Albumin Movement Out of the Subretinal Space After Experimental Retinal Detachment

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Purpose. The subretinal fluid of serous retinal detachments contains protein, but little is known about its origin and fate. The authors designed experiments to study the rate and route of albumin movement out of the subretinal space.

Methods. Experimental retinal detachments were made in Dutch rabbits by injecting Hanks' balanced salt solution containing serum levels (approximately 30 mg/ml) of fluorescein isothiocyanate (FITC) albumin into the subretinal space through a micropipette. Subretinal, vitreous, and serum fluid samples were withdrawn 0 to 4 hours later through a similar micropipette and were analyzed for osmolality, FITC albumin content (by fluorophotometry) and FITC + native albumin content (by gel electrophoresis). Sodium iodate was injected intravenously in some rabbits to damage the retinal pigment epithelium (RPE).

Results. Albumin injected into the subretinal fluid diffused steadily into the vitreous, and its concentration decreased by approximately 5% per hour. This rate was unaffected by RPE damage. Albumin did not move into the bloodstream unless the RPE was damaged with sodium iodate, and then it crossed the RPE at approximately 25% of the rate at which it moved into the vitreous. Subretinal fluid osmolality remained within the range of 293 to 294 mOsm/kg despite protein movement and the continual absorption of fluid from the detachments.

Conclusions. These results show that albumin in the subretinal space diffuses readily into the vitreous, and subretinal osmolality changes are rapidly equilibrated with the vitreous. Albumin does not cross normal RPE, and it crosses iodate-damaged RPE more slowly than it crosses retina. Thus, there must be a constant supply of albumin if high subretinal concentrations are to be sustained in clinical serous detachments. Invest Ophthalmol Vis Sci. 1995;36:1298-1305.

Serous retinal detachments occur in various chorioretinal disorders in which there is diffuse ischemic or inflammatory damage, or both, to the choriocapillaris and retinal pigment epithelium (RPE), such as in preeclampsia and Harada's disease. Central serous chorioretinopathy may belong to this category as well because fluid appears (by fluorescein angiography) to enter the subretinal space through focal defects in the RPE, but there is growing evidence that diffuse areas of choriocapillaris damage are actually responsible for the disease.1–4 Indeed, experimental serous detachments develop after choriocapillaris injury but not after focal RPE injury alone.5–10 We suspect that serous detachments form in central serous chorioretinopathy because there is widespread metabolic damage to the RPE (either from RPE disease or an underlying disease of the choriocapillaris) that compromises transport capabilities and possibly retinal adhesiveness but does not necessarily destroy the blood–retinal barrier.10 Under these conditions, defects in the RPE barrier will allow fluid to enter from the choroid and accumulate within the subretinal space over areas of nonleaking RPE that are poorly able to pump out the fluid.

The subretinal fluid of serous retinal detachments is commonly thought to contain a high concentration of protein (albumin) because choroidal exudation is the presumed source of fluid, but little is known about the origin and fate of this protein. We previously examined the rate and source of albumin entry into the
rabbit subretinal space after forming experimental retinal detachments using Hanks' balanced salt solution (HBSS). We found that albumin can diffuse into the subretinal space from vitreous and bloodstream, although entry from serum occurs only when the RPE has been damaged. These results suggest that subretinal fluid can be transported actively, passively, or both out of the subretinal space despite albumin entry or RPE damage and without major osmolar shifts. Because subretinal osmolality equilibrates rapidly with the vitreous and protein enters steadily from the vitreous, the subretinal space is not a "closed compartment" even over an intact RPE and the pathogenesis of serous detachment must be viewed in terms of a dynamic subretinal environment into which, and from which, there is a continuous flux of water, ions, and protein. The entry, persistence, and clearance of fluid will depend on a balance of these forces rather than any single source or sink. The present experiments were designed to study the rate and route of albumin movement out of the subretinal space after experimental, albumin-filled retinal detachment over normal and damaged RPE.

MATERIALS AND METHODS

These experiments conform to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experiments were performed on 30 pigmented Dutch rabbits, each weighing approximately 1.5 kg. The animals were sedated with acepromazine maleate (1 mg/kg, intramuscularly) and anesthetized with ketamine hydrochloride (20 mg/kg, intramuscularly) and xylazine (2 mg/kg, intramuscularly). The pupils were then dilated with 1% atropine sulfate and 10% phenylephrine.

Experimental Retinal Detachment

As described, retinas were detached by directing a micropipette with a tip diameter 50 to 80 μm through a small scleral slit 2 to 3 mm behind the limbus and across the vitreous until the tip penetrated the neurosensory retina to reach the subretinal space. The scleral slits were wide enough to keep the intraocular pressure close to zero. Hanks' balanced salt solution (Gibco, Grand Island, NY) and bovine fluorescein isothiocyanate (FITC albumin) (Sigma, St. Louis, MO) were injected into the subretinal space to raise detachments of 7.5-mm diameter, as measured using the eyepiece micrometer grid of an operating microscope. The micropipette was filled with a bolus of HBSS at the tip, followed by a small air bubble and then HBSS containing 40 mg/ml FITC albumin. It was easier to initiate retinal separation with the less viscous solution, and this technique insured that no FITC albumin leaked out during passage through vitreous. The resultant subretinal FITC albumin concentration using this procedure averaged 30.7 ± 5.4 mg/ml (standard deviation), which approaches the concentration of albumin in rabbit serum (36.7 ± 6.3 mg/ml). The injection volume was measured by marking on a connecting tube the initial volume and the final volume after a bleb diameter of 7.5 mm was reached. The average volume injected was 43.6 ± 4.0 μl (standard deviation).

Retinal Pigment Epithelium Damage

In experiments requiring RPE damage, sodium iodate (30 mg/kg) was injected intravenously immediately after making the detachment.

Fluid Withdrawal

Subretinal, vitreous, and serum fluid samples were withdrawn at 0, 1, 2, and 4 hours after detachment. Subretinal fluid was withdrawn by aspirating through a micropipette with tip diameter 70 μm to 100 μm. Vitreous fluid was withdrawn by a 25-gauge needle. After RPE damage with sodium iodate, fluid samples were withdrawn at 1 and 2 hours after detachment. As described, two methods of fluid withdrawal were used to measure the concentration and absolute quantity of albumin in the subretinal space, respectively. In the direct withdrawal method, fluid was aspirated from the absorbing detachment. This gave a measurement of albumin concentration. However, because some of the subretinal fluid had been absorbed and the volume of the detachment was not precisely known, the total amount of albumin in the subretinal space could not be determined by this method. In the reinjection method, HBSS was reinjected before withdrawing subretinal fluid to inflate the detachment as closely as possible to its initial volume. This allowed calculation of the total amount of albumin in the detachment and estimation of the amount of fluid absorbed up to that point. With both techniques, the subretinal fluid was stirred before withdrawal by repeatedly withdrawing approximately 10 μl of fluid from the subretinal space into the micropipette and reinjecting the fluid before the samples were obtained. If bleeding was seen at any point during the procedure, the data were omitted.

Measurement of Albumin Concentration and Osmolality

To determine the rate and route of albumin movement out of the subretinal space, subretinal, vitreous, and serum fluid samples were analyzed for concentration of FITC albumin by fluorophotometry. To measure the native albumin movement and to confirm the results using fluorophotometry, samples of subretinal and vitreous fluid also were analyzed for the combination of FITC + native albumin concentration by determin-
was calculated from the concentration and the volume of subretinal fluid. The amount of albumin in the vitreous was calculated from the concentration values using an estimated vitreous volume of 1 ml. To calculate the serum volume, we estimated blood volume to be 60 ml/kg, with a hematocrit of 40%. 15

Modification of Injection Hole
To evaluate the effect of the injection hole on results, detachments with 10 holes in the sensory retina were made in four rabbit eyes. Subretinal and vitreous fluid were withdrawn 2 hours later.

Prolonged Detachments
To evaluate the effect of longer periods of detachment (and relative ischemia) on the permeability of the retina, we studied six rabbit eyes in which a 7.5-mm detachment filled with HBSS was made 4 hours before injecting protein. At this point, the remaining subretinal fluid was withdrawn as much as possible, and HBSS with FITC albumin was injected to achieve a subretinal concentration of approximately 30 mg/ml and a diameter of 7.5 mm. Samples of subretinal and vitreous fluid were withdrawn 2 hours later.

Verification of Fluorescein Isothiocyanate Binding to Albumin
A 30-mg/ml sample of FITC albumin was placed in a test tube, and the same volume of 20% trichloroacetic acid was added. After centrifuging for 5 minutes, the protein became bound to trichloroacetic acid and aggregated to the bottom of the tube, and the fluorescence of the supernatant was measured by fluorophotometry. The concentration of FITC in the supernatant was only 1.25 ± 0.03 μg/ml (n = 4; 0.004% of the bound FITC albumin concentration), which indicates that there was little risk of our results becoming contaminated by free or dissociated fluorescein.

Sample Size and Statistical Analysis
Results from four to seven samples were averaged for each time point studied. Statistical significance was assessed with the Student's t-test.

RESULTS
Albumin Concentration
After injection of serum levels of FITC albumin into the subretinal space, the FITC albumin concentration in the subretinal fluid decreased steadily with time, from 30.7 mg/ml immediately after detachment to 26.4 mg/ml at 2 hours after detachment and then to 23.9 mg/ml at 4 hours after detachment (Fig. 1, left). In animals pretreated with sodium iodate injection, the rate at which albumin concentration decreased was hardly changed, suggesting that the RPE is not the major barrier to the removal of FITC albumin. Results were essentially the same when we measured the combination of FITC + native albumin, as shown in Figure 1 (right).

Concomitantly, the FITC albumin concentration in the vitreous increased steadily with time, from <0.1 μg/ml immediately after detachment to 157.5 μg/ml at 2 hours after detachment and to 280.1 μg/ml at 4 hours after detachment (Fig. 2). When the RPE was damaged, the rate at which vitreal FITC albumin concentration increased was hardly changed. The combination of FITC + native albumin concentration in the vitreous fluid increased at a similar rate over time, from an initial level of 254.0 μg/ml immediately after detachment to 389.5 μg/ml at 2 hours after detachment and to 536.4 μg/ml at 4 hours after detachment (Fig. 2). When the RPE was damaged, however, the concentration increased much more rapidly to 744.0 μg/ml at 2 hours after detachment, presumably because of serum leaking broadly across the RPE.

To determine whether our measurements of albumin movement from subretinal space to vitreous were influenced by the presence of the micropipette hole (used to make the detachments), we made detachments with ten holes instead of one. The changes in albumin concentration between subretinal fluid and vitreous in these multihole detachments were essentially the same as in detachments with a single hole (Figs. 1 (left), 2). This suggests that the pipette holes were not the major route of protein movement.

Because rabbit retina is avascular, it becomes relatively ischemic when detached from the choroid. To see whether longer durations of detachment (ischemia) would affect retinal permeability to albumin, we conducted a series of experiments in which the retina was detached (by HBSS) for 4 hours before placing FITC albumin in the subretinal space. The results (Figs. 1 (left), 2) were not statistically different from our regular experimental protocol.

Total Amount of Albumin
Figure 3 shows the total amount of FITC albumin in different compartments over time. The amounts in the subretinal fluid decreased from 1376.2 μg immedi-
Albumin Movement Out of the Subretinal Space

FIGURE 1. Concentration of fluorescein isothiocyanate (FITC) albumin (left) and FITC + native albumin (right) in the subretinal space, after filling the subretinal space with Hanks' balanced salt solution containing serum levels of FITC albumin. The subretinal albumin concentration decreased at approximately the same rate whether the RPE was intact or was damaged with sodium iodate, whether we measured FITC or the combination of FITC + native albumin, whether there were 1 or 10 micropipette holes in the retina, and whether the retina was detached for 4 hours before beginning the experiment. The values shown in this and subsequent graphs are mean ± standard error.

At 2 hours after injection, approximately 82.0% remained of the FITC albumin initially injected into the subretinal fluid, and approximately 11.0% had diffused into the vitreous (Fig. 3, left). These numbers add up to <100%, but it is possible that some albumin diffused into the anterior chamber and was removed with the aqueous humor. At 4 hours, 65.8% of the injected amount was still in the subretinal fluid, whereas 25.7% was now in the vitreous space. Serum levels were essentially 0% at both time intervals. At 2 hours after FITC albumin injection over damaged RPE, 79.4% of the injected amount remained in the subretinal fluid, whereas 12.3% was in the vitreous and 2.9% was in the serum.

These findings concerning subretinal fluid were essentially the same when we measured the combination of FITC + native albumin (Fig. 3, right).

Detachment Volume and Osmolality

With the reinjection method used to measure the albumin content of detachments, we simultaneously obtained measurements of fluid volume. The volume of protein-filled detachments over normal RPE fell to 91.9% of the initial volume at 2 hours after detachment and to 78.2% at 4 hours after detachment (Fig. 4). Damaging the RPE with sodium iodate had little effect on the absorption rate. This is in contrast to the situation when the subretinal space is filled with saline (nonproteinaceous) fluid, and RPE damage greatly accelerates fluid absorption.11,16

The osmolality of HBSS containing 30 mg/ml of FITC albumin was 289.3 mOsm/kg. The osmolality of subretinal fluid measured as soon as possible (approximately 1 minute) after forming detachments averaged 295.1 mOsm/kg (Fig. 5). It remained within the range of 295 to 294 mOsm/kg over the next several hours despite the steady absorption of fluid from the detachments. Vitreous osmolality in the rabbit measured 292.8 ± 3.0 mOsm/kg (n = 6).

DISCUSSION

The albumin concentration of subretinal fluid decreased steadily from the “serum levels” that we in-
Albumin Concentration in Vitreous

![Diagram showing albumin concentration in vitreous fluid over time with different conditions like normal RPE, damaged RPE, multiple retinal holes, and pre-existing detachment.](image)

FIGURE 2. Concentration of fluorescein isothiocyanate (FITC) albumin and of FITC + native albumin in the vitreous fluid. The vitreal FITC albumin concentration increased steadily with time after formation of albumin-filled detachments and at a similar rate (note the superimposed symbols) regardless of retinal pigment epithelium (RPE) damage, multiple retinal holes, or preexisting retinal detachment. When the RPE was normal, the combination of FITC + native albumin concentration rose at a similar rate (slope) from its baseline level. When the RPE was damaged, however, the combination of FITC + native albumin concentration in the vitreous rose much more rapidly, probably as a result of native albumin entry from the choroid across the nondetached retina.

We found in the present experiments that albumin entered the serum from the subretinal space only when the RPE has been damaged. However, the rate of albumin movement through an iodate-damaged RPE was only approximately 25% of the rate of albumin movement through the sensory retina into the vitreous. This result is intriguing, considering that the retina is a thick, multilayered structure whereas the RPE is monocellular, and it has implications for clinical conditions. Sodium iodate produces severe damage to the RPE barrier, as evidenced by the passage of fluorescein and protein tracers, yet the rate at which albumin crossed the RPE surface in our experiments was much lower than the rate of passage across retina. This suggests that the molecular channels opened by iodate damage, at least within the time frame of these experiments, remains small enough to hinder the passage of very large protein molecules.

The external limiting membrane has been reported to be the most resistant retinal structure to protein diffusion, whereas diffusion through the rest of the inner retina may not be as restricted. Intravitreal thorotrast, a molecule that has an effective diffusion radius of 50 Å, does not penetrate the external limiting membrane, but horseradish peroxidase (radius 30 Å) does to a variable degree. Fluorescein isothiocyanate dextran 70-S (radius 58 Å) in the subretinal space does not cross the retina easily, but FITC dextran 10-S (radius 22 Å) does. The effective diffusion radius of the FITC albumin used in our study was presumably approximately 35 Å, the radius of native bovine serum albumin, which is intermediate between the FITC dextran 70-S and the FITC dextran 10-S. This FITC albumin was able to cross the sensory retina easily under the conditions of our experiment.

Although we suspect that our results are relevant to humans, we caution that there are significant species differences in the retina and the vascular system. The rabbit retina is thinner than the human retina, which could allow for greater diffusion of protein.
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Total Amount of Albumin

![Graph showing albumin movement](image-url)

FIGURE 3. Total amounts of albumin in the subretinal fluid, vitreous fluid, and serum, expressed in micrograms (left axis) and as a percentage of the amount of fluorescein isothiocyanate (FITC) albumin initially injected into the subretinal space (right axis). The amount of subretinal albumin decreased over time, and the rate was the same whether we measured FITC albumin (left) or the combination of FITC + native albumin (right). Fluorescein isothiocyanate albumin moved into the vitreous at a steady rate independent of retinal pigment epithelium (RPE) damage; it entered the bloodstream (serum) only when the RPE had been damaged. The rate of albumin movement into the bloodstream after sodium iodate damage to the RPE was only approximately 25% of the rate of movement into the vitreous.

Retinal vessels in humans could contribute to protein movement, but most of the rabbit retina is avascular and is nourished primarily by the choroid. Thus, retinal separation makes the rabbit retina relatively ischemic, which in turn might cause cellular edema that affects retinal permeability to fluid or protein or the integrity of the outer limiting membrane. However, our experiments performed after 4 hours of preceding detachment showed no difference in the rate at which FITC albumin crossed the retina into the vitreous. We cannot tell whether ischemic damage within the first 2 hours of experimentation contributed to our primary results, but we know that the electroretinogram is well preserved in locally detached retina throughout this time.22,23

Our procedure for making experimental detachments necessitated producing a small hole in the sensory retina. It is theoretically possible that albumin moved out of the subretinal space through this hole during our experiments, which would confound our judgments about retinal permeability. However, it is unlikely that such movement was significant because small experimental pipette holes have been shown to be effectively self-sealing.24 and when we made detachments with multiple holes, there was no increase in the rate of albumin movement (which one would expect if the holes were a major conduit). Our procedure also caused intraocular pressure to be unphysiologically low, as a result of the scleral opening for the micropipette. This may have decreased modestly the rate of subretinal fluid absorption in our detachments25, or it may have favored the movement of fluid from the choroid through damaged RPE, but it does not alter the protein diffusion or osmotic effects that form the basis of our results and conclusions.

We found that the volume of protein-filled detachments decreased at a similar rate regardless of whether the RPE was intact. This is consistent with Negi’s report16 that the absorption of fluid from serum-filled detachments was only slightly slower over iodate-damaged RPE than over intact RPE. Because protein within the subretinal space neutralizes choroidal oncotic pressure, the driving forces left for fluid absorption will be metabolic transport across the RPE and hydrostatic pressure on the overlying retina. Over normal RPE, the main force will be metabolic transport, whereas over damaged RPE, the only force left is hydrostatic pressure. If the subretinal fluid has a low protein concentration, however, choroidal oncotic pressure becomes an additional absorptive force over a damaged RPE (which allows water movement). Under these conditions, detachments will absorb much...
more rapidly, as we have shown\(^{11,15}\) (see Fig. 4). The clinical implications of these observations is that protein in the subretinal fluid will delay fluid absorption primarily when the RPE is leaky, that is, when absorption would otherwise be faster than normal.

The osmolality of subretinal fluid (HBSS + albumin) grew within 1 minute after forming detachments to a level near vitreous osmolality (292.8 mOsm/kg) and stayed within the range of 293 to 294 mOsm/kg over the next several hours. The initial osmolar change is probably a result of rapid ionic and fluid equilibration with the vitreous, as we have observed.\(^1\) This suggests that the osmotic effects of protein that enters the subretinal space, or that becomes concentrated as fluid is absorbed, would be rapidly neutralized by equilibration with the vitreous. A small oncotic difference might persist, however, if there is a balance between fluid removal across the RPE and fluid entry from the vitreous.

The results of this study confirm our impression from experiments on protein movement into the subretinal space,\(^1\) that the subretinal space is a dynamic environment in which water, ions, and protein exchange continually across the retina and RPE. Fluid accumulation and retention will depend on influx from the choroid initially exceeding, and then balancing, the efflux of fluid across the RPE and the efflux of protein into the vitreous. It is possible, in some chronic exudative retinal disorders, that retina permeability decreases as a result of edema or lipoprotein deposition. However, to the extent that the retina is permeable in a chronic serous detachment, there must be a continual source of choroidal protein to balance the diffusion loss across the retina, as well as a marked obstruction to the fluid absorption across the RPE that would otherwise flatten the detachment. In diseases with diffuse ischemia or inflammation (e.g., Harada's disease), choroidal leakage occurs over a broad area and directly prevents the net absorption of fluid across the RPE. In disorders such as central serous chorioretinopathy, in which the leakage is focal, we postulate that compromise of RPE absorption must be diffuse (although the blood–retinal barrier will be intact except at the leakage sites). This transport abnormality may be a result of choroidal disease, metabolic dysfunction, or both, such as might occur with adrenergic stress.\(^{26,27}\)

**Key Words**

albumin, retinal detachment, rabbit, subretinal fluid

**References**


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