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

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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 Ophthalmol 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.1–6 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 epithelium7 and the formation of fluorescein glucuronide after intravenous injection.8 Carboxyfluorescein has much less affinity to the outward transport than fluorescein.9 0.1 mM probenecid completely inhibits the outward active transport of 0.06 mM carboxyfluorescein in the isolated dog retinal pigment epithelium–choroid preparation.9 Furthermore, carboxyfluorescein is only minimally converted to the glucuronide form.10 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.11 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.2 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.12 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...
in the vitreous cavity (Cv) and subretinal space (Csr) were measured with the fluorophotometer. The coefficient for the resolution, Csr/Fv, and the fluorescein distribution ratio in eyes with retinal detachment, Csr/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 (Kd) 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 μg/kg carboxyfluorescein with 25 mg/kg probenecid were injected intravenously. Sixty minutes after the injection, fluorophotometry was performed on the vitreous cavity in the fellow eyes, namely, 1.5 mm from the retina (PV), the center (MV), and 1.5 mm posterior to the lens (AV). Only MV (1.5 mm from the detached retina) and AV measurements were performed in eyes with retinal detachment. Blood samples were taken at 15 min and 75 min after injection using capillary tubes. The capillary tubes were centrifuged for 5 min to obtain plasma, which was diluted 1000 times with phosphate buffer. Total concentration of carboxyfluorescein in the plasma (Cp) was then measured with the fluorophotometer.

In a separate experiment, 2 ml of blood were taken from a cutaneous vein at 15–30 min, 60–75 min, and 120–135 min after intravenous injection of 20 mg/kg carboxyfluorescein in five monkeys, and at 30 min after the injection in the presence of probenecid in four monkeys. Two microliters of plasma were diluted 1000 times and the total concentration of carboxyfluorescein measured. The remainder of the plasma was centrifuged in an ultrafiltration membrane cone for 20 min. The ultrafiltrate was diluted 1000 times and measured with the fluorophotometer, so that the ratio of unbound to total carboxyfluorescein concentration in plasma was determined.

Part 4

One month later, following the preadministration of intraperitoneal 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 (Fav/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 KsVsv/Cv and the rate of loss not into the anterior chamber (presumably outward permeability across the blood–retinal barrier) is Kout) is Ksv - Ksv/Cv/Cv or Ksv - Ksv/Fv(Fv/Fv)(Cv/Fv)/(Cv/Cv), where Vsv is vitreous cavity volume and Cv/Cv, the ratio of concentration of carboxyfluorescein in the anterior chamber to vitreous following intravitreal injection. This expression is derived elsewhere.\(^{14}\) Vsv was assumed to be 2 ml.\(^{14}\) Csv/Fsv was assumed to be unity.\(^{12}\)

Assuming the carboxyfluorescein present in the vitreous cavity 1 hr after intravenous injection, (CvVsv)\(^{t=60}\), has entered across the blood–retinal barrier,

\[
P_{in} = \int_{0}^{60} C_{p} dt = (F_{v}/C_{v})^{t=60} (C_{v}/C_{v}) \int_{0}^{60} C_{p} dt,\]

where P in is the inward permeability of the blood–retinal barrier to carboxyfluorescein expressed in equivalent volumes of vitreous, and Cp is unbound carboxyfluorescein plasma concentration. This equation, another form of the Fick's first law, is true when Cv < Cp.\(^{7}\) All procedures conformed to the ARVO Resolution on the Use of Animals in Research.

Results

The ratio Csv/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, Csv/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 1). The mean value for Csv/Fsv was used in the
Table 1. Fluorescein distribution ratio (Csr/Cv) and resolution coefficient of fluorophotometry (Cv/Fv)

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Eyes with retinal detachment</th>
<th>Fellows eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Csr/Cv</td>
<td>Csr/Fv</td>
</tr>
<tr>
<td>1</td>
<td>0.2</td>
<td>1.87</td>
</tr>
<tr>
<td>2</td>
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<td>1.51</td>
</tr>
<tr>
<td>5</td>
<td>0.87</td>
<td>1.56</td>
</tr>
</tbody>
</table>

Mean ± SD

| Eyes with retinal detachment | 1.59 ± 0.15 | 1.68 ± 0.11 |
| Fellows eyes                |              |             |

* Mean disc diameter.

Cv = vitreous fluorescein concentration; Csr = 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 Csr following intravenous injection. Since the curve was nearly linear, Csr was simply expressed as Ae-^Bt. It was thus possible in subsequent experiments to determine the decay of Csr 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 Fv in the midvitreous was used to determine the total amount of carboxyfluorescein in the vitreous cavity (CvVv). Csr was larger than Fv 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. KavVs 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.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933362/)

![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.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933362/)
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 underestimate, 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, so that the effective vitreous volume \( V_\text{c} \) is the same in both eyes with retinal detachment and fellow eyes. \( K_{\text{v}}V_\text{s} \) 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 \( (C_v/F_v) \) has been measured. Since \( C_v/F_v \) is greater than unity, both \( P_{\text{n}} \) and \( P_{\text{out}} \) would be underestimated without the application of the coefficient. Fourthly, \( P_{\text{n}} \) has been determined with a method independent of \( P_{\text{out}} \) determination. In the previous study, \( P_{\text{n}} \) was calculated from \( P_{\text{out}} \) and the ratio \( P_{\text{out}}/P_{\text{n}} \) which was derived from “equilibrated” \( C_{\text{p}}/C_\text{s} \), following intravenous injection. However, since \( C_{\text{p}} \) declines as a function of time, it is difficult to determine the equilibrated \( C_{\text{p}}/C_\text{s} \). Furthermore, anterior segment contribution to the vitreous tracer concentration becomes more appreciable with time.

Carboxyfluorescein moves across the blood–ocular barrier by diffusion \( (J_\text{d}) \), transport \( (J_\text{t}) \), and the solvent drag of fluid flow, \( J_\text{f} \). Since the present dose of probenecid causes complete inhibition of carboxyfluorescein transport, the outward flow of fluid accounts for the significant difference between \( P_{\text{out}} \) and \( P_{\text{n}} \) in both retinal detachment and fellow eyes. This difference is larger in eyes with retinal detachment than fellow eyes, implying an overestimate of \( J_\text{f} \), thus resulting in the overestimate of \( J_\text{t} \). Second, solvent drag may affect carboxyfluorescein moving in both outward and inward directions, i.e., 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 permeation. \( J_\text{d} \) would be underestimated from \( P_{\text{n}} \) and \( J_\text{t} \) would be overestimated as a result. Assuming a value of 4.9 \( \text{cm}^2 \) 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 \( \mu\text{l/min-cm}^2 \) using the \( J_\text{v} \) 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. Probenecid in eyes with retinal detachment is 4.8 \( \times 10^{-7} \text{ cm/sec} \), which is consistent with the dog RPE–choroid in vitro (5.4 \( \times 10^{-7} \text{ cm/sec} \)).

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.

### Key words

retinal detachment, permeability, carboxyfluorescein, blood–retinal barrier, retinal pigment epithelium, cynomolgus monkey

### References


2. Cantrill HL and Pederson JE: Experimental retinal detachment.