example of no correlation between the two material properties, consider a bundle of macro scale capillaries with very thin walls between two salt solutions—the measured apparent diffusion coefficient (relating to diffusional permeability) is independent of capillary size, while the measured apparent hydraulic permeability varies as the capillary radius to the fourth power (which follows from the Hagen-Poiseuille equation). Another example is provided by aquaporins, which are very permeable to water while the diffusion of molecules larger than water is effectively zero. But even for water molecules flowing through an aquaporin channel under an osmotic gradient (p_f), versus migrating through an aquaporin channel by diffusion (p_d), the ratio of p_f/p_d is about 12 (Zhu et al., 2004), completely undermining Orr’s contention that if diffusion permeability of water is low so must the hydraulic conductivity be low.

3.2. Our attempt to resolve the conundrum

There are many processes occurring simultaneously in Maurice’s experiment with Na^{24}, Moseley’s experiment with tritiated water and Maurice’s posterior scleral needle hole experiment. To analyze these adequately really requires a computational model. We develop such a model as described below and report the findings for Na^{24} and the scleral needle-hole experiments. However, we first make some observations on the abovementioned experiments that do not require a more advanced analysis. We consider the Moseley et al. (1984) data first.

3.2.1. Moseley’s experiment

Despite first impressions, this data is actually compatible with a small fluid velocity through the vitreous humor (exiting across the RPE). For example, Dahrouj et al. estimated the in vivo subretinal sorption rates through RPE of New Zealand white rabbits as being about 6 μl/cm^2/hr at 10 mmHg and 15 μl/cm^2/hr at 15 mmHg. Assuming the posterior flow rate normally is about 10 μl/cm^2/hr and the RPE surface area is 6.1 cm^2 (Maurice, 1957), the estimated in vivo vitreal flow rate is
around 1 μl/min. Assuming a vitreal volume of about 1.5 ml, it will take about 25 h to replace the vitreal fluid. This means not even a significant advective transport necessarily makes a meaningful contribution to the removal of labelled water over the duration of Moseley et al.’s experiment. This is easily appreciated because in one half hour—which is the mean transit time for labelled water molecules to migrate from the site of injection in the vitreal compartment to the collection site at the vortex vein—provides time for fluid transport across the RPE of only 30 μl/1500 μl = 2% of the vitreal volume. In other words, advective transport is expected to contribute an error in the estimated mean transit time based on a diffusion-only analysis of about 2%, while the reported standard error of the mean transit time in Moseley et al.’s experiment is ±6% (i.e. 32 ± 2 min). This means advective transport of 1.0 μl/min across the RPE in the rabbit is not detectable in this experiment even when present, so in this case it is not surprising the experimental results appear to be explained ‘entirely’ by diffusive transport.

But Moseley et al. (1984) too, makes this very point. In the last sentence of their paper they comment: ‘The transfer of water to the choroid and anterior chamber is consistent with an explanation based on the movement of water by diffusion but does not rule out in addition active transport or a slow bulk flow’ (Moseley et al., 1984). We agree—but it is also apparent that this ‘small flow rate’ across the entire surface of the RPE can in fact amount to a significant fluid flow rate through the vitreous humor relative to the flow rate through the anterior chamber.

We mention here that the vast majority of water, transported by either diffusion or advection, leaving the posterior eye passes across the RPE and joins the interstitial choroidal fluid. Once in the choroidal interstitial space, water leaves this compartment according to Starling's force equation (Levick and Michel, 2010), to the choriocapillaris and then leaves the eye via the vortex veins. Only a very small fraction of water leaves via the scleral surface. In contrast, it is often assumed in some mathematical and engineering analyses of posterior flow through the vitreous that posteriorly directed fluid flow exits the eye via the scleral surface (Fatt and Hedbys, 1970; Xu et al., 2000; Missel, 2002; Stay et al., 2003). But the diffusive exchange of water at the choriocapillaris demonstrates that most of the labelled water in Moseley's experiments exits the eye via the vortex veins (see also the experiments reported in Foulds (1987)).

We also note that Smith et al. (2019) estimate the posterior vitreous flow rate in humans to be about 2.8 μl/min, while the average volume of vitreous in humans is about 6.2 ml (Silver and Geyer, 2000). This implies that it would take 37 h (6200 μl/2.8 μl/min × 60 min/h) for advective flow to replace the vitreous in humans. This is much slower than the time required to replace the anterior chamber fluid for the human eye. Assuming an anterior flow rate of 3 μl/min and an anterior chamber volume of about 180 μl (Labiris et al., 2009), the anterior chamber fluid is replaced on a timescale of about 1 h.

Now we consider the 1D transport analysis employed by Maurice to analyze his scleral needle puncture experiment.

### 3.2.2. Maurice's scleral needle puncture experiment

An important issue with Maurice's analysis is the assumption that the 1D analytic transport model only fits the data when transport is initially (i.e. prior to a needle hole being made in the rabbit sclera) diffusion only (Maurice, 1987). In other words, when the initial Peclet number is zero. In fact, we show that the experimental data (recall Fig. 1) can now be fitted just as well assuming an initial Peclet number of say 10, rather than zero, or any initial Peclet number between zero and 10 (see Fig. 4).

Assuming an initial Peclet number of 10, then using exactly the same assumptions as Maurice, the conclusion would be that the flow rate is about 6.5 times larger than the 8 μl/h estimated above (i.e. the posterior flow rate is about 52 μl/h). About 10% of the posterior flow is incremental flow, based on the increment in Peclet number following puncture, the 10 percent being equal to about 1.1 divided by the final

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**Fig. 4.** Comparison between experimental data shown in Fig. 1 and theoretical values based on Peclet numbers of zero and ten. For initial Peclet number zero and initial Peclet number ten, the semi-log plot shows the theoretical ratio of expected dextran FITC fluxes (control (F0)/after needle puncture (Fn)), from the vitreous into the posterior and anterior chambers of each eye in pigmented rabbits, versus number of needle holes in the posterior sclera of one of the eye pair. Numbers represent line of best fit for experimental data obtained from Fig. 1. Note that the initial Peclet number makes little difference to the goodness of fit between the experimental data and theoretical predictions, and that similarly good curve fits could be obtained for any Peclet number between zero and at least ten.

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Peclet number following needle puncture (about 11). So the incremental flow is equal to about 5.0 μl/h, which we note is about midway between Maurice's estimate of 8 μl/h and Araie et al.'s estimate of 2.6 μl/h. However the initial posterior vitreal flow rate, prior to a needle hole being made in the rabbit sclera, is estimated to be 0.77 μl/min.

Of course, these estimated initial and incremental flow rates could change significantly as additional assumptions made by Maurice (1987) are changed (i.e. as the values assumed by Maurice for v, L, D and surface area are changed). Nevertheless, the non-uniqueness of the data fit to the assumed initial Peclet number renders Maurice's conclusion based on his analysis of the scleral needle experiment to estimate initial posterior flow very weak. Additional uncertainty about the parameters in the analytic model makes the estimate further ill-constrained. We note that parameter uncertainty is in fact acknowledged explicitly in a later paper by Araie et al., a co-researcher with Maurice, who employed Maurice's analysis unaltered. However, Araie et al. did not mention the additional error associated with estimation of the Peclet number itself. For example, if the estimated D were three time larger, the estimated L three time smaller, and the estimated Peclet number twice as large as those chosen by Maurice, then the estimated v (and estimated flow rate) would be some 18 times larger. In other words, the data and 1D transport analysis described by Maurice (1987) is not reliably providing the magnitude of the initial posteriorly directed flow rate through the vitreous.

Maurice (1987) then argues that if one does assume that the flow rate across the normal in vivo RPE is as Cantrill and Pederson reports (i.e. 0.3 μm²/mm²/hr (Cantrill and Pederson, 1984)), this would lead to such a large flow velocity and consequently such a large Peclet number (i.e. 9), that it would: 'hold back any diffusional transfer of dextran FITC to the anterior chamber and be incompatible with the relative high concentration [in the anterior chamber] found by Johnson and Maurice (1984). We examine this argument further when we perform an analysis using our 3D flow model for the rabbit.

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### 3.2.3. The transepithelial potential across the retinal pigment epithelium

Marmor argues that because of substantial retinal resistance, the flow across the RPE is only small (Marmor, 1990). But as we have seen from the discussion above, the retina does not provide sufficient resistance to reduce the flow across the RPE to negligible or prevent it...
altogether (Fatt and Shantinath, 1971). But leaving this evidence to one side for the moment, we seek to answer a broader question: could it be true that net fluid flow through the RPE stops when retina contacts RPE? That is, perhaps the RPE transports water when it is not in contact with the retina, clearing subretinal fluid, but changes to negligible or no fluid transport when the tissues contact one another. One way to get some insight into this question is to consider measurements of the in vivo ‘transepithelial potential’, as the rate of fluid transport and transepithelial potential are tightly coupled (Edelman and Miller, 1991).

First, we recall the explanation provided by Edelman and Miller (i.e. ‘Since ion and fluid transport are tightly coupled, one would expect that net ion flux also is modulated by changes in TEP. This notion has been verified in frog and bovine RPE.’) (Edelman and Miller, 1991). We can see this clearly evidenced by the experimental data shown in Fig. 2 of Frambach et al. (1990). Gallemore et al.’s review says (Gallemore et al., 1997):

Under the open-circuit condition, which more closely resembles the situation for the RPE in the eye, there is a spontaneous transepithelial potential of the order of 5 to 15 mV, apical side positive [see also Fig. 2 in Strauss (2014)] ....

The comparison of flux and fluid transport measurements in control and cAMP stimulated tissues showed that the net ‘solute’ flux is linearly related to the measured fluid transport rate and that constant of proportionality is the osmolarity of the bathing solution, as expected for isotonic transport ....

The TEP [transepithelial potential] provides an electrical driving force causing the movement of Na in the retina-to-choroid direction through the paracellular pathway, which is presumably Na selective. In the open circuit, this passive absorptive flux of Na through the paracellular pathway exceeds the active secretory Na flux through the transcellular route, leading to net Na absorption. Thus, the net flux under the open circuit condition include both active transcellular transport as well as a paracellular flux driven by the TEP [see Fig. 7 in Gallemore et al. (1997)]...

We see that the extract from Gallemore et al. makes the crucial point that the transepithelial potential plays an important role in modulating the rate of net ion transport across the RPE, and so the net fluid transport across the epithelium. Strauss provides additional detail, explaining how regulating chloride ion transport across the RPE plays an important role in determining net ion transport and so fluid transport across the RPE (Strauss, 2014):

The RPE shows a constant water transport from the subretinal space to the blood stream of the choroid between 1.4 and 11 μm$^2$ cm$^{-2}$ hr$^{-1}$ depending on the species.... The transepithelial CI- transport osmotically drives the transport of water across the RPE cell through aquaporin water channels. The required CI- conductance originates from a variety of different CI- channels which are localized in the basolateral membrane. The CI channel ClC-2 seems to provide a basic CI- conductance. The ClC-2 knock-out results in a loss of transepithelial potential and a retinal degeneration comparable to that of retinitis pigimentosa. In addition to the ClC-2 the RPE expresses Ca$^{2+}$ dependent Cl channels and cAMP-dependent Cl channels. The activity of the latter two CI channels is linked to intracellular signaling systems and can therefore be adapted to change the transepithelial CI- and water transport to different metabolic needs. The intracellular regulation of Cl channel activity in the basolateral membrane of the RPE is likely responsible for the light-dependent adaptation of the water transport and can be monitored in the electro-oculogram.

In this account net ion transport is modulated by both transepithelial potential and intracellular signaling (i.e. regulated intracellularly by intracellular calcium and cAMP concentrations).

In addition, there is bicarbonate transport through retinal pigment epithelial cells (Hamann et al., 2003; Adjanto et al., 2009), and this apparently also plays a significant role in water transport across the RPE, as it does at the ciliary epithelium (Shahidullah et al., 2009; Lee et al., 2011). Because bicarbonate production is linked to light-dark conditions experienced by photoreceptors in the retina, this helps to modulate light-dark dependent fluid transport across the RPE (Strauss, 2005):

The light-induced changes in ion transport do not only maintain ion homeostasis in the subretinal space. The changes in the transport direction also imply light dependent changes in water transport. This effect is based on the fact that the activity of the apical Na–HCO$_3$ cotransporter is dependent on the membrane potential. Light-induced hyperpolarization of the apical membrane results in a decrease of its transport activity, which subsequently leads to intracellular acidification. This increases Cl efflux through the basolateral membrane and results in an increase in Cl and water transport from subretinal space to choroid. The light-induced increase in water absorption seems to be of importance to control subretinal space volume during changes in illumination.... In the dark, the apical membrane of the RPE is depolarized. Now the activity of the Na–HCO$_3$ cotransporter rises causing intracellular alkalization. In consequence, less CI leaves the cell through the basolateral membrane. This reduces fluid absorption in the dark.

From this we can conclude that ion transport across the RPE is modulated by the transepithelial potential, as well as local ion concentrations and intracellular signaling molecules. Let us now consider more closely electric potentials that can be recorded in the posterior eye. This can be a confusing subject, though fortunately clarity is restored in the hands of Steinberg et al. (1985).

The normal direct current electroretinogram (dc-ERG) measures the potential between an electrode placed in the vitreous and an electrode immediately behind the eye. In other words, it is (primarily) the sum of the transepithelial potential and the neural (or retinal) potential. By placing an additional electrode in the subretinal space, it is possible to show these two potentials are quite large and are opposite in polarity (see Fig. 5 in Steinberg et al. (1985)—so it is only the difference in the retinal transepithelial potential and the neural potential that is measured by a dc-ERG.

Upon short-term exposure to light (i.e. measured in seconds), a so-called ‘c-wave’ is measured by the dc-ERG. In the cat eye, the transepithelial potential is always greater than the neural potential, and this difference creates a clearly defined c-wave. In humans, the transepithelial and neural potentials are more closely matched, and so in some people it can be difficult to detect a c-wave. We note that because the transepithelial and neural potentials are large relative to their difference, small changes in transepithelial or retinal potentials lead to large changes in measured c-waves (see Fig. 12 in Steinberg et al. (1985)). There are also additional changes in the dc-ERG upon longer exposure to light—the so-called ‘light rise’ is a slow increase of the dc-ERG following eye illumination over minutes (Steinberg et al., 1985), which then slowly oscillates with a period of about 40 min (probably due to feedback processes within RPE and neural cells), before settling to a steady state over a couple of hours (Marmor, 1991).

Here we are interested in the transepithelial potential only, as it is only that potential which is connected to modulating fluid transport across the RPE. For the cat eye, the transepithelial potential in vivo is about 6.0 mV when dark adapted (see Fig. 13 in Steinberg et al. (1985). Upon receiving a 4 s light flash of magnitude 8.3 log quanta deg$^{-2}$ s$^{-1}$, the transepithelial potential increases by about another 6 mV, to around 12 mV, while the c-wave rises only about 1.5 mV (see Fig. 9 in Steinberg et al. (1985)). See also Fig. 26 for the ‘light rise’ response of the cat eye over 4 min of illumination, and Fig. 28 for 5 min of illumination followed by dark, revealing the so-called ‘dark trough’ (Steinberg et al., 1985). Importantly, qualitatively similar responses are seen in all mammalian eyes, though the magnitudes of resistance and potential changes may be somewhat different (Steinberg et al., 1985; Frishman and Wang, 2011).

Given that the transepithelial potential is connected to
transepithelial fluid flow (Edelman and Miller, 1991; Gallemore et al., 1997; Strauss, 2005, 2014), and given the measured changes in transepithelial potential changes from dark to light (Steinberg et al., 1985), it is not too great a step to suggest that the light-dependent transepithelial fluid flow change described by Strauss (2005) is also associated with changes in transepithelial potential. While recognizing that the transepithelial potential changes are variable over time along with the transepithelial fluid flow, it is nevertheless clear from the discussion above on transepithelial potential changes in the cat that it is entirely possible that in a time averaged sense, the transepithelial fluid flow during illumination may be about twice that in darkness.

Now we recall the well-known circadian rhythm reported for aqueous production by the ciliary body (Brubaker, 1991; Sit et al., 2008; Goel et al., 2010; Nau et al., 2013). The reported circadian variation in the rate of aqueous humor formation from night to day is around two. Here we simply observe that the abovementioned light-dependent transepithelial fluid sorption across the RPE may provide a plausible synchronous variation in outflow that complements the circadian rhythm in aqueous fluid production at the ciliary body.

Typically, the reported transepithelial potential in vivo is in the range of 5–15 mV depending on the species tested (Gallemore et al., 1997; Strauss, 2014). While there are doubts some differences in the transepithelial potential (and fluid flow) when the retina and RPE are in contact compared to when they are separated, for normal in vivo conditions there is almost certainly a non-zero transepithelial potential that remains when they are in contact (Steinberg et al., 1985; Gallemore et al., 1997; Strauss, 2014). From this we conclude that there would almost certainly be a significant net fluid resorption across the RPE when retina and RPE are in contact. But is there any evidence for fluid resorption when retina and RPE are in contact?

Probably the best experimental evidence available confirming the existence of net fluid flow when retina and RPE are in contact, as well as the measured difference in fluid flow for detached retina relative to retina and RPE in contact, is the experiment reported by Cantrill and Pederson on cynomolgus monkeys (Cantrill and Pederson, 1984). Specifically, one eye was treated with vitrectomy and retinal detachment (for the extent of the retinal detachment, see Fig. 2 in Cantrill and Pederson (1982)), while the fellow control eye was treated with vitrectomy and retinal detachment (i.e. retina and RPE remain intact and so in contact). The inferred rate of fluid transport across the RPE in the control eye (i.e. intact retina and RPE) was 2.89 μl/min, while for stable retinal detachment the rate of fluid transport across the RPE is approximately doubled, at 6.38 μl/min. This data suggests that retinal detachment leads to a larger fluid flow rate (i.e. about double the flow rate of intact tissues in this case), but importantly, significant net fluid resorption remains when retina and RPE are in contact. This sits nicely with the data on changes in transepithelial potential, which may well increase when retinal and RPE are detached, but thereby remains a substantial transepithelial potential when retina and RPE are in contact.

Cantrill and Pederson (1984) state that the reason for the marked increase in flow across the RPE with stable retinal detachment was unclear to them, however they speculated about the hydraulic resistance provided by the retina (Cantrill and Pederson, 1984). But we know from our earlier discussion above that the hydraulic resistance of the retina is not the primary explanation, though it may play a secondary role. The most likely explanation for the increase in fluid resorption is the increase in the transepithelial potential associated with stable retinal detachment, which probably can be explained by the influence of different local ion and signaling environments experienced by the RPE when the retina is detached from the RPE.

Taking all the data above into account, the above discussion on transepithelial potential means that fluid resorption across the RPE does not simply ‘disappear’ when the retina contacts the RPE, but rather resorption continues. However, the rate of fluid resorption continually changes over time with changes in environmental conditions, that is, with darkness and light, with the partial pressure of carbon dioxide, with local extracellular potassium ion concentration, with pH and with extracellular and intracellular concentrations of signaling molecules.

Finally, we mention that in addition to light-dark exposure, changes in potassium and carbon dioxide concentrations, and pH changes, significant changes in transepithelial potential are caused by various endogenous molecules, as well as drugs. Examples of drugs causing changes in transepithelial potentials include azide (Steinberg et al., 1985), mannitol and acetazolamide (Yonemura and Kawasaki, 1979), and adrenergic blockers and agonists (Edelman and Miller, 1991; Frambach et al., 1990)). In addition, various endogenous molecules such as epinephrine (Edelman and Miller, 1991), inflammatory cytokines (Shi et al., 2008) and signaling molecules such as VEGF (Dahrouj et al., 2014), can also modify the transepithelial potential and so the rate of fluid resorption. We keep this in mind, as we later consider the experiments by Gaul and Brubaker (1986) and Aral et al. (1991) when mannitol and acetazolamide are used on treatment groups.

3.2.4. Contributions of retinal water sources to RPE fluid resorption?

While the RPE is undoubtedly capable of transporting very large quantities of water (Chihara and Naoi, 1985; Dahrouj et al., 2014), a question remains as to the source of this fluid. In the foregoing, we have assumed that fluid flowing across the RPE is sourced from aqueous production by the ciliary body. However it is possible that secondary sources of fluid in the retina, either capillary filtration (i.e. fluid leakage by retinal capillaries), or metabolic water production (i.e. water produced as a result of energy production from glucose within the retina), might make significant contributions to the fluid transported across the RPE—in which case not all the fluid transport across the RPE can be attributed to aqueous production by the ciliary body. So to better understand if these secondary sources are large enough to warrant being taken into account in a steady-state fluid balance for a normal eye, we now consider the magnitude of each secondary source in turn.

3.2.4.1. Retinal vascular filtration. The blood-retinal barrier is part of the blood-brain barrier, which is generally reported to be impermeable to most molecules (Paulson, 2002; Wong et al., 2013). Here we assume a good approximation for the blood-retinal barrier is the blood-brain barrier, so we look to the blood-brain data. The question we need to consider here is not how permeable blood-brain barrier is to water diffusion (in fact there is continuous diffusion of water molecules in both directions across all cells and through cell membranes all the time (Candia and Alvarez, 2008)), but rather, we need to evaluate filtration (advective water transport) across the blood-brain barrier.

To calculate the fluid loss from retinal blood-brain barrier we can use Starling equation for fluid filtration (Yuan and R.R., 2010), but to do this we need to estimate driving pressures and the hydraulic conductivity for the blood-brain barrier (in units of cm3 of transudate/s/cm H2O of driving pressure/cm2 of capillary surface area). Fenstermacher et al. measured the ‘filtration coefficient’ of the rabbit blood-brain barrier in vivo, and found it to be 3.2 × 10−10 cm3/s/cm H2O/cm2 (Fenstermacher and Johnson, 1966). Note that Fenstermacher et al.’s ‘filtration coefficient’ is actually the hydraulic conductivity for the blood-brain barrier, as it has units for surface hydraulic conductivity. Paulson et al. measured in vivo the ‘filtration coefficient’ for the human blood-brain barrier in healthy adults (Paulson et al., 1977), and found it to be the same value as reported by Fenstermacher.

The capillary surface area is reported to be 190 cm2/g for gray matter in the brain (Paulson et al., 1977; Wong et al., 2013). The wet weight of adult human retinal tissue in each eye is reported to be 0.32 g (Werkmeister et al., 2015). Discussing driving pressures to be used in Starlings force balance equation across capillaries in retinal tissue, Cunha-Vaz reports (Cunha-Vaz, 2017):

At a first approximation, protein osmotic pressure equals zero in the retinal tissue because protein is negligible in the vitreous and retinal extracellular space. Normally, capillary hydrostatic and protein osmotic
pressures dominate the force term in the [Stirling force] equation. \( \pi_{\text{tissue}} \) [plasma oncotic pressure] is 25 mm Hg higher than \( \pi_{\text{tissue}} \) [retinal tissue oncotic pressure] because of protein oncotic pressure. \( \pi_{\text{plasma}} \) [fluid pressure in retinal capillaries] can be estimated by assuming that arteriolar resistance reduces carotid artery pressure from 65 mm Hg by about 50%. If \( \pi_{\text{plasma}} = 30 \) mm Hg, the driving force for filtration is less than 5 mm Hg [about 7 cm H2O], depending on the value of \( \pi_{\text{tissue}} \) [fluid pressure in the tissue].

Finally we can then multiply these number together (i.e. surface hydraulic conductivity \( \times \) driving pressure for filtration \( \times \) capillary surface area/gram tissue \( \times \) grams of tissue) to estimate the retinal filtration rate. The driving pressure is said to be less than 5 mm Hg, so the filtration rate is less than 0.0082 \( \mu \)l/min. This is equivalent to less than 0.3% of the 2.8 \( \mu \)l/min of aqueous estimated to flow through the human vitreous and across the RPE (Smith et al., 2019b).

It might be argued that Cunha-Vaz (2017) did not take into account the IOP, which would reduce the net mechanical driving pressure across retinal capillaries from 30 mm Hg to about 15 mm Hg (interestingly, we mention that for normal eyes the 15 mm Hg pressure difference between retinal capillaries and IOP is almost independent of IOP (Guidoboni et al., 2014)). Taking into account a 10 mm Hg resorptive pressure (25 mm Hg–15 mm Hg), the rate of fluid resorption by retinal capillaries would then be around 0.6% of the 2.8 \( \mu \)l/min of aqueous estimated to flow through the human vitreous and across the RPE (Smith et al., 2019b).

In either scenario, capillary resorption or capillary leakage, the rate of fluid movement across retinal capillaries in the normal human eye is found to be negligible (i.e. less than 1% of the estimated aqueous flow through the vitreous humor). We note these estimates would not apply for the diseased eye, as the blood-retinal barrier may be compromised (Stitt et al., 2016; Daruich et al., 2018).

Next we consider metabolic water production within retinal tissue. Much like we did when estimating the retinal capillary filtration rate, we calculate the metabolic water production in several ways, thereby ensuring our final estimate is more reliable.

3.2.4.2. Metabolic water production. Retinal tissue is believed to mainly use glucose as an energy source (Petit et al., 2018), though when stressed the retina may use fatty acids (Joyal et al., 2016). In the following, we assume there is glucose metabolism only. Glycolysis and aerobic metabolism of glucose both produce metabolic water. Aerobic metabolism processes one glucose molecule and 6 oxygen molecules to produce 36 ATP molecules and 6 water molecules. In contrast, glycolytic metabolism produces 2 pyruvate molecules (with the 2 pyruvate molecules subsequently converted to 2 lactate molecules), 2 ATP molecules and 2 water molecules from the consumption of one glucose molecule. This means that for the same amount of energy production, obtaining the energy from glycolytic metabolism produces 6 times as much metabolic water as aerobic metabolism. Typically, in the mammalian retina both aerobic and glycolytic metabolism occur simultaneously, a phenomenon known as ‘aerobic glycolysis’ or the ‘Warburg effect’ (Ng et al., 2015).

To calculate the amount of metabolic water produced by the retina, it is most convenient to measure the amount of oxygen consumed during the metabolism of glucose, from which the amount of metabolic water produced by aerobic metabolism within the retina can first be calculated using the basic principles of chemistry. If the fractions of glucose consumed by aerobic and glycolytic metabolism are also known, then the total metabolic water produced by metabolism of glucose can be estimated.

It is found experimentally that the fractions of glycolytic and aerobic metabolism measured within the retina differs from species to species, and from inner retina to outer retina. Indeed the balance between aerobic and glycolytic metabolism in photoreceptors (located in the outer retina) of the human eye is unknown, though photoreceptors are known to convert most of their glucose to lactate (Petit et al., 2018).

Therefore to make appropriate estimates of metabolic water production in the human retina, we need to rely on data from animal models. Unlike retinas in many species, the pig retina is a fully vascularized retina like the human retina, and data on fractions of aerobic and anaerobic metabolism for the whole retina are available. For the pig retina, Wang et al. reports (Wang et al., 1997b):

> From the data in the present study and that in the accompanying one (Wang et al., 1997a), in the whole retina, oxidation in darkness and light accounted for 16% and 21% of the glucose consumption, respectively, lactate formation for 59% and 42%, respectively, and other pathways for 25% and 36% [of glucose consumption], respectively.

It is important to realize that even though most glucose may be converted to lactate under certain conditions, because aerobic metabolism is much more efficient at producing ATP, most of the energy required by the retina is usually derived from aerobic metabolism. So based on the data provided above by Wang et al., for the whole pig retina in darkness, 83% of the ATP is derived from aerobic metabolism and 17% from glycolysis, while in light, 90% of the ATP is derived from aerobic metabolism and 10% from glycolysis. In the following, we assume the human eye is similar to the pig eye in darkness (i.e. 83% aerobic and 17% anaerobic metabolism), which is the ‘worst case’ scenario for metabolic water production.

We can do our first estimate of metabolic water production very approximately using the following data. Assuming an adult has a daily energy expenditure requiring a diet of 2500–3000 kcal per day, it is reported that the metabolic water produced is between 250 and 350 ml/s per day (i.e. about 8%–10% of the adult daily water requirement) (Sawka et al., 2005). For a rough estimate of metabolic water production by the human retina, let us assume that the average fraction of metabolic water produced by the tissue is proportional to the fractional blood flow to that tissue. The total blood flow to all tissue for normal adult human is 5000 ml/min, while the normal human retina has a blood flow of about 0.75 ml/min (Williamson and Harris, 1994). Applying our fractional apportionment to the retina, this implies the metabolic water produced by the eye is approximately 0.026 and 0.036 ml/min. Dividing by 2.8 μl/min, the estimated RPE flow in each human eye (Smith et al., 2019b), leads to an estimated average fraction of metabolic water attributed to each eye as 0.9%–1.3%.

Now we can adjust for the fractions of aerobic and glycolytic metabolism. If we assume that 83% of the total ATP production is produced via the aerobic pathway and another 17% via the glycolytic pathway, then the rate of total metabolic water production increases from X to 2.2X (i.e. 2.2X = X \( \times \) \( [1 + 17/83 \times 6] \)), where X is the water production under aerobic metabolism. So for this rough approximation, our final estimate for the rate of metabolic water production is 0.057 and 0.079 μl/min. Dividing by 2.8 μl/min (estimated RPE flow (Smith et al., 2019b)), leads to an estimated fraction of metabolic water as 2.0%–2.8%.

We can now do a second, more accurate approximation for metabolic water production in the human retina, based on the measured oxygen consumption in gray matter of the brain. Pantano et al. report that the oxygen consumption of the brain gray matter in young adults is about 4.0 ml(O2)/100 g tissue/min (Pantano et al., 1984). Assuming the whole adult brain (1400 g) is gray matter, oxygen consumption for the whole brain is 56 ml(O2)/1400 g tissue/min, which is 2.2 mmoles/1400 g/min. Converting mmoles of O2 to ml of metabolic water (conversion factor 18 μl/mmol), this results in an estimated production of metabolic water of 40 μl/min.

Assuming retinal tissue is similar to gray matter in the brain, and we now take into account that the human retinal tissue weighs only 0.32 g (Werkmeister et al., 2015). Then the rate of metabolic water production in the human retina by aerobic metabolism alone is predicted to be 0.0091 μl/min (i.e. about 13.2 μl/day). Dividing by 2.8 μl/min (estimated RPE flow (Smith et al., 2019b)), the estimated fraction of metabolic water as 0.3%.
Now we again take into account that 83% the total ATP production is produced via the aerobic pathway and another 17% via the glycolytic pathway, then the rate of metabolic water production is about 0.020 μl/min, and dividing by 2.8 μl/min (estimated RPE flow (Smith et al., 2019b)), the estimated fraction of metabolic water as 0.7%.

We can now do our final third calculation, based on the best available oxygen consumption data as measured in young adult retinas (Werkmeister et al., 2015). Measurements are made in light. The rate of oxygen consumption for the inner half of the retinal tissue was found to be 1.42 ml/O2/100 g tissue/min (Werkmeister et al., 2015). The authors say: “Our values for retinal blood flow are, however, in the same range as those obtained by other authors using a variety of different methods.” However, this measured oxygen consumption rate is only about 36% of that reported for gray matter in the brain by Pantano et al., as assumed in the second calculation. Because of this, one can simply scale the results to find the rate of metabolic water production is about 0.0072 μl/min, and dividing by 2.8 μl/min (estimated RPE flow (Smith et al., 2019b)), the estimated fraction of metabolic water as 0.3%.

We now tabulate the estimates for the three methods, as shown in Table 1. Method 1 is based on an estimated whole body metabolic water production weighted by blood flow to the eye, and modified by the fractions of aerobic and glycolytic energy production, gives the highest estimate. Method 2 is based measured oxygen consumption in gray matter of the human brain, the weight of retinal tissue in one eye, and modified by the fraction of aerobic and glycolytic energy production, gives a lower estimate. The difference between these two estimates is largely explained by the lower oxygen extraction ratio for uveal blood compared to the whole body (i.e. about oxygen extraction ratio for the whole body is about 25%, but it is only 3%–7% for uveal blood flow (Elgin, 1964; Alm and Bill, 1970)). In other words, the eye is comparatively well supplied with blood.

The Method 3 is based on actual measurements of oxygen consumption for the inner retina of adult humans. The measured oxygen supplied is considerably less than the measured supply to gray matter in the brain, which explains the discrepancy between the last two estimates of metabolic water production.

Based on the estimates shown in Table 1, we conclude that the rate of production of metabolic water in the normal human is negligible. It is probably less than 0.02 μl/min (about 29 μl/day), which is less than 0.7% of the 2.8 μl/min, the estimated amount of aqueous humor directed posteriorly through the vitreous humor (Smith et al., 2019b). And because a similar conclusion was reached for fluid movement associated with the retinal vascular filtration, we conclude that for steady-state conditions in a normal eye, almost all of the estimated 2.8 μl/min of fluid that exits across the RPE and leaves the eye via the vortex veins, is sourced as aqueous produced by the ciliary body. This conclusion is consistent with what we have previously assumed to be the case in this paper.

3.3. Reanalysis of data on posterior flow through the vitreous

The 1D transport analysis proposed by Maurice (1987) is inadequate for the task of analyzing the data relating to posterior vitreal flow obtained for the rabbit, because the actual problem is more complicated than the underlying assumptions relating to a 1D analytic solution to the transport equation. In this section, we explain this statement more fully and then demonstrate more accurately that the data on rabbits collected by Maurice (1957), Gaul and Brubaker (1985), Maurice (1987) and Ariaie et al. (1991) are all consistent with the significant posterior flow through vitreous. To do this, we employ the geometry of the rabbit eye reported in (Hughes, 1972), and develop a flow model based on the one developed for the human eye by Smith et al. (2019b). We then do a flow and transport analysis of Na2424 injected in rabbit vitreous humor as reported in the Maurice (1957) experiment, followed by a flow and transport analysis of dextran 70 kDa FITC injected into the rabbit vitreous reported in the Gaul and Brubaker (1986) experiment.

3.3.1. Governing equations, initial and boundary conditions

To do these transport analyses through a porous medium with fixed solid phase and a mobile fluid phase, we use a diffusion-advection equation for the fluid phase incorporating a generalized flux equation. It is instructive to first look carefully at the transport equation as it can help us better understand the adequacy of the experimental data itself and help us identify some additional limitations in Maurice’s approach when analyzing the available data on vitreal transport.

Our fluid transport analysis is based on solving the following transport equations, subject to appropriate boundary and initial conditions. The conservation of a chemical species in the fluid phase during advective and diffusive transport throughout eye tissue can be generally expressed as (Rowe et al., 2004; Smith et al., 2019a):

\[
\frac{\partial n_c}{\partial t} = -\nabla \cdot (J + S)
\]

where \( n_c \) (unitless) is the connected fluid porosity in the tissue, \( c \) (mol/m³) is the total concentration of molecules per unit volume of fluid (mol/m³), \( t \) (s) is time, and \( S \) (mol/m³/s) is a source/sink term. The source/sink term \( S \) may represent for example, a zero of first order chemical reaction consuming the chemical being transported. While a zero-order chemical reaction is simply a constant, a first order chemical reaction is represented by \( S = -k_c \), where \( k \) is the first order rate constant. If one molecule transforms into another (e.g. fluorescein may be transformed in fluoroscein glucuronide within the vitreous (Blair et al., 1986)), then the sink in the first equation for fluorescein is matched by a source term a second equation for fluorescein glucuronide. In this way, fluorescein mass is conserved.

Sometimes a chemical may temporarily bind to the stationary solid phase (e.g. due to electrostatic interactions between charged solute and oppositely charged solid phase), and so the chemical may be temporarily immobilized (Ekani-Nkodo and Fygenson, 2003; Moen and Quinn, 2012; Kastorf et al., 2015; Eriksen et al., 2017). Equilibrium ‘reversible binding’ of a chemical onto the solid phase within the porous media may be represented by the equation \( S = -\rho_c K_c n \), where \( \rho_c \) is the density of the solid phase and \( K_c \) is the ‘partitioning coefficient’ (Rowe et al., 2004). We note that the equilibrium reversible binding has the effect of slowing down the initial transport rate, and so extending the ‘breakthrough’ time in a transient analysis. The so-named ‘retardation coefficient’ \( R \) is equal to \((1 + \rho_c K_c n_i)\). If the binding sites are saturated by the chemical, then retardation only occurs below a certain threshold concentration. Non-equilibrium reversible binding may also be implemented by modeling the forward (i.e. binding) and reverse (i.e. unbinding) reactions. In the limit of fast forward and fast reverse reactions, relative to the transport rates, these equations reduce to the equilibrium reversible reactions. But if the binding is fast and dissociation of the complex is slow, then the rate of release of the chemical may be rate limiting rather than the transport of the chemical. This serves to highlight some of the possible ways by which the transport of a molecule can interact with the chemical behavior of the molecule, which in turn will influence the observed spatial and temporal distribution of the chemical within the eye.

The term \( J \) (mol/m²/s) in equation (5) is the flux of molecules through the tissue. For tissue represented as a porous media, the appropriate constitutive equation is defined for the fluid phase to be (Rowe et al., 2004; Smith et al., 2019a):

\[
J = -n_i \tau D_f Vc + \beta Vc
\]
the tortuosity, and the hindrance coefficient all vary between zero and one. In practice, $n_f D_f$ is often bundled together and said to be equal to the apparent diffusion coefficient and denoted simply as $D$.

But this apparent diffusion coefficient $D$ is influenced by many factors including the concentration, temperature, local fluid viscosity, and the ‘background matrix’ the molecule is diffusing through (that is, diffusing through distilled water is not the same as diffusion through isotonic saline, which is not the same as diffusion through a complex extracellular matrix such as vitreous humor). And perhaps surprisingly, this apparent diffusion coefficient is influenced by the spatial concentration gradient. It can be shown that for concentrated solutions, the gradient in the chemical potential of the molecule with respect to its concentration can influence the diffusion coefficient significantly (Laurent et al., 1976; Comper et al., 1986). This apparent diffusion coefficient is known as the mutual diffusion coefficient $D_{mn}$, which depends on the absolute concentration, the magnitude of the concentration gradient, as well as the ‘background matrix’ the molecule is diffusing through. Generally, any decrease in the self-diffusion coefficient with increasing concentration is more than offset by the increase in non-ideal solution effects. In other words, there is an interplay between ‘hydrodynamics effects’ and ‘thermodynamic effects’. The net result of this interplay on the mutual diffusion coefficient is that usually the mutual diffusion coefficient increases with increasing concentration (Laurent et al., 1976).

There may also be a ‘background matrix’ effect due to ‘multi-component diffusion’ in concentrated systems (i.e. diffusion in solutions with a variety of different molecules present). Again, a similar interplay between hydrodynamic and thermodynamic effects occurs, but in this case the different shapes and charges on the background (i.e. the extracellular matrix molecules) and the diffusing molecules can have a profound effect on the apparent diffusion coefficient, resulting in extraordinarily large diffusion coefficients (Laurent et al., 1979). Clearly our vitreous tissue is a multicomponent system, so it would be preferable to measure the diffusion coefficient of molecules (e.g. fluorescein, dextran FITC and ions such as Na$^{24}$) though vitreous humor itself (denoted here $D_{mn}$), rather than through a ‘simple’ fluid (e.g. distilled water). Though the number of experiments is very limited, fortunately some diffusion measurements for vitreous humor do exist (Dias and Mitra, 2000; Gajraj, 2012; Shafaei et al., 2018), as we later discuss.

Finally, the governing equations are solved subject to initial and boundary conditions. Usual boundary conditions are Neumann, Dirichlet or Robin boundary conditions.

Having discussed our general governing transport equations and boundary conditions, we can particularize these to specific circumstances. For example, we can now set time dependent concentration changes to zero, and setting all sources and sinks to zero in equation (5), and assuming all the parameters representing material properties may be taken as constant in equation (6), then we see that for our simplified 1D problem considered above in equations (1)–(4), the Peclet number is more appropriately represented as $\beta v L / D_{mn}$, rather than $v L / D$. Though these Peclet numbers appear superficially to be similar, for a particular application they may be quite different.

3.3.2. Estimating the diffusion coefficient

Let us first consider the diffusion coefficient. Ideally, we would like to know the diffusion coefficient for say dextran FITC, diffusing through vitreous humor at 37 °C. Fortunately there has been a detailed set of experimental data presented by Gajraj (2012). The reader is referred to Gajraj for details of the diffusion cell apparatus. Gajraj estimated the diffusion coefficients based on the ‘breakthrough time’ between two compartments separated by vitreous humor. However it is known that dextran of a notional mean molecular weight actually contains a range of dextran sizes (it has a polydispersity index Mn/Mw < 1.25 (Andrieux et al., 2002)), and so breakthrough time may not be the most appropriate estimate of a representative diffusion coefficient for the mixture. For this reason, we recomputed steady-state diffusion coefficients by fitting all the data presented by Gajraj. For tests on diffusion through phosphate buffer solution, we also considered the delay due to the membrane employed to confine the dextran. Gajraj’s estimates and our estimates based on Gajraj’s data (our estimates are generally smaller than those estimated by Gajraj), as well as some selected measurements by Laurent et al. and Dias and Mitra are presented in Table 2.

Inspection of Table 2 reveals there is a large disparity (around 10 fold) between the ‘self-diffusion coefficient’ for dextran FITC in water at infinite dilution and 20 degrees C (corrected to 37 degrees C by us) as reported by Laurent, and the reported diffusion coefficient for dextran FITC at average concentration 10 mg/ml in isotonic ‘phosphate buffer solution’ at $pH = 7.4$, known at PBS, reported by Gajraj (2012). We note that the dextran FITC in PBS experiment diffusion coefficient reported by Gajraj employed a ‘breakthrough’ analysis, while the authors performed a ‘steady-state diffusion analysis’ of Gajraj’s data (denoted Gajraj* in Table 2). This substantial difference in the estimated diffusion coefficient may be at least partly attributable to test conditions. Gajraj’s experiment measures the mutual diffusion coefficient at average concentration of 10 mg/ml in a steep concentration gradient, while Laurent measures the self-diffusion coefficient at infinite dilution and zero concentration gradient. The difference in diffusion coefficient may be partly attributable to concentration and gradient differences, as well as solution pH differences, which strongly influence the fraction of diffuse double layers formed by fluorescein isothiocyanate (FITC). FITC has a $pK_a$ is around 6.5, meaning the weak acid is mostly ionized when in a solution buffered at $pH 7.4$, as is PBS.

Further, it is also apparent from Table 2 that there is about a 2.5 to 3-fold decrease in the diffusion coefficient of dextran FITC in vitreous humor (pig or rabbit vitreous) compared to PBS. That is, the ratio of the diffusion coefficient in isotonic saline or PBS to that in vitreous is between 0.3 and 0.4. For dextran 70 FITC, the net result is the estimated diffusion coefficient in vitreous humor ($D_{mn_{DFITC}}$), as shown in Table 2, is about 3 times larger than the diffusion coefficient estimated by Maurice (1987).

3.3.3. Estimating the advective transport hindrance factor

Consider now the advection hindrance factor $\beta$, which is equal to the ratio of solute velocity to fluid velocity. The hindrance factor depends on molecular size, molecule type (linear or branched), shape (spherical or oblate or deformable) and net charge (i.e. charge number per molecule), as well as the properties of the gel or tissue through which it is being transported (gel viscosity, average pore size, pore size shape and distribution, interconnectedness of the pore space, heterogeneity of all relevant parameters, etc.).

One way to develop a hindered transport theory is to idealize the molecule (perhaps to a perfectly spherical, uncharged molecule) and idealize the pore spaces through which it moves (perhaps as cylindrical tubes) (Kost and Deen, 2005). But this idealized molecule travelling through an idealized pore space is a very long way from what we need to consider to model the transport through vitreous humor. In short, there is not really a theory to help us determine an appropriate hindrance factor, including for the vitreous humor, so we were obliged to use experimental data. But the difficulty here is that there is only one report of hindrance factors relating to advective transport through vitreous humor. This report contains no quantitative data at all, only the statement that Gd-albumin and 30 nm nanoparticles moved ‘somewhat slower’ than the carrier fluid (Penkova et al., 2013). On the basis of these qualitative results, Penkova et al. proposed the inclusion of an advective hindrance factor in transport analyses for vitreous humor as $1-\sigma$, incorporating a coefficient $\sigma$ (Penkova et al., 2013).

3.3.4. Reanalysis of Maurice (1957) $Na^{24}$

Maurice (1957) reports on experiments using pigmented rabbits, describing the intravitreal injection of radiolabeled sodium (as 0.9% saline solution), and measuring the subsequent decrease in labelled
To do this, we undertook a diffusion-advection analysis of Na\textsuperscript{24} loss following vitreous injection into the rabbit eye. Further details of the model are given in the Appendix. We performed three analyses: (i) a diffusion-only transport analysis using our model while employing the parameters estimated by Maurice (1957), (ii) a diffusion-only transport analysis using our model with parameters estimated by us to fit the measured data, and (iii) a diffusion-advection transport analysis using our model, which assumes one third of the total aqueous production moves posteriorly through the vitreous, and parameters are then estimated to fit the measured data. A summary of the results of this analysis are shown in Table 3.

The last column is a ‘model fit score’ based on our judgement of how well the model fits the measured experimental data. We see all three models used a total aqueous flow rate of 5.4 μl/min, which is close to Maurice's estimated aqueous flow rate of 5.0 μl/min. However, we observe that for the diffusion-advection analysis, one third of the total flow (1.8 μl/min) is directed posteriorly through the vitreous humor.

All three models predicted differing amounts of Na\textsuperscript{24} ions passing from vitreous to aqueous and flowing through the anterior chamber (60% for Maurice's diffusion only model, 40% for the author's diffusion only model, and 25% for the diffusion-advection model), and so each model predicts different amounts of Na\textsuperscript{24} passing across the RPE into the choroid (i.e. 40% for Maurice's diffusion only model, 60% for the author's diffusion model and 75% for the diffusion-advection model).

Comparing the concentration contours at 10 h, and the spatially average concentrations in vitreous humor, aqueous humor and plasma compartments over time for two models (diffusion only and diffusion-advection models, both with author fitted parameters), it is apparent that there is very little difference in modeling results (compare Fig. 5 and Fig. 6). Careful observation shows the concentration contours in the diffusion-advection analysis are slightly displaced posteriorly compared to diffusion only analysis. And because the fitted diffusion coefficient is slightly smaller in the diffusion only analysis compared to that in the diffusion-advection analysis, the maximum concentration in the vitreous humor after 10 h is slightly larger in the diffusion only analysis compared to the diffusion advection analysis (18.1 g/m\textsuperscript{3} compared to 17.4 g/m\textsuperscript{3}). But the spatially average concentration profiles over time for the different compartments are seen to be virtually identical. This is enabled by using a slightly larger diffusion permeability for sodium 24 across the RPE in the diffusion-advection analysis compared to the diffusion-only analysis, meaning more sodium 24 is removed from the vitreous humor across the RPE for the diffusion-advection analysis than for the diffusion only analysis.

A relevant output to indicate which model is more correct is the ratio of the average concentration in the anterior chamber divided by the average concentration in the vitreous humor. The measured ratio is 0.2 (see Fig. 2 in Maurice (1957)). The diffusion only model employing Maurice's parameters estimates this ratio as 0.35 (a 75% error), while our diffusion model estimates this ratio as 0.2 (0% error). Our diffusion-advection model also estimates a ratio of 0.2 (0% error). This suggests our diffusion model and diffusion-advection models are more likely to be correct models. Our diffusion-advection model is slightly closer to the measured half-life of Na\textsuperscript{24} ions in the vitreous than Maurice's diffusion only model.

The only other model prediction that we can compare is the model estimated diffusion coefficient. Maurice (1957) estimated the diffusion coefficient in vitreous to be 0.9 of that for isotonic saline, while our

### Table 3

<table>
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<th>Model</th>
<th>Diffusion coefficient ( \times 10^{-9} ) m\textsuperscript{2} s\textsuperscript{-1}</th>
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<th>Diffusion only</th>
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<tr>
<td>Author's diffusion-advection model</td>
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<td>3.6</td>
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</tr>
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</table>

Comparison of parameter estimates for diffusion coefficient in the vitreous body for a diffusion only analysis and a diffusion-advection analysis using parameters estimated by Maurice (1957) and those estimated by our model.
diffusion only and diffusion-advection models predict the diffusion coefficient for Na\(^{24}\) in the vitreous is 0.45 (0.86/1.9) and 0.53 (1.0/1.9) of that for isotonic saline respectively. We now investigate the diffusion coefficient predictions more carefully in an attempt to find the most likely value in vitreous humor.

Consider the diffusion coefficient for water and sodium ions in saline solution at 37 degrees C (Table 4). The diffusion coefficient for water in isotonic saline is about 2.84 \(\times\) 10\(^{-9}\) m\(^2\)/s according to Mills and Lobo (1989), while Moseley (1984) estimated the diffusion coefficient for water in vitreous to be about 0.6 of this value (Moseley et al., 1984). Moseley’s analysis would suggest the viscosity of fluid in the vitreous is about 1.7 times greater than that of isotonic saline. The diffusion coefficient of sodium ions in isotonic saline at 37 degrees C, is 1.9 \(\times\) 10\(^{-9}\) m\(^2\)/s (Vitagliano and Lyons, 1956), less than that of water molecules. Maurice (1957) estimated the diffusion coefficient for sodium ions in vitreous humor to be 0.92 of Vitagliona and Lyons estimate. Fitting the data to our diffusion-only model, we found the diffusion coefficient ratio (vitreous over saline) to be 0.45 (Table 4). Fitting sodium diffusion while assuming posteriorly directed aqueous flow through the vitreous, we found the diffusion coefficient ratio (vitreous over saline) for sodium to be 0.53.

Maurice (1957) made his estimate for the sodium diffusion coefficient for vitreous humor based on electrical conductance measurements, rather than estimating the diffusion coefficient from a diffusion (or diffusion-advection) transport analysis of his data, possibly because the difficulty of doing the type of numerical analysis done here was significant at that time. Maurice experimentally found the electrical conductance for vitreous humor was 3%–20% less than the conductance for aqueous humor, so he concluded from this data that the diffusion coefficient for sodium in vitreous would be about 10% less than for sodium diffusion through isotonic saline.

But ion conductance is not the same thing as the ion diffusion coefficient. The electrical conductance is influenced by the solution composition (aqueous humor includes an isotonic mixture of sodium, potassium, chloride and bicarbonate ions, among other ions including hydrogen and hydroxyl ions (To et al., 2002)), ion concentrations (i.e. mainly of sodium, chloride, bicarbonate and potassium), and it also depends on the ‘transference number’ for each ion in solution (i.e. two ions can be at the same concentration and have the same charge but may carry different fractions of the current through the solution because they have different transference numbers).

Further, because there is a fixed negative charge in vitreous humor that have counterions in solution, one might expect the conductance to increase in vitreous humor, but Maurice reports the electrical conductance of vitreous decreased. In addition to geometric tortuosity in the vitreous humor, a likely partial explanation for observed conductance differences is anion exclusion and cation inclusion due to the diffuse-double layers on the macromolecules in the vitreous humor (Smith et al., 2004). The so named ‘Randle’s circuit’ used in modern electrical conductance measurements takes into account the influence of diffuse-double layers in solution (Silue et al., 2017). We also note that the reported electrical conductance of vitreous humor is variable. The conductance has been reported to be equal to that of aqueous humor (Oksala and Lehtinen, 1959), and using a more sophisticated electrical circuit that takes into account the behavior of diffuse-double layers, it is reported to be about 0.7 of the electrical conductivity of isotonic saline (Silue et al., 2017). But the main point to be made here is that changes in the electrical conductance are not directly related to
changes in the diffusion coefficient of sodium ions, as assumed by Maurice, because of the complexity of the non-ideal solution being tested.

The non-uniformity and material complexity of vitreous humor makes it difficult to estimate an appropriate diffusion viscosity for a particular molecule (Bos et al., 2001; Silva et al., 2017). There are so many factors that may be important, it is very difficult to know a priori what may happen. For example, a large diffusing branched molecule like dextran 70 FITC may become mechanically entangled in the macromolecular networks making up the gel, but there again a diffusing branched molecule may deform and make its way through or around obstacles. However, it is interesting to note that Silva et al. find the vitreous humor liquid had a fairly constant viscosity of about 0.002 Pa·s, declining towards 0.0009 Pa·s as the shear rate increased by about an order of magnitude (see Fig. 4a in Silva et al. (2017)). Given the viscosity of water at 37 degrees C is about 0.00069 Pa·s, if these measured viscosities are relevant to molecules and ions diffusing in the vitreous humor, then we might expect the ratio of diffusion coefficient in vitreous humor to that in isotonic saline would be between 0.35 and 0.75, depending on the relevant local shear rate for the diffusing molecule.

But given the difficulties in estimating electrical conductance, vitreal viscosity, the presence of diffuse double layers, and possible interactions of molecules with macromolecular networks, it seems to us most appropriate to look at the actual experimental data on measured diffusion coefficients through vitreous humor for a variety of molecules (Table 5). This data shows the ratio of the diffusion coefficients in saline and vitreous for small molecules of a few hundred molecular weight is in the range of about 0.2-0.5.

Taking all this data together, we expect that the ratio of diffusion coefficients for sodium ions in solution to be somewhat less than that for water molecules (estimated above at 0.6), and towards the top end of the range for small molecules (estimated range 0.2-0.5). Therefore, the ratio of our estimated diffusion coefficient for vitreous (on the basis of our transport models) to that in isotonic saline, 0.45 (diffusion only model) or 0.53 (diffusion-advection model), appear to be plausible estimates, while the estimate made by Maurice of 0.9 on the basis of electrical conductance for an alternating current, is likely to be an overestimate.

We tentatively conclude from our analysis of the Maurice data that our diffusion only and diffusion-advection transport models are most likely more correct than Maurice’s model. The implication of our re-analysis of Maurice’s (1957) data is that it supports the idea that there could be a posteriorly directed flow through the vitreous, but because the diffusion only analysis also provides a good fit to the data, it does not rule out that the vitreous is stagnant. In other words, both solutions are equally likely given the experimental data available.

### Table 4

<table>
<thead>
<tr>
<th>Isotonic saline or vitreous</th>
<th>Mills and Lobo (saline)</th>
<th>Mosley (vitreous)</th>
<th>Reference Vitagliano and Lyons (saline)</th>
<th>Reference Maurice (vitreous)</th>
<th>Reference Author (vitreous)</th>
<th>Ratio of diffusion coefficients (vitreous over saline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion coefficient ($\times 10^{-9}$ m²/s)</td>
<td>2.84</td>
<td>1.73</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>0.6</td>
</tr>
<tr>
<td>Diffusion coefficient ($\times 10^{-9}$ m²/s)</td>
<td>na</td>
<td>na</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
<td>0.92</td>
</tr>
<tr>
<td>Diffusion coefficient for A-D model ($\times 10^{-9}$ m²/s)</td>
<td>na</td>
<td>na</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
<td>0.53</td>
</tr>
<tr>
<td>Diffusion coefficient for diffusion only model ($\times 10^{-9}$ m²/s)</td>
<td>na</td>
<td>na</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
<td>0.86</td>
</tr>
</tbody>
</table>

### 3.3.5. Reanalysis of Gaul and Brubaker (1986) dextran 70 FITC

Recall Gaul and Brubaker employed two fluorometric methods to measure aqueous flow, with both methods employed in each eye pair in four pigmented rabbits: method A, the ‘corneal deposit method’, and method B, the ‘vitreal deposit method’ (Gaul and Brubaker, 1986). Gaul and Brubaker observed that administration of mannitol (an osmotic diuretic) and acetazolamide (a carbonic anhydrase inhibitor) resulted in a substantial decrease in aqueous flow measured by method A (i.e. about a 50% reduction), but only a comparatively small change in the flow rate when measured by method B (13% reduction). This was surprising, because it would be expected that the two methods would detect the same change in aqueous flow.

Employing the same experimental protocol but with water loading for the treatment group, the eye system is driven in the direction opposite to that of mannitol treatment. Aqueous flow was measured to increase by method A (plus 33%), but also to lead to a so-named ‘paradoxical reduction’ when measured by method B (13% reduction) (Gaul and Brubaker, 1986). Gaul and Brubaker suggested the differences in the two methods of measuring aqueous flow could be attributed to eye volume changes associated with changes in intraocular pressure. This volume change probably does cause a short-term effect (probable time scale of minutes), but the findings of Gaul and Brubaker are similar to those of Araie et al., where acetazolamide was administered at the beginning of the experiment and every 1 h for 3 h (Araie et al., 1991), and for this longer experiment Araie et al. obtained similar results to that reported by Gaul and Brubaker (for example, see Fig. 2 in Araie et al., which suggests about a 10–15% reduction in aqueous flow rate over a couple of hours (Araie et al., 1991)). This rules out eye volume change as an explanation for the experimental data.

Our first computational modeling aim is to test if these experimental results can be explained by a decrease in aqueous production accompanied by an increase in posteriorly directed flow of aqueous, and our second computational modeling aim is to explore if this data can tell us if there is a permanent posteriorly directed flow through the vitreous, or otherwise.

In the following figures and tables, we show that it is possible to computationally model the experimental data by a decrease in aqueous production and a simultaneous increase in posteriorly directed flow through the vitreous humor causing a decrease in the amount of dextran FITC entering the aqueous. And once again, a model with either initially diffusive transport acting alone, or a model that has initially both diffusive transport and advective transport acting together can both explain the experimental data satisfactorily.

However, an examination of Table 6 or Table 7 show these two models have different diffusion coefficients, different fractions of dextran FITC exiting via the aqueous humor and different predicted posteriorly directed flow rates. The diffusion coefficient for the diffusion...
Table 5
Diffusion coefficients at for fluorescein, acridine orange and dextran 70 FITC in saline solution and vitreous humor. All diffusion coefficients at 37 °C, unless otherwise noted. Author denotes steady-state diffusion estimates made by the authors using Gajraj’s data, rather than based on breakthrough time, a method employed by Gajraj.

<table>
<thead>
<tr>
<th>Isotonic saline or vitreous</th>
<th>Gajraj (saline)</th>
<th>Gajraj (vitreous)</th>
<th>Shafai (PBS)</th>
<th>Shafai (vitreous)</th>
<th>Xu et al. (vitreous)</th>
<th>Gisladottir et al. (vitreous)</th>
<th>Reference Author (saline)</th>
<th>Reference Author (vitreous)</th>
<th>Ratio of diffusion coefficients (vitreous over saline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion coefficient (× 10⁻⁹ m²/s) Na Fluorescein</td>
<td>16 ± 7.0</td>
<td>6.0</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>0.4</td>
</tr>
<tr>
<td>Diffusion coefficient (× 10⁻⁹ m²/s) Na Fluorescein at 34 °C</td>
<td>na</td>
<td>na</td>
<td>23 ± 4.5</td>
<td>4.6</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>0.2</td>
</tr>
<tr>
<td>Diffusion coefficient (× 10⁻⁹ m²/s) Na Fluorescein</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>10</td>
<td>4.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Diffusion coefficient (× 10⁻⁹ m²/s) Acridine Orange</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>3.4</td>
<td>na</td>
<td>0.5</td>
</tr>
<tr>
<td>Diffusion coefficient (× 10⁻⁹ m²/s) Dexamethasone</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>1.8</td>
<td>na</td>
<td>0.25</td>
</tr>
<tr>
<td>Diffusion coefficient (× 10⁻⁹ m²/s) Dextran 70 FITC</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>10</td>
<td>4.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 6
Transport analysis of Gaul and Brubaker data, with initial diffusion transport only. The initial aqueous and vitreous flow rates are taken to be 1.8 and 0.9 μl/min respectively (total aqueous flow rate 2.7 μl/min). Model fit score based on degree of primarily on accord between model and Gaul and Brubaker data, but also spatially averaged concentration ratio reported in Johnson and Maurice.

<table>
<thead>
<tr>
<th>Treatment type and measurement or prediction</th>
<th>Diffusion coefficient (× 10⁻¹⁰ m²/s) Dextran 70 FITC</th>
<th>Fraction dextran exiting via aqueous with treatment</th>
<th>Half-life (days) of dextran in vitreous before treatment</th>
<th>Ratio of average dextran conc. vitreous over aqueous before and after treatment</th>
<th>Ratio of aqueous flow rates after and before treatment</th>
<th>Ratio after treatment vitreous to aqueous flow rates</th>
<th>Model fit score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetazolamide and mannitol -expt</td>
<td>na</td>
<td>na</td>
<td>7</td>
<td>20/22</td>
<td>50%</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>water loading -expt</td>
<td>na</td>
<td>na</td>
<td>7</td>
<td>20/22</td>
<td>50%</td>
<td>133%</td>
<td>na</td>
</tr>
<tr>
<td>-model</td>
<td>0.35</td>
<td>30%</td>
<td>7</td>
<td>20/22</td>
<td>50%</td>
<td>15%</td>
<td>100%</td>
</tr>
<tr>
<td>-model</td>
<td>0.35</td>
<td>42%</td>
<td>7</td>
<td>20/22</td>
<td>133%</td>
<td>96.5%</td>
<td>100%</td>
</tr>
</tbody>
</table>
only model (Table 6), was fitted to ensure that the ratio of the average concentration of dextran FITC in the vitreous to that in the anterior and posterior chambers was close to 20, as reported for dextran 66 FITC in Table 1 of Johnson and Maurice (1984). The rate of removal of dextran FITC across the RPE was then adjusted to ensure the correct half-life for dextran FITC in the vitreous, as can be inferred from the in vivo data reported in Table 6 of Gaul and Brubaker (1986). Some iteration is required to find the best fit, but once the rate of removal of dextran FITC across the RPE was approximately correct, these two parameters are largely independent of one another.

From Table 2 we see a reasonable estimate for the self-diffusion coefficient in water at 37 degrees C for dextran 70 FITC would be about $0.7 \times 10^{-10}$ m$^2$/s, so the diffusion-only model (i.e. no vitreal flow) fitted value of $0.35 \times 10^{-10}$ m$^2$/s for vitreous humor is half that value (Table 6). This is a plausible ratio between diffusion coefficients in water and vitreous given the data shown in Table 2. For a diffusion-advection analysis we found the mutual diffusion coefficient in vitreous humor for dextran 70 FITC calculated from Gajraj’s data (and then interpolated based on molecular weight) to be about $1.5 \times 10^{-10}$ m$^2$/s, which is very close to the model fitted diffusion coefficient of $1.6 \times 10^{-10}$ m$^2$/s (Table 7).

For the acetazolamide and mannitol treatment cases, the diffusion only model (i.e. with no initial vitreal flow) has half the anteriorly directed flow rate, which is experimentally measured. This model then predicts the posterior directed flow is about 15% of 0.9 μl/min = 0.135 μl/min (or 8.1 μl/h) (Table 6). We can compare this with Araie et al.’s prediction that the posteriorly directed flow is 2.6 μl/h. This suggests Araie et al.’s estimate is an underestimate by a factor of about three, even though we have employed a 30% smaller diffusion coefficient in our model than the diffusion coefficient employed in Araie et al.’s analysis.

Interestingly, for the acetazolamide and mannitol treatment cases the diffusion-advection model (the model has an initial posterior directed flow through the vitreous of 0.9 μl/min) halves the rate of anteriorly directed flow rate (so agreeing with the experimentally measured reduction by method A), yet the model predicts the posterior directed flow is increased by about 33% of 0.9 μl/min = 0.3 μl/min (or 18.0 μl/h) (Table 7). We observe the increment in posteriorly directed flow is more than twice as large as that predicted by the model initial posterior flow. If the initial vitreal flow were larger, say doubled to 1.8 μl/min, the increment in posteriorly directed flow would also be commensurately larger i.e. it would approximately double to 36 μl/h. This is interesting because Negi and Marmor report that acetazolamide given intravenously at 50 mg/kg to rabbits (i.e. the same dose used by Gaul and Brubaker) increased the rate of subretinal resorption by about 75% (i.e. 0.16 ± 0.06)/(0.09 ± 0.04); see Table 1 in Negi and Marmor (1986). If the initial rate of resorption across the RPE is 54 μl/h (i.e. equal to 0.9 μl/min times 60 min), then increasing the resorption rate by 18 or 36 μl/h represents a 33% or 66% increase respectively, which is certainly in the range of measurement uncertainty reported by Negi and Marmor, and so is compatible with Negi and Marmor’s experimental data.

Though the two models (i.e. one with initially diffusion only and one initially with a posterior flow) both fit the data very well and so cannot be distinguished on the basis of Gaul and Brubaker’s data, the data of Negi and Marmor does lend support to the model that has an initial posteriorly directed vitreal flow. Importantly, we observe that the model with the initial posterior flow, agrees with the experimentally measured 50% decrease in the anterior flow rate, but this model only has a 22% decrease in aqueous production (i.e. a $(1 - \frac{(0.9 \times 0.3) + (1.8 \times 2)}{2})$ decrease in aqueous production). We highlight that this new model prediction contrasts with the standard fluid flow model, which predicts a 50% decrease in the anterior flow rate is caused by a 50% decrease in aqueous production.

Importantly Araie et al. (1991) provides us with additional data for
a similar experiment to that by Gaul and Brubaker. The IOP is reported
to decrease by about 33%, from 20 mmHg to about 13 mmHg (see
Figs. 3 and 5 in Araie et al. (1991)). According to our pressure de-
pendent out
fl
ow facility model, with
\alpha = 0.07
5 , this corresponds to a
27% decrease in aqueous production, which reasonably closely ap-
proximates our di
ff
usion-advection model estimated 22% decrease in
aqueous production. In contrast, the model with no initial posterior
flow predicts a 50% decrease in aqueous production, which appears to
be too much on the basis of this IOP data. Again, this data lends support
to the new model with an initial posteriorly directed vitreal
flow.
So we conclude from this that the model with posteriorly directed vitreal
flow better
fits the known data sets.

However, although the averaged concentration of dextran FITC
variation over time predicted by the diffusion only model and the dif-
fusion-advection models are very similar (compare Figs. 7 and 8),
the concentration profiles within the vitreous humor may be noticeably
different. For example, compare the predicted dextran FITC distribu-
tions in the eye at 24 days shown in Figs. 7 and 8. The observed dif-
ference is clearly driven by the advective component of dextran 70
transport, which becomes relatively more important as the magnitude
of the diffusion coefficient decreases. Tan et al. report on the dis-
tribution of dextran 150 along the optical axis of the eye, with both
intact and partially liquefied vitreous present (Tan et al., 2011):

The plot derived from the ocular fluorophotometry suggests there was a
temporary forward flux in the partially liquefied vitreous model during
the first few hours after injection. The same observation was not noted
for in normal vitreous. This result indicates that the flow processes in the
liquefied vitreous generated this movement.

Overall, the fluorophotometry plots shown in Tan et al. do not show
the same increase in dextran 70 FITC concentrations posteriorly, as
predicted in Fig. 8. Why there is disagreement between the experiments
and the model prediction is uncertain at this stage. However we note
that Johnston and Maurice report that (Johnson and Maurice, 1984):

When the fluorescent dextran was injected into an eye with a dilated
pupil, it was seen initially to remain as a discrete volume mainly in mid-
vitreous. As it spread by di
ff
usion, it also sank in the cavity and much of
it formed a pool at the bottom, so that at first the lower levels were more
heavily stained that the upper.

It seems most likely the 'sinking bolus' is density driven, and this
needs to be included in a more re
fi
ned transport model for dextran
within the vitreous humor, than the model attempted here. In addition,
we expect the distribution of dextran FITC fluorescence could be sig-
ificantly modified by a type of reversible binding of dextran FITC to
vitreous macromolecules, which incorporates a threshold due to sa-
turation of binding sites. It is also possible that pH e
ff
ects on FITC
fluorescence may need to be taken into account close to the retina,
where pH is usually reduced.

4. Discussion

4.1. The balance of evidence

It is widely held that aqueous produced by the ciliary body exits the
eye anteriorly, with little if any of the aqueous production travels
posteriorly through the vitreous humor, exiting across the RPE. In this
paper we have reanalyzed the foundational research on which this
assumption is based, and found the evidence supporting this view to be weak.

First, we demonstrated that diffusion of trititated water from the vitreous humor occurs rapidly compared to vitreal advective flow, so that even though the data is said to have been explained by diffusion alone, the experiment is not of sufficient accuracy to rule out significant posterior flow through the vitreous humor. In fact, this point was made previously by Mosley et al. (1984). Furthermore, although Maurice concluded that diffusion alone can explain the loss of radioactive sodium ions from vitreous humor (Maurice, 1957), we have shown it to be based on an unrealistically large diffusion coefficient. While a more realistic diffusion coefficient also results in a solution that can explain the experimental data, so too can a solution involving both diffusive and advective transport with a posteriorly directed flow. In other words, concluding on the basis of this experiment that there is no posterior flow is not justified.

We then examined Maurice’s scleral puncture experiment (Maurice, 1987), which involves the creation of needle holes in the posterior sclera to augment posteriorly directed flow. After assuming no initial posteriorly directed vitreal flow, Maurice employed an analytic solution of a 1D diffusion-advective equation and found it could fit the experimental data. Maurice then concluded that the needle hole created a very small posteriorly directed flow, and there is no initial posteriorly directed vitreal flow (Maurice, 1992) despite it being a modeling assumption (Maurice, 1987). Maurice also argued if the initial posteriorly directed flow was as large as measurements of subretinal fluid absorption said it was, it would create an initial Pecllet number of about nine, which was so large that no dextran FITC would reach the anterior chamber, and this is incompatible with the experimental data (Maurice, 1987). However, using Maurice’s own 1D analysis and assuming an initial posteriorly directed flow, we have shown that a model that does assume a posteriorly directed vitreal flow can fit the data he collected just as well as the model that assumes no initial posteriorly directed vitreal flow. We explain that the 1D transport model is not an appropriate model to use in any case, given the non-linear transport behavior of the eye system. Finally, we confirm using a 3D diffusion advection model that these model predictions are compatible with Maurice’s experimental data, again, with or without a significant posterior flow. In other words, concluding there is no posteriorly directed vitreal flow on the basis of this experiment is also not justified.

We reanalyzed Gaul and Brubaker’s data (Gaul and Brubaker, 1986), and showed that the measurements following administration of acetazolamide, mannitol and water loading can be explained by both a diffusion only model and a model with a significant initial posteriorly directed vitreal flow. However, the model with a significant initial posterior vitreal flow is more consistent with the increment in posterior vitreal flow, as measured by the increment in subretinal resorption following intravenous administration of acetazolamide, as reported in Negi and Marmor (1986).

Further, using a similar experimental protocol to that employed by Gaul and Brubaker, Araie et al. collected data that is similar to that of Gaul and Brubaker, but Araie et al. also measured changes in IOP (Araie et al., 1991). The reported change in IOP accompanying treatment with acetazolamide is consistent with our pressure dependent outflow model which predicts a posteriorly directed flow (i.e. the reduction in aqueous production of 22% predicted by the model with an initial posteriorly directed vitreal flow is consistent with the measured decrease in IOP (Smith et al., 2019b)). Our analysis of Gaul and Brubaker’s data, Negi and Marmor’s data, and Araie et al.’s data, shows it is not justified to conclude that there is negligible or no initial posteriorly directed vitreal flow. In other words, key experimental data used as evidence in support of the notion that there is no posteriorly directed flow is very weak, and in fact Negi and Marmor and Araie et al.’s data provides some limited evidence supporting a posteriorly directed vitreal flow.

On the other hand, there is a very substantial amount of independent data supporting the notion there is a significant fluid flow across the RPE. This conclusion is supported by studies on epithelial sheets in cell culture (Shi et al., 2008; Li et al., 2009; Adjianto et al., 2009; Baetz et al., 2012) and numerous ex vivo experiments on RPE tissue and numerous in vivo experimental measuring the rate of resolution of subretinal blebs (Marmor et al., 1980; Miller et al., 1982; Hughes et al., 1984; Negi and Marmor, 1986; Tsuibo, 1987; Tsuibo and Pederson, 1988; Kawano and Marmor, 1988; Marmor, 1990; Dahrouj et al., 2014; Edelman and Miller, 1991). There is also data on the rates of resolution of subretinal blebs in humans (Chihara and Nao-i, 1985), which suggests a flow rate of about 2.5 μl/min (Quintyn and Bresser, 2004). Interestingly the in vivo rate of fluid transport reported by Chihara and Nao-i is comparable to those reported by Adjianto et al. for cultured sheets of human RPE (Adjianto et al., 2009).

There is also a substantial body of independent evidence suggesting the rate of fluid transport across the RPE is coupled to net ion transport across the RPE (Gallemore et al., 1997), and that net ion transport is modulated by the transepithelial (electrical) potential (Frambach et al., 1990; Edelman and Miller, 1991; Gallemore et al., 1997; Strauss, 2005, 2014). It is known that the transepithelial potential is nearly always non zero (normally between 5 and 15 mV) (Gallemore et al., 1997; Strauss, 2014). This potential is modulated by the amount of light exposure (Strauss, 2005), by various autocrine, paracrine and hormonal substances (Edelman and Miller, 1991, 1992; Dahrouj et al., 2014), as well as by various drugs (Yonemura and Kawasaki, 1979; Gaul and Brubaker, 1986; Frambach et al., 1990; Araie et al., 1991). The fluid flow across the RPE (Cantrill and Pederson, 1984) and the transepithelial potential are modified by the separation from the overlying retina, but how this potential and flow varies with size and duration of the detachment remains to be experimentally quantified.

Based on the conflict between their interpretation of their own data suggesting little or no posteriorly directed flow (Maurice, 1957, 1987; Araie et al., 1991), and the data mentioned in the previous two paragraphs suggesting a substantial posteriorly directed flow, Maurice and Marmor concluded that there was a conundrum—how could both sets of data be correct (Maurice, 1992; Marmor, 1990)? Marmor tried to resolve the conflict by suggesting that retinal resistance to fluid flow was substantial, and so apposition of the retina and RPE essentially blocked flow across the RPE. But this interpretation is not supported by the evidence, as the data Marmor offered in support of this contention actually says the opposite. On every count, we observe that the data said to support no posteriorly directed fluid flow is shown to be weak or very weak, while the data supporting significant fluid flow across the RPE is comparatively strong.

More recently, Smith and Gardiner developed a pressure dependent outflow model of the human eye that predicted about half of the aqueous production is removed from the eye via anterior routes (i.e. trabecular meshwork and the uveoscleral routes) and the other half via the RPE (Smith and Gardiner, 2017). A subsequent 3D model of the human eye based on this pressure dependent outflow model Smith and Gardiner could explain the rise in the IOP observed when a silicon oil tamponade is inserted following vitrectomy, while the same model could also explain the rise in IOP observed in Schwartz-Matsuo syndrome (Smith et al., 2019b). Importantly, the standard fluid flow model of the human eye, which assumes no significant posteriorly directed vitreal flow, predicts no increment in IOP associated with introduction of a silicon oil tamponade, which is inconsistent with the clinical data (Jonas et al., 2001). Therefore our new fluid flow models provide further support that there is a significant posteriorly directed flow of aqueous humor.

For the normal human eye, we estimate that the total retinal capillary leakage and metabolic water production is less than a couple of percent of the posteriorly directed aqueous flow estimated by Smith et al. (2019b), so these secondary fluid sources make a negligible contribution to total RPE flow. We also observed that light-dependent increases in transepithelial potential and fluid flow may be synchronous with known circadian variations in aqueous production at the ciliary
body, though this has not been experimentally investigated at the present time.

Based on the foregoing discussion, let us assume that there is in fact a significant posteriorly directed vitreal flow, and ask the question: what are the implications?

4.2. What are the implications for the new outflow model?

The main implications of this new outflow model of eye physiology have to do with vitreal transport modeling, the interpretation of measured drug effects on fluid transport, and the interpretation of outflow facility measurements on the in vivo eye. We consider each in turn.

4.2.1. Vitreal transport

We have considered vitreal transport in some detail in Section 3.3.1. What comes through strongly is the complexity of vitreal transport modeling. One source of significant uncertainty is the diffusion coefficient, which is strongly influenced by the non-uniform viscosity of the eye, the diffuse double-layers between the structural components of the vitreous and the tortuosity imposed by relatively immobility of the structural components of the vitreous. In addition, the diffusion coefficient at infinite dilution for a molecule in isotonic saline may be considerably different from its mutual diffusion coefficient, which is modified by the interplay between hydrodynamic and thermodynamic factors (associated with the concentration of the molecule and its concentration gradient (Laurent et al., 1976; Comper et al., 1986)). And when structural macromolecules in the vitreous are taken into account, the mutual diffusion coefficient is modified again, due to the multi-component nature of the background matrix (Laurent et al., 1979). This complexity means that accurate diffusion coefficients can only be reliably estimated from in vitro vitreous diffusion experiments, of which there are relatively few studies for a limited number of molecules (Gajraj, 2012; Shafaei et al., 2018).

Another source of uncertainty is hindrance factors that are appropriate for advective transport through the vitreous humor. To date, there is only one preliminary qualitative study on hindered vitreal transport, but this paucity of data is understandable given the prevailing view that advective transport is not needed because there is negligible or no posterior vitreous flow.

Another very significant transport modeling uncertainty is reversible chemical reactions between the transported molecule and the background matrix. It is apparent that if there is reversible binding of the molecule to the vitreous molecules (of which there are thousands), then the transient rate of molecular migration through the vitreous is slowed, and the subsequent ‘washout’ of the molecule will be prolonged following intravitreal injection. The extent of possible intersections between various transported molecules and the vitreous is largely unexplored.

4.2.2. Interpretation of measured drug effects

The interpretation of the effects of an intravenous injection of acetazolamide on eye fluid physiology, as considered in Section 3.3.5, provides an appropriate example to illustrate how the interpretation of drug effects on the eye are modified by taking into account outflow across the RPE. For if it is assumed that the vitreous is stagnant and fluid only leaves the eye via anterior pathways, then a change in anterior flow rates is equal to the change in aqueous production at the ciliary body, and we are led to Brubaker and Gaul's and Araie et al.'s interpretation of the effect that intravenous injection of acetazolamide has on eye fluid physiology. Brubaker and Gaul report that ‘aqueous production’ decreases approximately 50% as measured by the corneal deposit method, as does Araie et al. (1991).

But if it is assumed there is initially a significant posterior vitreal flow due to outflow across the RPE, then the reduction in anterior flow may be attributed to some combination of a reduction in aqueous production and an increment in outflow across the RPE. For our rabbit model with a posterior flow, the reduction in aqueous production is estimated to be about 22%, while the redirection of aqueous through the vitreous humor due to the increase in RPE outflow explains the other 28%. This increment in posterior flow through the vitreous is consistent with the reported effect of acetazolamide on fluid transport across the RPE (Negi and Marmor, 1986). We note that the standard fluid flow model for the eye is not consistent with the reported effect intravenous acetazolamide has on the RPE.

According to the outflow model presented here, this means that the effect of intravenous acetazolamide on fluid flow is of roughly similar magnitude at the ciliary body and at the RPE (i.e. the 22% reduction in aqueous compared to the 33% increase in RPE flow). If this is true for acetazolamide, then clearly the implication is that other drugs may have effects at both the ciliary body and the RPE (e.g. timolol (Kazemi et al., 2019)). This means that each of the reported drugs effects on fluid flow through the eye need to be reinterpreted in terms on our new outflow model for the eye.

4.2.3. Interpretation of measured outflow facility

We have developed a new pressure-dependent outflow model for the whole eye, which we calibrated using data on humans and animals that is available in the literature (Smith and Gardiner, 2017). The model has three important parameters: the hydraulic conductivity for the whole eye \( C_{L}^{p} \), a no outflow pressure \( p_{n} \), and an exponential decay constant, \( \alpha \), which reduces increments in outflow as IOP increases. Assuming a hydraulic conductivity of 1 \( \mu \text{LMH/m} \text{mHg} \) at zero mmHg, a no outflow pressure of 3 mmHg, and employing an \( \alpha = 0.075 \) we found that the calibrated outflow model predicted an aqueous outflow (which at steady state is always equal to the rate of aqueous production) to be about 6.3 \( \mu \text{LMH/m} \text{mHg} \) at 15 mmHg (see Fig. 9).

This is about twice the usual reported value for aqueous production, which is
typically reported to be 2.5–3.0 μl/min via anterior outflow pathways (Brubaker, 1991; Toris et al., 1999; Goel et al., 2010).

The way the model predicted outflow varies as it varies is shown in Fig. 9. Of course, the α varies from person to person, as do the other model parameters. Nevertheless, it is apparent from Fig. 9 that as α (mm Hg−1) becomes larger, the aqueous outflow (μl/min) plateaus at lower magnitudes. In other words, as α increases and maximum outflow reduces, the model predicts the IOP becomes unstable, as then a small change in aqueous production leads to a large change in IOP. Such instability in IOP is known to be an independent risk factor for glaucoma (Song et al., 2014; Agnifili et al., 2015).

An interesting property of this new outflow model is that it is consistent with many of the current estimates of outflow facility (μl/min/mm Hg), despite the aqueous production rate being more than twice that normally quoted, because the outflow through anterior pathways is of similar magnitude to the standard outflow model at 15 mm Hg. And we see that the average outflow facility between 15 and 30 mm Hg (denoted Cfl 15−30 i.e. slope of a secant over a pressure range between 15 and 30 mm Hg) for the standard outflow model and the new outflow model are similar (see parallel solid lines shown in Fig. 9). However, there is clearly an upward ‘translation’ in the secant location for the new outflow model compared to the standard outflow model, as the outflow rates from the eye predicted by the two models are quite different.

Referring to Fig. 9, we note that Toris et al.’s data (Toris et al., 1999) is obtained both fluorometrically (using the corneal deposit method) and pneumatography, while the Kazemi et al.’s data (Kazemi et al., 2017) is obtained by using pneumatography and digital Schiötz tonography. We observe the data of Toris et al. and Dijkstra et al., and the data of Kazemi et al. are obtained over different pressure ranges. Toris et al.’s fluorometric data is obtained over an estimated pressure range 10 mmHg−15 mmHg (Toris et al., 1999), while Dijkstra’s anterior segment outflow data is obtained over the pressure range 0 mmHg−15 mmHg (Dijkstra et al., 1996) (shown in Fig. 9). Kazemi et al.’s tonography data is obtained between 15 mmHg and 30 mmHg (Kazemi et al., 2017)) (shown in Fig. 9).

For the standard flow model, the similarity of the measured outflow facilities over these two pressure ranges, zero to 15 mmHg, and 15 mmHg−30 mmHg, is taken as evidence that the outflow facility is constant (i.e. outflow facility is independent of IOP), as assumed by the Goldmann equation (Brubaker, 2004) and modified Goldmann equation (Brubaker, 2004; Kazemi et al., 2017).

Now the reason for the translation in location of the solid line for the standard outflow model compared to the new model (as shown in Fig. 9), is interesting. The new outflow model takes account of pressure dependent outflow facility (Moses, 1977; Ericksonlamy et al., 1991), while the standard model does not, and the new outflow takes account of outflow across the RPE, which the standard model does not. It is the interplay between the increased outflow and pressure dependence of the outflow for the new model that causes the upward translation in the slope for the Kazemi et al. data. So the new outflow model that takes into account pressure dependent outflow facility, is consistent with Kazemi et al.’s tonography data, just as the standard fluid flow model is, but the new outflow model is consistent with the Kazemi et al. data in a completely different way to that for the standard outflow model. And while the standard outflow model is consistent with Toris et al.’s fluorometric data, just as the new outflow model is, the outflow facility for the anterior pathways over the pressure range 0 mmHg−15 mmHg is approximately 0.4/2 = 0.2 because the new fluid model for the human eye has approximately half the total outflow exiting via the anterior chamber (see Fig. 9).

Despite the similarities in the two models, the crucial point to be made here is that the new outflow model is consistent with a greater range of data than the standard outflow model. For example the new outflow model is consistent with pressure dependent outflow reported by Brubaker for enucleated eyes (Brubaker, 1975). Brubaker reports data showing an outflow for the whole eye of 6.0 μl/min at 15 mmHg, equivalent to a mean outflow facility over the pressure range zero to 15 mm Hg (Cfl 0−15) of about 0.4. Similar experimental findings are reported by Dijkstra et al. for outflow from whole enucleated eyes (which also shows about 6 μl of outflow at 15 mmHg; see dashed line shown in Fig. 9 (Dijkstra et al., 1996)). However, the standard flow model is not consistent with Brubaker’s data and Dijkstra et al.’s data (i.e. at 15 mm Hg the outflow for the standard model is believed to be about 0.2 μl/min/mm Hg × 15 mm Hg equals around 3.0 μl/min, not 6.0 μl/min as measured by Brubaker and Dijkstra et al.).

The new outflow model is also consistent with crucially important in vivo data on the human eye. Prior to cataract surgery, Dastiridou et al. (2013) and Karyotakis et al. (2015) measured pressure-volume and pressure-time data for in vivo human eyes. From this data they (unusually) calculated the in vivo ‘local outflow facility’ for the human eye (i.e. the ‘local’ or ‘tangent’ outflow facility, denoted Cfl). Karyotakis found that the local outflow facility for the whole eye decreased substantially from 0.27 μl/min/mm Hg at 20 mmHg to just 0.067 μl/min/mm Hg at 40 mmHg, almost a fourfold decrease in the local outflow facility (Karyotakis et al., 2015). While the new pressure dependent outflow model is consistent with this data (see excellent agreement between model and data (Smith and Gardiner, 2017), as depicted in Fig. 10), the standard outflow model is not consistent with this data.

And Friberg et al. measured IOP of young adult subjects, first in the supine position, and then again upon assuming a head-down inverted position (Friberg et al., 1987). Friberg et al. reports that the Pearson correlation coefficient (i.e. slope of regression line) between the measured change in EVP and the measured change in IOP is 0.83 ± 0.21, and that IOP approximately doubles in the inverted position relative to normal IOP. With a small change in the no flow pressure (pfl) from 3 mm Hg to 2.5 mmHg, we found excellent consistency between the new outflow model and this data set (Smith and Gardiner, 2017), as depicted in Fig. 10. However, the standard outflow model is not consistent with this data. We also mention that the new outflow model is consistent with pressure-dependent outflow measurements reported in Ericksonlamy et al., while the standard outflow model is not (Ericksonlamy et al., 1991).

Finally we again point to the success of the new outflow model in being able to predict both the IOP rise and fall following insertion and removal of a silicon oil tamponade, and the rise and fall in IOP associated with Schwartz-Matsuo disease and its treatment (see Section 2.4). In summary, we see that the new outflow model for the eye is consistent with more high quality data sets than the standard outflow model. We observe that this difference in model predictions becomes clinically important when it is recognized that the silicon oil tamponade investigation suggests it may be possible for ocular hypertension or a glaucoma type to arise from a reduction in outflow facility across the RPE.
5. Conclusions and future directions

Though it is widely held that there is little or no aqueous fluid transport through the vitreous, we conclude the evidence supporting this contention is weak. On the contrary, evidence supporting fluid flow through the RPE is comparatively strong. Though it is difficult to make pronouncements on the existence of significant fraction of aqueous production flowing posteriorly through the vitreous humor and across the RPE, on the evidence available today, we believe it is on balance more likely there is significant posterior directed flow of aqueous through the vitreous humor and across the RPE than there is not. This conclusion has significant research and clinical implications; for the interpretation of outflow facility measurements, for understanding the origin of changes in IOP with the introduction of silicone oil tamponade and for Schwartz-Matsuo syndrome, for drug distribution in the vitreous and for the interpretation of drug effects on aqueous dynamics. It also has significant implications for understanding some types of glaucoma.

However it is clear from the above that defining quantitatively the amount of posteriorly directed aqueous flow presents a difficult problem about which to make definite statements, so further research is warranted. This uncertainty is due to the variability of vitreous humor, and the non-uniformity of this extracellular matrix that has complex material properties that make it difficult to predict or choose on the basis of experimental data appropriate model parameters for transport analyses to predict drug distributions within the vitreous humor. Hindrance coefficients appropriate for advective transport analysis in the vitreous humor are unknown at present, and there is no experimental data currently available and no theory with which to predict an appropriate parameter value. In the immediate future, this suggests that experiments to detect posteriorly directed flow may be more straightforward to interpret when performed following vitrectomy. Solute tracers would then preferably be contained within negatively or neutrally charged ‘nanospheres’, that are neutrally buoyant, and of such a size they ensure a very small diffusion coefficient. This system would provide the best opportunity to visualize posteriorly directed advective transport of the labelled nanospheres.

There also appears to be scope for further experiments on the change in transepithelial potential when the retina is in contact with the RPE, compared to when it is detached from the RPE. Further research is required to ascertain if the transepithelial potential has a circadian variation. And further research is needed to determine if fluid transport across the RPE in vivo is uniform, as assumed for the new outflow model discussed here.

It would be valuable and revealing if new technologies for injecting or withdrawing fluid from the eye at a constant rate, while rapidly establishing a steady state IOP (Stockslager et al., 2016; Dattilo et al., 2019) were applied to the human eye. For then it should be possible to establish if our new outflow model correctly predicts changes in IOP with outflow, particularly at IOPs lower than the normotensive pressure. And if the new outflow model is not correct, such data should suggest how the new outflow model needs to be modified so it does agree more closely with such data.

It is also clear that there are negative feedback processes operating in the eye, both at anterior outflow pathways (Acott et al., 2014) and at the RPE (Hao et al., 2016; Bousquet et al., 2019), suggesting models developed using data from short-term tests on the eye may not be representative of the eye’s longer term behavior. Either negative feedback mechanisms could operate locally (Acott et al., 2014), or they could involve paracrine signaling via the aqueous humor (Shin et al., 2012; Rogers et al., 2013; Reina-Torres et al., 2017), and this regulation may involve a variety of signaling molecules being transported across the vitreous humor.

It is apparent that the computational model we have proposed could be improved in a variety of ways: (i) by including an individual model for each outflow pathway within the eye, with the outflow behavior of the whole eye being the sum of these individual outflow models (Smith and Gardiner, 2017), (ii) by exploring different mathematical relationships between rate of change of IOP with respect to the reference pressure other than an exponential relationship employed to date (Smith and Gardiner, 2017), and (iii) by including negative feedback processes.

We conclude by agreeing with Maurice that it is almost certain that ‘the vitreous still has mysteries to reveal’, as he observed almost 30 years ago (Maurice, 1992).

Appendix. 3D eye flow model with pressure dependent outflow

The rabbit eye is represented as an elliptic structure with a major and minor axes of 20 and 19 mm (see Fig. 11 and Table 8). The geometry of the rabbit eye and its lens are based on the schematic eye geometry depicted in Fig. 1 of (Hughes (1972)). Fluid flows from the ciliary body into the posterior chamber of the eye, and then to outflow pathways anteriorly. But in addition, fluid flows from the ciliary body into the posterior chamber of the eye, and then in a posterior direction through the vitreous and across the RPE. The very small flow along the optic nerve is neglected from this analysis (Smith et al., 2019b). The lens and iris are treated as impermeable for simplicity.

We mention that the relatively simple eye geometry chosen here means the anterior chamber is larger than it should be in the rabbit. While this makes little difference to average concentration of the anterior chamber when changes occur slowly (as they do in the dextran 70 FITC analysis), they do have a significant effect when the changes are relatively fast, as the in the sodium 24 analysis. For this reason, in the sodium 24 analysis only, we averaged the anterior and posterior chamber concentrations on the region with the vertical axis greater than minus 0.0058 m (see Fig. 11).. Further, we are told that the volume of the vitreous humor chamber is 1.7 ml (Maurice (1957)), so we averaged the vitreous chamber over the region with the vertical axis greater than 0.0 m and multiplied by 2.3/1.7 to take account of the somewhat greater volume of the vitreal chamber in the model.

The parameters employed in the flow model are shown in.

Table 9, which are basically the same as those parameters employed in (Smith et al. (2019b)) for the human eye. The only change is the hydraulic conductivity across the RPE (C_PV), which is reduced in proportion to the reduction in surface area (i.e. C_PV reduces from 0.5 to 0.275 because the surface area of the rabbit retina for the rabbit model geometry is 55% of the surface area of the retina for the human model geometry). We also mention that the thickness of the retina in the rabbit eye model remains unchanged from the human model at 200 μm, though the rabbit retina is around 100–150 μm in thickness. This is not expected to materially influence rabbit model predictions.

SI units are employed in the computational model for all analyses, but to make the presentation of information more familiar we report in units more often used in eye physiology, as deemed appropriate (for examples, we report IOP in mmHg, and the aqueous production rate μl/min). The 2D geometry cross-section of the model rabbit eye is shown in Fig. 11, and a 3D perspective of the model rabbit eye model is shown in Fig. 11. Further geometric details of the model rabbit eye are given in Table 8.
Fig. 11. Axisymmetric 2D cross-section of 3D rabbit eye model. AC is anterior chamber, PC is posterior chamber, VH is vitreous humor, MVD is region of mid-vitreal chemical deposit and L is lens. (see measurements in Table 1; scale meters).

Table 8
Geometry for model of rabbit eye.

<table>
<thead>
<tr>
<th>Anatomic Structure</th>
<th>Model Geometry</th>
<th>Experimental Measurement</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor (anterior-posterior) axis</td>
<td>19 mm</td>
<td>19 mm</td>
<td>Hughes (1972)</td>
</tr>
<tr>
<td>Major (inferior-superior) axis</td>
<td>20 mm</td>
<td>20 mm</td>
<td>Hughes (1972)</td>
</tr>
<tr>
<td>Depth of lens</td>
<td>7.9 mm</td>
<td>7.9 mm</td>
<td>Hughes (1972)</td>
</tr>
<tr>
<td>Distance posterior of lens surface to retina</td>
<td>6.9 mm</td>
<td>6.7 mm</td>
<td>Hughes (1972)</td>
</tr>
<tr>
<td>Width of lens</td>
<td>9.8 mm</td>
<td>In vivo eye, 9–10 mm</td>
<td>Hughes (1972)</td>
</tr>
<tr>
<td>Retinal surface area</td>
<td>7500 mm²</td>
<td>6100 mm³</td>
<td>Maurice (1957)</td>
</tr>
<tr>
<td>Vitreal volume</td>
<td>2300 μl</td>
<td>2400 μl</td>
<td>Bakri et al. (2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1500 μl</td>
<td>del Amo et al. (2017)</td>
</tr>
</tbody>
</table>

Table 9
Model parameters for rabbit eye.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Symbol</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous Production Rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole eye surface hydraulic conductivity</td>
<td>C_s²</td>
<td>0.775</td>
<td>μl/mmHg/min</td>
</tr>
<tr>
<td>RPE surface hydraulic conductivity</td>
<td>C_r²</td>
<td>0.275</td>
<td>μl/mmHg/min</td>
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<tr>
<td>Anterior pathways surface hydraulic conductivity</td>
<td>C_a²</td>
<td>0.5</td>
<td>μl/mmHg/min</td>
</tr>
<tr>
<td>Exponential decay constant</td>
<td>α</td>
<td>0.075</td>
<td>1/mmHg</td>
</tr>
<tr>
<td>No flow pressure</td>
<td>Pr</td>
<td>3</td>
<td>mmHg</td>
</tr>
<tr>
<td>Whole eye back pressure</td>
<td>P_back</td>
<td>0</td>
<td>mmHg</td>
</tr>
<tr>
<td>Hydraulic conductivity anterior chamber</td>
<td>k_a</td>
<td>1.0×10⁻⁴</td>
<td>m²/Pa.s</td>
</tr>
<tr>
<td>Hydraulic conductivity vitreous humor</td>
<td>k_v</td>
<td>8.4×10⁻¹¹</td>
<td>m²/Pa.s</td>
</tr>
<tr>
<td>Hydraulic conductivity retina</td>
<td>k_ret</td>
<td>5.0×10⁻¹⁴</td>
<td>m²/Pa.s</td>
</tr>
</tbody>
</table>

References


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Kaplan, J.R., Michel, C.C., 2010. Microvascular fluid exchange and the revised Starling references between the peripheral and the central nervous system in permeability to sodium fluorescein. J. Physiol. 513, 133–147.


Mauric, D.M., Salmoiraghi, J., Zouhal, H., 1971. Subretinal pressure and retinal adhe-
siveness. Eye. 12, 212–217.
Smith, D.W., Gardner, B.S., 2017. Estimating outflow facility through pressure depen-
Tan, L.E., Orilla, W., Hughes, P.M., Tsai, S., Burke, J.A., Wilson, C.G., 2011. Effects of vitreous liquefaction on the intravitreal distribution of sodium fluorescein, fluor-
Tuiboi, S., Pederson, J.E., 1987b. Permeability of the blood retinal barrier to carbokxy-
Tuiboi, S., Pederson, J.E., 1988. Volume flow across the isolated retinal pigment epi-

