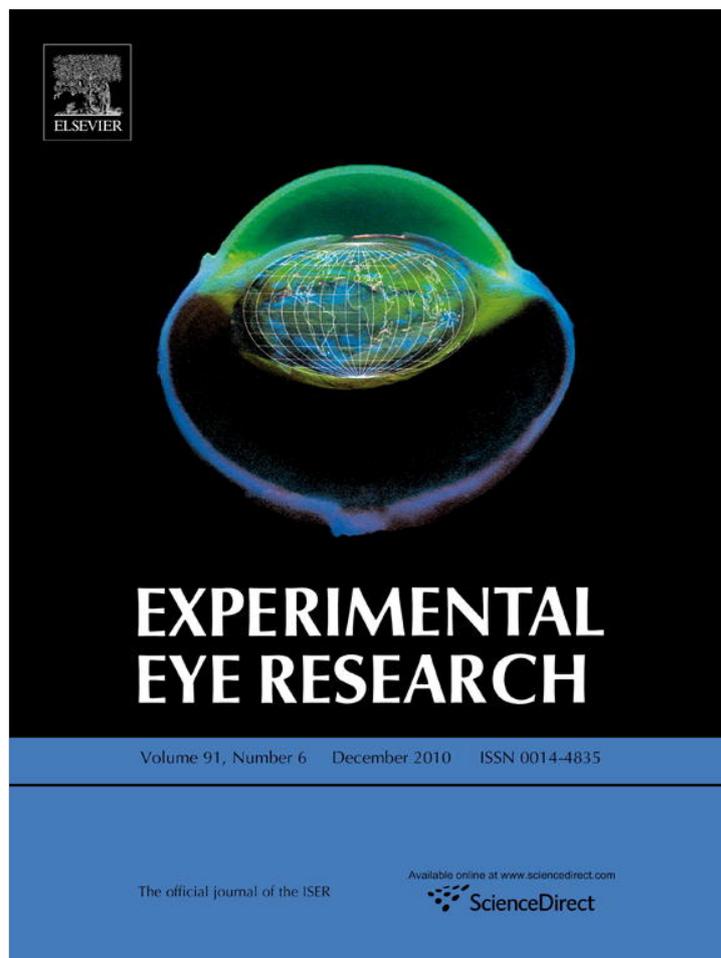


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Adequacy of exchanging the content of the anterior chamber[☆]

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ABSTRACT

Perfusion studies of the anterior segment of the eye frequently involve an exchange of the contents of the anterior chamber. We here determined how much fluid was necessary to pass through the upstream tubing and anterior chamber such that the contents of the anterior chamber were adequately exchanged. We used fluorescein dextran (500 kD) to assess the adequacy of exchange of enucleated porcine eyes that were washed out with varying volumes of buffer. The results were compared with two theoretical models, one that accounted for the convective dispersion that occurs in the upstream tubing while in the other, more simple model, it was assumed that the upstream tubing and anterior chamber behave essentially as a well-mixed chamber. We found that the experiment results were bounded by these two models, with the well-mixed model giving a lower bound on the rate at which the anterior chamber can be cleared. We found that exchange of the anterior chamber to reduce the concentration of a drug or tracer by 20-fold required a perfused volume three times the combined volume of the upstream tubing and anterior chamber.

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1. Introduction

Perfusion studies of the anterior segment of the eye frequently involve a step whereby the contents of the anterior chamber (AC) are exchanged in order to find the effect of a drug on tissues of the anterior segment. Such experiments occasionally also involve a step whereby this drug is then removed from the anterior segment by again exchanging the content of the AC. The question arises as to how much fluid need be passed through the AC such that an adequate exchange is achieved. Such consideration must naturally also concern the volume of the tubing whereby these agents are perfused into the AC.

In this study, we used fluorescein dextran to assess the adequacy of exchange of enucleated porcine eyes that were washed out with varying volumes of buffer. The results are compared with two theoretical models.

2. Methods

Following a baseline measurement of outflow facility (to ensure that the eye was behaving normally), the AC of each porcine eye was exchanged with 4–8 ml of fluorescein dextran in buffer to replicate the conditions by which drugs are usually introduced to the AC.

Then a variable quantity of buffer (without fluorescein dextran) was exchanged through the eyes and at the conclusion of the experiment, the concentration of fluorescein dextran remaining in the AC was determined. The effects of quantity of buffer exchanged and volume of tubing used were determined.

2.1. Materials

A total of 22 porcine eyes were obtained from Park Packing Company (Chicago, IL). The eyes were kept chilled during transport and all experiments were started within 6 h post-mortem.

Dulbecco's phosphate-buffered saline solution (DPBS: Sigma Corporation, St. Louis, MO) with 5.5 mM glucose added was prepared and filtered through a 0.2 μ m filter (Pall Life Sciences, East Hills, NY). Fluorescein dextran (500 kD, 10 mg, Sigma Corporation, St. Louis, MO) was prepared for the exchange studies at a concentration of 0.17 mg/ml and sonicated (Model 50T, VWR, West Chester, PA) at 40 kHz for 20 min to prevent clumping. Standards for comparison were prepared at concentrations of 0.2 mg/ml, 0.02 mg/ml, 0.002 mg/ml, and 0.0002 mg/ml.

2.2. Experimental setup

The eyes were placed in gauze, wetted with saline and placed in beakers. The beakers were placed in a water bath maintained at 34 °C. Eyes were cannulated with a 25 gauge needle attached to 12" long butterfly tubing (Becton Dickinson, Franklin Lakes, NJ) with

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the needle tip place in the posterior chamber of the eye. The butterfly tubing was attached to tubing of 12"–36" in length (disposable pressure tubing, 0.05" ID, Mallinckrodt, Hazelwood, MO) that in turn was attached to a three-way valve (Mallinckrodt 91044). The three-way valve connected directly to a perfusion reservoir containing fluid to be introduced to the AC, and also to tubing that connected to a computer controlled syringe pump and pressure transducer.

The tip of a second 25 gauge needle was placed into the AC of the eye. This needle attached to 12" butterfly tubing that directed the fluid to a waste reservoir.

2.3. Experimental procedure

Porcine eyes were perfused with DPBS at a constant pressure of 10 mm Hg for roughly 30 min to obtain a baseline facility using our previously described methods (Sit et al., 1997). Once a stable facility was achieved, the AC was normally exchanged with 4 ml of the fluorescein dextran solution using tubing of length 12", 24" or 36" (in one experiment, 6 ml of the fluorescein dextran solution was exchanged through 12" of tubing, while in another, 8 ml was exchanged through 36" of tubing). The exchange procedure followed our previously described methods (Sit et al., 1997): the upstream (exchange) reservoir was raised to an initial pressure of 12 mm Hg while the downstream (waste) reservoir was lowered to an initial pressure of 8 mm Hg with the pump off. The volume exchanged was monitored by observing the decreased in level of the fluid in the upstream reservoir. Once the exchange was complete, any remaining fluorescein dextran solution was removed from the exchange reservoir, and it was then filled with DPBS.

The AC was then exchanged again, this time with DPBS. The amount of DPBS exchanged ranged from 0.6 ml to 11 ml depending on the experiment. At the conclusion of the experiment, fluid from the AC was extracted by cutting the butterfly tubing exiting the AC just above the needle, and collecting one to two drops (30–50 μ l) of fluid from the anterior chamber exiting this needle into a micro-centrifuge tube. It was found in our preliminary studies that this procedure gave more repeatable results than our original procedure of extracting fluid from the AC using a separate syringe and needle.

The sample was weighed to determine the exact amount of fluid removed from the eye. The concentration of fluorescein dextran in these samples was determined using a spectrofluorometer (see below).

2.4. Volume determinations

A total of 8 porcine eyes were perfused with DPBS at 10 mmHg for 20 min. Fluid from the AC was then extracted using a 1 ml syringe and a 25 gauge needle until the AC just collapsed. The syringe was weighed before and after extraction to determine the volume of the AC. The AC volume of the porcine eye was found to be $260 \pm 11 \mu$ l (mean \pm standard error).

The volume of the tubing used was assessed by weighing the tubing before and after filling it with water. Table 1 shows the measured volume of the tubing types (mean \pm standard error). It should be noted that the tubing volume was not exactly proportional to nominal tubing length, an observation we confirmed with

additional measurements. This was due to each type of tubing being approximately 1" longer than its nominal length, and to the luer-lock fittings on the end of each tubing set that added a fixed volume to each tubing set. However, the values were repeatable on different tubing sets.

2.5. Measurement of fluorescein dextran concentration

The fluorescence measurements were taken using a spectrofluorometer (ISS PC1: Champaign, IL, USA) with an excitation wavelength of 410 nm and an emission wavelength of 520 nm (the peak of the emission spectrum). AC samples were diluted to a total volume of 1.5 ml for measurement. The results were compared to standard curves (see typical example in Fig. 1).

3. Theory

The simplest description of the process of dilution in the AC would treat the AC as a well-mixed chamber, and allow this mixing to start once the upstream tubing has been clear of fluorescein dextran by an equivalent volume of diluent (buffer). Such a simplistic model would suggest that the required perfusion volume for adequate dilution would include the upstream volume of the tubing and the volume of the anterior chamber.

However, such a model ignores the Poiseuille velocity profile in the tubing. Several important consequences arise from this. First, the dilution process of the anterior chamber begins earlier than the above simplistic considerations suggest. Since the maximum fluid velocity at the center of the tubing is twice the average velocity for a Poiseuille flow, the dilution process in the AC begins more quickly. More importantly, even after a buffer volume equivalent to the volume of the tubing has passed through, the tubing will still contain a significant quantity of fluorescein dextran. This is because the fluorescein dextran nearest the wall of the tube is moving much slower than the mean fluid velocity (see Fig. 2). In fact, the fluid closest to the wall will not pass out of the tube except by molecular diffusion (in the radial direction) whereby it diffuses to a faster streamline and then flows out.

Such processes are well-known and are described as convective dispersion and Taylor diffusion (Taylor, 1953; Probstein, 1989). In the case of the former, transport and dispersion of a tracer in the flow are dominated by convection whereby tracer on different

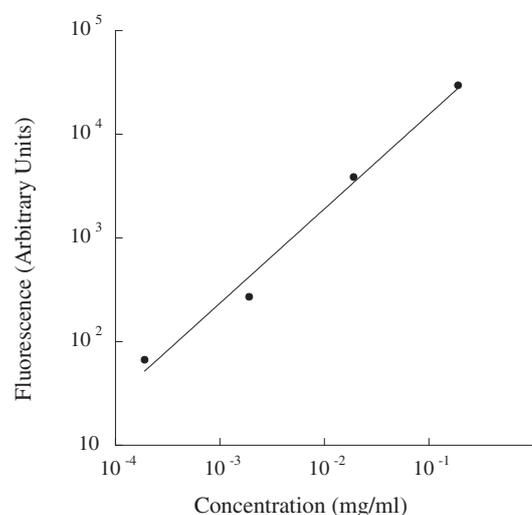


Fig. 1. Typical standard curve showing fluorescence of fluorescein dextran as a function of concentration.

Table 1
Volumes of tubing used in experiments.

12" tubing	0.59 \pm 0.02 ml (n = 4)
24" tubing	0.92 \pm 0.01 ml (n = 4)
36" tubing	1.36 \pm 0.03 ml (n = 4)
12" butterfly tubing	0.39 \pm 0.05 ml (n = 5)

streamlines travels at different velocities through the tube due to the Poiseuille velocity profile; diffusion plays no role. In the latter case, the process is more complicated as both radial diffusion and axial convection interact to affect the transport process. For short time scales, convective dispersion dominates while for long time scales, the process is better described by Taylor dispersion. The time scale (τ) that differentiates which of these processes dominates the dispersion process is (Chatwin, 1977):

$$\tau = \frac{0.15R^2}{D} \quad (1)$$

where R is the tube radius and D is the diffusivity of the tracer in the flow; for time scales longer than this, Taylor dispersion is important. For the problem at hand, the relevant tube inner diameter is 0.05" and the molecular diffusivity of the fluorescein dextran is $2 \times 10^{-7} \text{ cm}^2/\text{s}$ (Gribbon and Hardingham, 1998) yielding a time-scale of approximately 1 h. As the exchange processes of interest here are always faster than this (typically 10–20 min), we consider convective dispersion as it applies to the exchange process.

We model flow in the tubes as Poiseuille, and allow this flow to passively transport the tracer particles. This flow enters the AC where it is assumed to be well-mixed. Under these circumstances, we show in the Appendix that if the anterior chamber is initially at a concentration of C_0 and the concentration of tracer in the buffer is zero, then the AC concentration of tracer, $C(V)$, as a function of perfused volume of buffer (V) is found to be:

$$\frac{C(V)}{C_0} = \exp(-V^*) + \frac{\beta}{2} \left\{ \exp(-V^*) - \frac{1}{\left(\frac{2V^*}{\beta} + 1\right)} \right\} + \frac{\beta^2}{2} \exp(-V^*) \left\{ Ei\left(V^* + \frac{\beta}{2}\right) - Ei\left(\frac{\beta}{2}\right) \right\} \quad (2)$$

where $V^* = (V - V_{\text{tubing}}/2)/V_{\text{AC}}$, $\beta = V_{\text{tubing}}/V_{\text{AC}}$, V_{tubing} is the volume of the tubing upstream of the AC, and V_{AC} is the volume of the AC. Ei is the exponential integral function. The subtraction of $V_{\text{tubing}}/2$ from V arises because for perfused volumes less than this, no buffer has reached the AC; the first fluid arriving at the AC that is free of tracer is at the centerline of the tubing where the velocity is twice the mean.

A simpler model that accounts for effect of the tubing is to assume that the AC concentration behaves as a single well-mixed chamber of volume $V_{\text{AC}} + V_{\text{tubing}}$ while still allowing that the AC concentration cannot decrease until the perfused volume is greater than $V_{\text{tubing}}/2$. For such a model, we find that a simple exponential decay model gives the following result:

$$\frac{C(V)}{C_0} = \exp\left(-\frac{V^*}{1+\beta}\right) \quad (3)$$

In the calculations thus far presented, it has been assumed that at the start of the wash-out process, the AC was at a concentration of C_0 . However, because the volume used to fill the AC is not much, much larger than $V_{\text{tubing}} + V_{\text{AC}}$, the same considerations involved in the wash-out process apply to the process of filling the AC with the fluorescein dextran. To correct for this, we used equation (3), the lower-bound for the rate at which the AC is exchanged (see Fig. 3), to estimate the initial concentration of fluorescein dextran, C_{initial} , in the AC before the exchange with buffer. We included in the perfused volume both the volume of fluorescein dextran perfused before buffer (V_{pre} in Table 1) and that volume of fluorescein dextran in the upstream tubing that would enter the AC before the buffer first arrived at the AC ($V_{\text{tubing}}/2$). These results will be presented separately below (Fig. 4). Note that because C_{initial} was always close to C_0 (see Results), this correction had only a minor impact on the results.

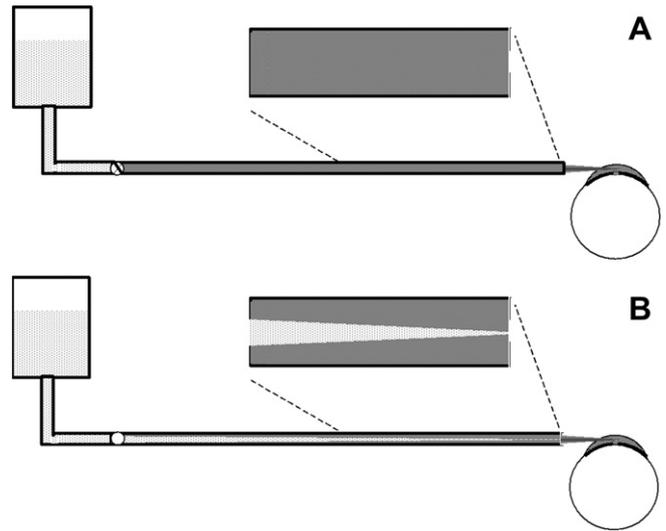


Fig. 2. Schematic showing process of exchanging fluid in tubing. A: valve is closed and downstream of the valve is filled with tracer; upstream of valve is buffer. B: valve is open and buffer has just reached that AC. Blowups shows region near end of tubing; note that in B, the buffer is in center of tube but not at edges.

4. Results

The experimental results are shown in Table 2 and plotted in Fig. 3. As expected, even for perfused buffer volumes (V) greater than the combined tubing and AC volume, the concentration of fluorescein dextran remaining in the AC was significant. Roughly the perfused volume needed to be greater than three times this combined volume before the AC could be considered to be exchanged.

Also shown in Fig. 3 are the results of theoretical predictions for C_{final}/C_0 . Equation (2), which does not include the effects of molecular diffusion, appears to give an upper bound for the rate at which the AC can be cleared. Equation (3), which is developed assuming that the AC and tubing is a single well-mixed chamber, gives a lower bound to this rate.

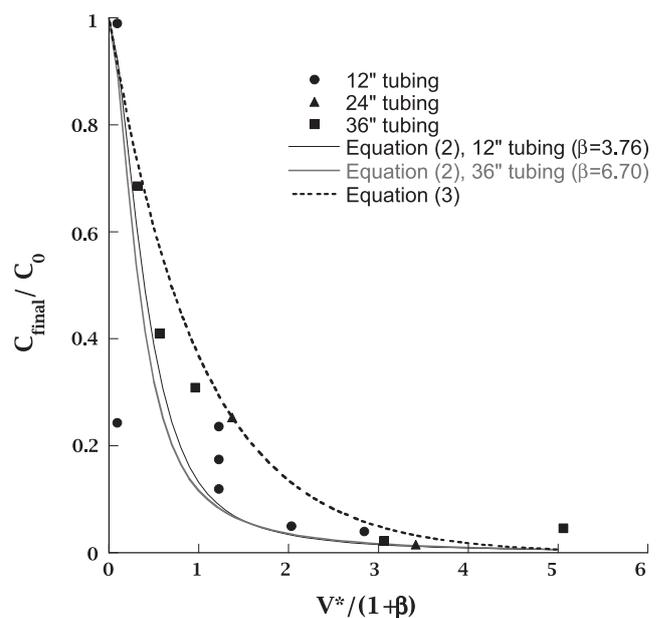


Fig. 3. C_{final}/C_0 of fluorescein dextran as a function of $(V - V_{\text{tubing}}/2)/(V_{\text{tubing}} + V_{\text{AC}})$. $\beta = V_{\text{tubing}}/V_{\text{AC}}$.

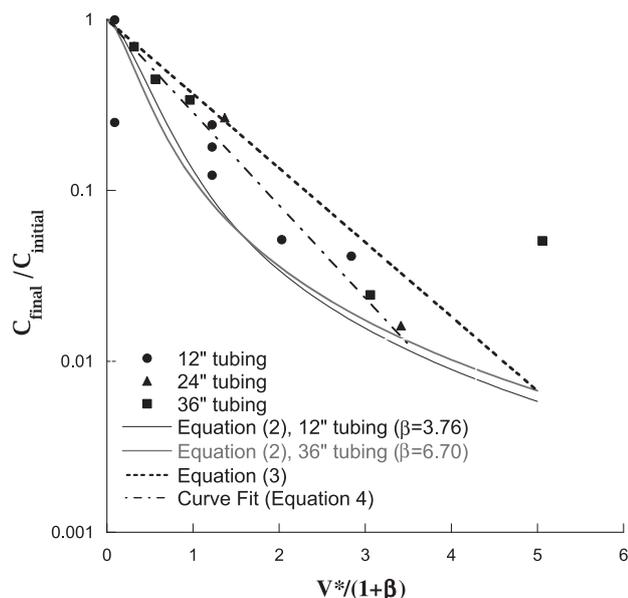


Fig. 4. $C_{final}/C_{initial}$ of fluorescein dextran as a function of $(V - V_{tubing}/2)/(V_{tubing} + V_{AC})$.

As mentioned in the methods section, $C_{initial}$ in the experiments was always lower than C_0 . Estimates for the initial AC concentration of fluorescein dextran before the exchange were all greater than 90% of C_0 , and most were greater than 95% of C_0 , consistent with a preliminary experiment in which AC concentration was measured before exchange (using a different methodology). Fig. 4 shows the results for $C_{final}/C_{initial}$. The same trend is apparent as seen in Fig. 3, with Equation (2) as an upper limit on wash-out rate and Equation (3) as a lower limit.

Note in Fig. 4 that use of the log scale for the ordinate makes clear that two of the data points are outliers. One of these (the first row in Table 2) was the first data set taken. With these two data points excluded, the remaining data points show an exponential decline. A exponential least square fit to this data (with $C(t=0)/C_{initial} = 1$) yields

$$\frac{C(V)}{C_{initial}} = \exp\left(-\frac{1.25V^*}{1 + \beta}\right) \quad (4)$$

($r = 0.983$). This curve-fit is also shown in Fig. 4. We can use this result to estimate that to have $C(V)/C_{initial} < 0.05$,

Table 2

Measured concentration, C_{final} , of fluorescein dextran for the different test conditions. L : length of tubing upstream of butterfly tubing entering eye; V_{tubing} : volume of tubing upstream of eye, including butterfly tubing; V_{AC} : volume of AC; V_{pre} : volume of fluorescein dextran perfused into tubing and eye before buffer; V : volume of buffer exchanged.

L (inches)	$V_{tubing} + V_{AC}$ (ml)	V_{pre} (ml)	V (ml)	C_{final}/C_0
12	1.24	4	0.6	0.244
12	1.24	4	2	0.175
12	1.24	4	2	0.120
12	1.24	4	2	0.236
12	1.24	4	3	0.050
12	1.24	4	4	0.040
12	1.24	6	0.6	0.994
24	1.57	4	2.8	0.253
24	1.57	4	6	0.015
36	2.00	4	2	0.409
36	2.00	4	2.8	0.309
36	2.00	4	7	0.022
36	2.00	4	11	0.046
36	2.00	8	1.5	0.686

$$\frac{V^*}{1 + \beta} = \frac{V - \frac{V_{tubing}}{2}}{V_{AC} + V_{tubing}} > 2.4 \quad (5)$$

while to have $C(V)/C_{initial} < 0.01$,

$$\frac{V^*}{1 + \beta} = \frac{V - \frac{V_{tubing}}{2}}{V_{AC} + V_{tubing}} > 3.7 \quad (6)$$

To put these criteria in terms of the combined volume of the upstream tubing and the anterior chamber, two limits can be considered: for $V_{tubing} \ll V_{AC}$, the criterion for $V/(V_{tubing} + V_{AC})$ is the same as that found for $V^*/(1 + \beta)$ in equations (5) and (6); if $V_{tubing} \gg V_{AC}$, then the results above can be used to find that $V/(V_{tubing} + V_{AC}) > 2.9$ for $C(V)/C_{initial} < 0.05$ and $V/(V_{tubing} + V_{AC}) > 4.2$ for $C(V)/C_{initial} < 0.01$. As this latter criterion for $V_{tubing} \gg V_{AC}$ is more conservative than equations (5) and (6) (and it is often the case that $V_{tubing} \gg V_{AC}$), this latter criterion is recommended.

5. Discussion

Many studies have examined the effects of drugs introduced into the AC on aqueous humor outflow. A natural part of these experiments involves a subsequent clearance of the drug from AC to determine whether any measured drug effect is reversible. We have here addressed the question of how much buffer needs to be passed through the AC to be sure that a drug has been adequately removed from the AC.

The results of the current study suggest that only by exchanging a substantial volume of fluid can a drug be cleared from the AC. To reduce the concentration by 20-fold (5% of the initial concentration) requires perfusion of a buffer volume approximately three times the combined volume of the upstream tubing and the AC; to reduce the concentration 100-fold, over four times the combined volume of the upstream tubing and the AC is required. We suspect that few studies meet these standards.

Two theoretical models were developed to compare with these data. More rapid clearance was predicted with the model that accounted for convective dispersion in the tubing than the more simple model in which the entire volume of upstream tubing and AC was modeled as a single well-mixed chamber. While molecular diffusion in the tubing was not included in the convective dispersion model, this was not responsible for the more rapid clearance predicted by this model. Molecular diffusion in the tubing would allow the tracer to diffuse from the wall where it is effectively trapped by the slow flow there, and move onto more streamlines in the center of the tube where it would be more rapidly cleared. Diffusion within the AC would slow clearance from the AC, but neither model correctly accounted for this.

Both models correctly predicted that adequate dilution would not be achieved if the perfused buffer volume included only the upstream volume of the tubing and the volume of the anterior chamber. Instead, several times this volume is required. Comparison of the experimental data with the two theoretical models showed that the data fell between the predictions of these models. In summary, adequate exchange of the anterior chamber requires a perfused volume 3–4 times the combined volume of the upstream tubing and the AC.

Appendix

We here determined the average concentration of tracer remaining in a well-mixed chamber that is initially at a concentration C_0 . Upstream of the chamber is tubing of length L and radius R that is initially also filled with tracer at a concentration C_0 . At $t = 0$, fluid free of tracer enters this upstream tubing at a flow rate of Q .

We first need to determine the rate at which tracer enters the chamber as a function of time. For $t < L/v_{\max}$, this is simply QC_0 as none of the tracer-free fluid entering the tube will have yet reached the end of the tube. $v_{\max} = 2Q/(\pi R^2)$. Since the rate of tracer leaving the chamber will be identical to this, the concentration of tracer in the chamber is C_0 for $t < L/v_{\max}$.

For times longer than this, there will be a radius $a(t)$ at the end of the tube for which the fluid is tracer-free for radii smaller than this. Using the Poiseuille velocity profile, $v(r) = v_{\max}(1 - r^2/R^2)$ and letting $t = L/v(a)$ (the time at which the tracer free fluid first reaches the chamber), we can solve to find that:

$$a(t) = R\sqrt{1 - \frac{L}{v_{\max}t}} \quad (A1)$$

The rate at which tracer is then entering the chamber at any time $t > L/v_{\max}$ is then found as:

$$\int_{a(t)}^R C_0 v_{\max} \left(1 - \frac{r^2}{R^2}\right) 2\pi r \, dr = \frac{QC_0 L^2}{V_{\max}^2 t^2} \quad (A2)$$

To find the concentration in the anterior chamber as a function of time, a mass balance is applied to the tracer. Assuming that the

concentration of tracer leaving the chamber is equal to the average concentration of the chamber (a well-mixed assumption), the following differential equation arises (using the result from (A2)):

$$V_{AC} \frac{dC(t)}{dt} = \frac{QC_0 L^2}{v_{\max}^2 t^2} - QC(t) \quad (A3)$$

with an initial condition that $C(t = L/v_{\max}) = C_0$.

A somewhat laborious solution of equation (A3) leads to equation (2), where the answer has been put as a function of perfused volume $V = Qt$.

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