Permeability of the Blood–Retinal Barrier to Carboxyfluorescein in Eyes With Rhegmatogenous Retinal Detachment

Shunji Tsuboi* and Jonathan E. Pederson

Outward and inward permeability of carboxyfluorescein across the blood–retinal barrier were measured fluorophotometrically in seven cynomolgus monkey eyes with experimental rhegmatogenous retinal detachment. Probenecid was used to inhibit outward transport of carboxyfluorescein. The outward permeability was 1.98 ± 0.31 µl/min in eyes with retinal detachment and 0.84 ± 0.15 µl/min in control eyes with vitrectomy alone (P < 0.01). The inward permeability, determined separately following intravenous injection, was significantly lower than the outward permeability: 0.14 ± 0.02 µl/min for eyes with retinal detachment and 0.04 ± 0.01 µl/min for control eyes. Since the outward permeability minus the inward permeability in the presence of probenecid represents that fraction of tracer moving due to fluid flow, it may be concluded that outward flow of fluid across the blood–retinal barrier is a substantial contributor to carboxyfluorescein loss from the vitreous cavity following intravitreal injection. Invest Ophthamol Vis Sci 28:96–100, 1987

Numerous studies have strongly suggested the existence of fluid movement across the retinal pigment epithelium in eyes with rhegmatogenous retinal detachment. Studies using fluorescein as a tracer to evaluate fluid exchange across the retina have been limited by the high affinity of fluorescein to the outwardly-directed active transport system in the retinal pigment epithelium and the formation of fluorescein glucuronide after intravenous injection. Carboxyfluorescein has much less affinity to the outward transport than fluorescein. 0.1 mM probenecid completely inhibits the outward active transport of 0.06 mM carboxyfluorescein in the isolated dog retinal pigment epithelium–choroid preparation. Furthermore, carboxyfluorescein is only minimally converted to the glucuronide form. These characteristics of carboxyfluorescein permit the rate of fluid flow across the retinal pigment epithelium to be quantitated more precisely.

The present study was undertaken to determine the outward and inward permeability of carboxyfluorescein across the blood–retinal barrier in the presence of probenecid in eyes with or without retinal detachment.

Materials and Methods

Rhegmatogenous retinal detachments were created in one eye of each of 12 cynomolgus monkeys. A total vitrectomy was performed on the fellow eyes. The details of the surgical procedure are described elsewhere. At least 2 months were allowed for the detachment to become stabilized.

Part 1

Uneven distribution of fluorescent tracer in the vitreous cavity and subretinal space after intravitreal injection may cause an error in determining the outward permeability in eyes with retinal detachment. Furthermore, optical resolution of the lens and cornea in a fluorophotometrical measurement of vitreous should be known, since there is a difference between measured fluorescence (Fv) and concentration (Cv) in the vitreous cavity. Five monkeys with a variety of retinal hole sizes were thus used to clarify these problems.

Under intraperitoneal sodium pentobarbital anesthesia, a 20-µl solution of phosphate buffer containing 10⁻⁴ g/ml fluorescein was injected into the vitreous cavity of both eyes. Three hours later, fluorophotometry was performed on the midvitreous (Fv). Shortly thereafter, 200 µl of fluid were withdrawn from the subretinal space in eyes with retinal detachment, followed by vitreous cavity aspiration in both eyes. This was accomplished with a 27-gauge needle introduced via the pars plana under binocular ophthalmoscopy. From these fluid samples, the fluorescein concentration

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in the vitreous cavity (Cv) and subretinal space (Cs) were measured with the fluorophotometer. The coefficient for the resolution, Cv/Fv, and the fluorescein distribution ratio in eyes with retinal detachment, Cs/Cv, were then calculated.

Part 2

For the following experiments, seven monkeys with a retinal hole greater than six disc diameters in size were used to ensure adequate subretinal fluid-vitreous exchange. Under intraperitoneal sodium pentobarbital anesthesia, 20 μl of 10^-5 g/ml carboxyfluorescein were injected into the anterior chamber through a small self-sealing incision made in the peripheral cornea with a needle knife. Anterior chamber fluorophotometric measurements were made hourly for 7 hr, and the exponential decay constant (Ke) was determined by linear regression. The anterior chamber volume (Va) was determined photographically.

Part 3

Two months after the experiment described in part 2, the same seven monkeys were used. One hour after intraperitoneal administration of 150 mg/kg probenecid and 300 mg/kg pentobarbital, 20 μl of phosphate buffer containing 10^-4 g/ml carboxyfluorescein and 10^-2 M probenecid were injected into the central vitreous cavity in both eyes of the same seven monkeys. Fluorophotometry of the vitreous cavity and anterior chamber were then performed hourly for 7 hr. The exponential decay constant of the vitreous (Kv) was determined with linear regression. The ratio of fluorescence in the anterior chamber to vitreous cavity (Fa/Fv) was also determined from the mean of seven measurements. Expressed in equivalent volumes of vitreous, the rate of loss of carboxyfluorescein from the vitreous cavity into the anterior chamber is KsVgCg/Cv and the rate of loss not into the anterior chamber (presumably outward permeability across the blood-retinal barrier, Pout) is KsVg - KsVgCg/Cv, or KsVg - KsVg(Fa/Fv)(Cg/Fg)(Fa/Cg), where Vg is vitreous cavity volume and Cg/Cv the ratio of concentration of carboxyfluorescein in the anterior chamber to vitreous following intravitreal injection. This expression is derived elsewhere.14 Va was assumed to be 2 ml.14 Cp/Fa was assumed to be unity.12

Assuming the carboxyfluorescein present in the vitreous cavity 1 hr after intravenous injection, (CvVg)^t^t=60, has entered across the blood-retinal barrier, the inward permeability of the blood-retinal barrier to carboxyfluorescein expressed in equivalent volumes of vitreous, and Cg/Fg is unbound carboxyfluorescein plasma concentration. This equation, another form of the Fick's first law, is true when Cg ≤ Cp.7 All procedures conformed to the ARVO Resolution on the Use of Animals in Research.

Results

The ratio Cs/Cv following intravitreal injection of fluorescein in eyes with retinal detachment was much less than unity when the retinal hole was small. However, when the mean diameter of the hole was larger than five disc diameters, Cs/Cv was close to unity, indicating uniform distribution of fluorescein in the vitreous cavity and subretinal space. There was no statistical difference between the resolution coefficient (Cv/Fv) of eyes with retinal detachment and fellow eyes (Table I). The mean value for Cg/Fg was used in the
Table 1. Fluorescein distribution ratio (Cvr/Cv) and resolution coefficient of fluorophotometry (Cv/Fv)

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Size of hole*</th>
<th>Cvr/Cv</th>
<th>Cv/Fv</th>
<th>Cvr/Fv</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>0.28</td>
<td>1.87</td>
<td>1.81</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.33</td>
<td>1.44</td>
<td>1.61</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0.73</td>
<td>1.58</td>
<td>1.51</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>1.06</td>
<td>1.51</td>
<td>1.75</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>0.87</td>
<td>1.56</td>
<td>1.72</td>
</tr>
</tbody>
</table>

Mean ± SD  

<table>
<thead>
<tr>
<th>Eyes with retinal detachment</th>
<th>Fellow eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.59 ± 0.15</td>
<td>1.68 ± 0.11</td>
</tr>
</tbody>
</table>

* Mean disc diameter.

Cv = vitreous fluorescein concentration; Cvr = subretinal fluorescein concentration; Fv = measured fluorescence in the midvitreous; SD = standard deviation.

calculation of P_in and P_out in the subsequent experiments.

Figure 1 shows the exponential decay of C_p following intravenous injection. Since the curve was nearly linear, C_p was simply expressed as Ae^{-Bt}. It was thus possible in subsequent experiments to determine the decay of C_p from two blood samples taken 15 min and 75 min after injection. The unbound to total concentration ratio of carboxyfluorescein in the plasma was relatively constant: 42 ± 4% at t = 15-30 min, 41 ± 1% at t = 60-75 min, and 39 ± 4% at t = 120-135 min (mean ± SD). However, the ratio was significantly higher in the presence of probenecid (54 ± 3%). The ratio was thus taken as 0.54 for the P_in calculations. Carboxyfluorescein in the vitreous cavity 1 hr after intravenous injection was distributed evenly (Table 2), so that F_v in the midvitreous was used to determine the total amount of carboxyfluorescein in the vitreous cavity (C_vV_v). C_p was larger than F_v by more than two orders of magnitude for the first hour after intravenous injection (see Figure 1 and Table 2).

Figure 2 shows a representative time course of the carboxyfluorescein concentration in the midvitreous and anterior chamber following intravitreal injection. The exponential decay of the vitreous was significantly lower in the fellow eye than the eye with retinal detachment. The concentration in the anterior chamber was always lower than that in the vitreous. Table 3 summarizes the fluorophotometric results obtained from experiments 2-4. Although P_in was significantly larger in eyes with retinal detachment than fellow eyes, it was only 5-7% of P_out, even in the presence of probenecid. Therefore, (P_out − P_in) was also significantly larger in eyes with retinal detachment than fellow eyes. K_vV_v was significantly lower in eyes with retinal detachment, indicating decreased anterior chamber aqueous flow, consistent with previous results.

Discussion

The present study is a reevaluation and expansion of a previous report, where fluorescein was used to estimate posterior flow of fluid across the blood–retinal

![Fig. 1. Plasma carboxyfluorescein concentration after intravenous injection. Data points are from triplicate measurements in five monkeys. Note linearity of exponential decay.](image1.png)

![Fig. 2. Carboxyfluorescein concentration in the midvitreous (MV) and anterior chamber (AC) in eyes with retinal detachment and fellow eyes following intravitreal injection in the presence of probenecid.](image2.png)
barrier in eyes with or without retinal detachment. The values in the present study are approximately one-fourth as large as previously reported. The former values are likely an overestimate, for several reasons. First, carboxyfluorescein is not converted to the glucuronide metabolite, as is fluorescein. Second, the present study shows that the tracer is not well mixed across the retinal hole following intravitreal injection, unless the hole is larger than five disc diameters. Therefore, in the present study, only eyes with a retinal hole greater than six disc diameters were used. In these eyes the vitreous cavity and subretinal space join into one compartment, in which the effective vitreous volume in both eyes with retinal detachment and fellow eyes. K \text{\textsubscript{c}}V \text{\textsubscript{c}} would be overestimated in eyes with a retinal detachment and a small retinal hole, since the effective vitreous volume would be overestimated.

Third, the resolution coefficient (C \text{\textsubscript{c}}/F \text{\textsubscript{c}}) has been measured. Since C \text{\textsubscript{c}}/F \text{\textsubscript{c}} is greater than unity, both P \text{\textsubscript{in}} and P \text{\textsubscript{out}} would be underestimated without the application of the coefficient. Fourthly, P \text{\textsubscript{in}} has been determined with a method independent of P \text{\textsubscript{out}} determination. In the previous study, P \text{\textsubscript{in}} was calculated from P \text{\textsubscript{out}} and the ratio P \text{\textsubscript{out}}/P \text{\textsubscript{in}} which was derived from "equilibrated" C \text{\textsubscript{p}}/C \text{\textsubscript{v}} following intravenous injection. However, since C \text{\textsubscript{p}} declines as a function of time, it is difficult to determine the equilibrated C \text{\textsubscript{p}}/C \text{\textsubscript{v}}. Furthermore, anterior segment contribution to the vitreous tracer concentration becomes more appreciable with time.

Carboxyfluorescein moves across the blood–ocular barrier by diffusion (J \text{\textsubscript{d}}), transport (J \text{\textsubscript{t}}), and the solvent drag (J \text{\textsubscript{f}}). Since the present dose of probenecid causes complete inhibition of carboxyfluorescein transport, outward flow of fluid accounts for the significant difference between P \text{\textsubscript{out}} and P \text{\textsubscript{in}} in both retinal detachment and fellow eyes. This difference is larger in eyes with retinal detachment, implying an increased fluid flow. Why posteriorly directed fluid flow increases in eyes with retinal detachment, without is well understood, but the low flow conductivity of the intact sensory retina may be an explanation. Resistance to fluid movement across the sensory retina is eliminated in eyes with retinal detachment, so that the retinal pigment epithelium is the only remaining barrier. In the present experiments, J \text{\textsubscript{f}}, ie, (P \text{\textsubscript{out}} – P \text{\textsubscript{in}}), is 2.3 times larger in eyes with retinal detachment than fellow eyes.

Although expressed in µl/min, J \text{\textsubscript{f}} may not precisely equal the amount of volume flow, but represents the movement of carboxyfluorescein due to fluid movement (solvent drag). The volume flow may be underestimated if the reflection coefficient of carboxyfluorescein across the blood–retinal barrier is not zero. On the other hand, the volume flow may be overestimated for several reasons. First, the unstirred layers on both the vitreous and choroidal sides of the blood–retinal barrier may cause an underestimate of J \text{\textsubscript{d}}, thus resulting in the overestimate of J \text{\textsubscript{f}}. Second, solvent drag may affect carboxyfluorescein moving in both outward and inward directions, ie, carboxyfluorescein diffusing across the retinal pigment epithelium after intravenous injection may be “swept” in the opposite direction by fluid flow, depending on the exact path of solute and solvent penetration. J \text{\textsubscript{f}} would be underestimated from P \text{\textsubscript{in}} and J \text{\textsubscript{f}} would be overestimated as a result.

Assuming a value of 4.9 cm\textsuperscript{2} for the surface of retinal pigment epithelium in the monkey, fluid transport across the retinal pigment epithelium in vivo may be calculated as 0.38 µl/min-cm\textsuperscript{2} using the J \text{\textsubscript{f}} value in eyes with retinal detachment. This figure is approximately three times larger than the fluid flow measured in vitro using frog retinal pigment epithelium–choroid but similar to the resorption rate of subretinal fluid from nonrhegmatogenous retinal detachments in rabbits and monkeys. P \text{\textsubscript{in}} in eyes with retinal detachment is 4.8 × 10\textsuperscript{-7} cm/sec, which is consistent with the dog RPE–choroid in vitro (5.4 × 10\textsuperscript{-7} cm/sec). An increased fluid flow posteriorly across the retinal pigment epithelium has also been shown in human retinal detachment eyes with tears. This flow of fluid may be responsible for the spontaneous resorption of subretinal fluid after surgical closure of the retinal break. The flow in the normal eye, if small in amount, may keep the sensory retina attached firmly to the retinal pigment epithelium.

### Table 3. Inward (P \text{\textsubscript{in}}) and outward (P \text{\textsubscript{out}}) permeability of the blood–retinal barrier to carboxyfluorescein (with probenecid)

<table>
<thead>
<tr>
<th></th>
<th>RD eye</th>
<th>Fellow eye</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P \text{\textsubscript{in}}</td>
<td>0.14 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>K \text{\textsubscript{c}}V \text{\textsubscript{c}}</td>
<td>2.10 ± 0.32</td>
<td>1.10 ± 0.15</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>K \text{\textsubscript{v}}V \text{\textsubscript{c}}</td>
<td>0.39 ± 0.08</td>
<td>0.83 ± 0.10</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>C \text{\textsubscript{p}}/C \text{\textsubscript{v}}</td>
<td>0.34 ± 0.07</td>
<td>0.30 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>K \text{\textsubscript{v}}V \text{\textsubscript{c}}/C \text{\textsubscript{v}} = P \text{\textsubscript{out}}</td>
<td>1.98 ± 0.31</td>
<td>0.84 ± 0.15</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>P \text{\textsubscript{out}} – P \text{\textsubscript{in}}</td>
<td>1.84 ± 0.29</td>
<td>0.80 ± 0.14</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Values (except C \text{\textsubscript{p}}/C \text{\textsubscript{v}}) are µl/min (mean ± SE).

NS = not significant; RD = retinal detachment; K \text{\textsubscript{c}} = anterior chamber exponential decay constant; K \text{\textsubscript{v}} = vitreous exponential decay constant; V \text{\textsubscript{c}} = anterior chamber volume; V \text{\textsubscript{v}} = vitreous cavity volume; C \text{\textsubscript{c}} = concentration of carboxyfluorescein in the anterior chamber; C \text{\textsubscript{v}} = concentration of carboxyfluorescein in the vitreous.

### Key words: retinal detachment, permeability, carboxyfluorescein, blood–retinal barrier, retinal pigment epithelium, cynomolgus monkey

### References
2. Cantrill HL and Pederson JE: Experimental retinal detachment.
VI. The permeability of the blood–retinal barrier. Arch Ophthal- 


VIII. Retinochoroidal horseradish peroxidase diffusion across the 


in eyes with rhegmatogenous retinal detachments. Am J 


5. Tsuboi S and Pederson JE: Experimental retinal detachment. X. 

Effect of acetazolamide on vitreous fluorescein disappearance. 


XI. Furosemide-inhibitable fluid absorption across retinal pig- 


and Manabe R: Measurement of retinal permeability to sodium 


8. Araie M, Sawa M, Nagataki S, and Mishima S: Aqueous humor 

dynamics in man as studied by oral fluorescein. Jpn J Ophthalmol 


pigment epithelium to carboxyfluorescein. Invest Ophthal- 

mol Vis Sci, in press. 

10. Neault TR, McLaren JW, Brubaker JH, and Brubaker RF: Spec- 

tral shift of fluorescein and carboxyfluorescein in the anterior 

chamber of the rabbit eye following systemic administration. 


11. Pederson JE, Cantrill HL, and Cameron JD: Experimental retinal 

detachment. II. Role of the vitreous. Arch Ophthalmol 100:1155, 

1982. 


aqueous humor flow with intravitreal fluoresceinated dextrans. 


13. Johnson SB, Coakes RL, and Brubaker RF: A simple photo- 

grammetric method of measuring anterior chamber volume. Am 


III. Vitreous fluorophotometry. Arch Ophthalmol 100:1810, 

1982. 

15. Pederson JE: Experimental retinal detachment. IV. Aqueous 

humor dynamics in rhegmatogenous detachments. Arch 


16. Tsuboi S. Problems in analyzing vitreous fluorophotometry. Folia 


17. Katchalsky A and Curran PF: Nonequilibrium Thermodynamics 


18. Fatt I and Shantinath K: Flow conductivity of the retina and its 


19. Taylor E and Jennings A: Calculation of total retinal area. Br J 


20. Hughes BA, Miller SS, and Machen TE: Effects of cyclic AMP 

on fluid absorption and ion transport across frog retinal pigment 


21. Frambach DA, Weiter JJ, and Adler AJ: A photogrammetric 

method to measure fluid movement across isolated frog retinal 


22. Negi A and Marmor MF: Quantitative estimation of metabolic 

transport of subretinal fluid. Invest Ophthalmol Vis Sci 27:1560, 

1986. 

23. Pederson JE and MacLellan HM: Experimental retinal detach- 

ment. I. Effect of subretinal fluid composition on reabsorption 