Purpose. To investigate the rate and source of albumin entry into experimental nonrhegmatogenous detachments.

Methods. Detachments were made in Dutch rabbits by injecting Hanks’ balanced salt solution into the subretinal space through a micropipette. Subretinal fluid was withdrawn 0 to 4 hours later through a similar micropipette and analyzed for osmolality and albumin content (by gel electrophoresis). Sodium iodate was injected intravenously in some rabbits to damage the retinal pigment epithelium (RPE). In some rabbits fluorescein isothiocyanate albumin (FITC-albumin) was injected intravitreally or intravenously to measure its entry into the subretinal fluid by fluorophotometry. Results from 4 to 8 eyes were averaged for each data point.

Results. The albumin concentration and total amount of albumin in the subretinal fluid increased steadily over 4 hours in retinal detachments initially filled with Hanks’ solution. Pretreating rabbits with sodium iodate injection resulted in a 50-fold increase in the rate of albumin entry, although the levels were still low relative to those of serum. Intravitreal FITC-albumin entered the subretinal fluid at a rate independent of sodium iodate damage, but intravenous FITC-albumin only entered the subretinal space after RPE damage. Subretinal fluid osmolality remained within the range of 291 to 294 mOsm/kg, irrespective of sodium iodate damage or differences in the rate of fluid absorption.

Conclusions. These results indicate that albumin can diffuse into the rabbit subretinal space from both vitreous and bloodstream, although entry from serum requires damage to the RPE. Subretinal fluid appears to be transported actively (control eyes) or passively (iodate-damaged eyes) out of the subretinal space, despite albumin entry and without major osmolar shifts.
and persist as long as they do. The primary pathology may include choriocapillary damage and elevated choroidal fluid pressure, as well as a diffuse compromise of RPE fluid transport. A pathologic increase in the protein content of subretinal fluid may also be a factor in causing the fluid to accumulate and persist, by raising the oncotic pressure of the subretinal space to a level that minimizes or even reverses the oncotic absorption from the choroid. As a step towards clarifying the role of albumin in serous retinal detachment, we have examined the rate and source of albumin entry into the subretinal space, after making experimental saline-filled retinal detachments, in the presence and absence of RPE damage.

**MATERIALS AND METHODS**

These experiments conform to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experiments were conducted on 65 pigmented Dutch rabbits weighing approximately 1.5 kg each. 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. As described previously, experimental retinal detachments were made by directing a micropipette with tip diameter 40 μm into the vitreous through a small scleral slit 2 to 3 mm behind the limbus. The scleral slits were wide enough to keep the intraocular pressure at a low level. Hanks’ balanced salt solution was injected slowly into the subretinal space until the dome-shaped detachments had a diameter of 7.5 mm, as measured using the eyepiece micrometer grid of an operating microscope (calibrated with a standard object placed surgically on the retina). 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. An average of 43.9 ± 8.6 μl (mean ± SD) of Hanks’ solution was injected to make each bleb. Sodium iodate (30 mg/kg) was injected intravenously in some rabbits immediately after making the detachment.

Fluid was withdrawn from the subretinal space 0, 1, 2, and 4 hours after detachment using a vacuum through a micropipette with tip diameter 50 μm to 100 μm. After RPE damage with sodium iodate, fluid was withdrawn 1 and 2 hours after detachment. Different methods of fluid withdrawal were used to measure the concentration and absolute quantity of albumin, respectively, in the subretinal space (Fig. 1). In the direct withdrawal method, fluid was aspirated from the absorbing detachment. This yielded 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, Hanks’ solution was reinjected before withdrawal of subretinal fluid to reinflate the detachment back as closely as possible to its initial diameter and shape. This allowed calculation of the total amount of albumin in the detachment and yielded an estimate of the amount of fluid that had been absorbed up to that point in time. 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. The average volume of fluid withdrawn was 30.0 μl using direct withdrawal and 42.2 μl using reinjection. For the reinjection method, this amounted to approximately 90% of the volume. With all experiments, results from 4 to 8 samples were averaged for each time point studied.

To determine whether the injection hole in the sensory retina had an effect on albumin entry from the vitreous space, we made detachments with multiple (approximately 10) holes in the sensory retina of 4 rabbit eyes and withdrew subretinal fluid 2 and 4 hours later using the direct withdrawing method. To determine the baseline albumin content of vitreous and serum, samples were withdrawn from normal rabbits.

Samples of fluid were analyzed for albumin concentration by determining the albumin content of a 10-μl aliquot with gel electrophoresis in a 10% polyacrylamide SDS gel according to Laemmli et al. To visualize separated bands, the gel was stained using a BioRad silver stain kit (Hercules, CA). Pure rabbit albumin was diluted in quantitative steps, and 10-μl aliquots were run on the gel to produce a scale of density against which the albumin content (in μg) was measured (Fig. 2). The osmolarity of samples was measured using a Wescor 5100C vapor pressure osmometer (Logan, UT).

Two sets of experiments were performed using bovine fluorescein isothiocyanate albumin (FITC-albumin; Sigma, St. Louis, MO). In the first experiments, 500 μg FITC-albumin was injected into the vitreous space immediately after bleb formation with Hanks’ solution. One and 2 hours after retinal detachment, subretinal fluid was withdrawn as described above, and the concentration of FITC-albumin was measured using a fluorophotometer. Sodium iodate (30 mg/kg) was used to damage the RPE in some rabbits. The concentration of FITC-albumin in the vitreous was verified by withdrawing fluid from the vitreous space immediately after the samples of subret-
direct withdrawing method

1. Injection of Hanks' solution into subretinal (SR) space to make retinal detachment
2. Absorption of SR fluid 0-4 hours after detachment
3. Direct withdrawal of SR fluid

reinjection method

1. Injection of Hanks' solution
2. Withdrawal of SR fluid
3. Reinjection of Hanks' solution
4. Withdrawal of SR fluid

FIGURE 1. Methods of withdrawing subretinal fluid. In the direct withdrawal method, fluid is aspirated from the absorbing detachment, allowing measurement of albumin concentration. In the reinjection method, Hanks' solution is reinjected to inflate the detachment back to its approximate initial volume before withdrawal of subretinal fluid. This allows calculation of the total amount of albumin in the detachment.

inal fluid were obtained. In the second experiments, approximately 50 mg FITC-albumin was injected intravenously 30 minutes before bleb formation with Hanks' solution. Subretinal fluid was withdrawn 1 and 2 hours after retinal detachment, and sodium iodate was used to damage the RPE in some rabbits. The concentration of FITC-albumin in the serum was measured 2 hours after intravenous injection.

All statistical analyses used Student's t-test.

RESULTS

We made our experimental detachments with Hanks' solution, a balanced salt solution containing no protein, to measure the rate and source of albumin entry into the subretinal space. As a point of reference, in 5 normal rabbits, the albumin concentration measured 244 ± 31 µg/ml in vitreous and 36.7 ± 6.3 mg/ml in serum. Figure 3 shows that the albumin concentration of the subretinal fluid increased steadily with time from less than 1.0 µg/ml immediately after detachment to 9.7 µg/ml 2 hours after detachment and 28.7 µg/ml 4 hours after detachment. In animals pretreated with sodium iodate injection, the concentration of albumin increased much more quickly (P < 0.001) to 688.9 µg/ml 2 hours after detachment.

The albumin concentration in detachments prepared with multiple retinal holes increased at essentially the same rate as that in detachments made with a single hole, to 13.1 µg/ml 2 hours after detachment and 27.8 µg/ml 4 hours after detachment. This suggests that the pipette hole is not the major route of protein entry.

The total amount of albumin in detachments increased over time from less than 0.04 µg immediately after detachment to 0.25 µg 2 hours after detachment and 2.59 µg 4 hours after detachment (Fig. 4). After sodium iodate injection, the total amount of albumin
Figure 3. Concentration of albumin in detachments filled initially with Hanks' solution. The samples were obtained using the direct withdrawal method at different times after detachment. The values shown in this and subsequent graphs are mean ± SEM. In animals pretreated with sodium iodate injection to damage the RPE, the concentration of albumin increased much more quickly.

FITC-albumin concentration in subretinal fluid obtained immediately after retinal detachment was less than 0.1 µg/ml but rose to 20.3 µg/ml 2 hours after detachments made over normal RPE and to 24.8 µg/ml over iodate-damaged RPE (Fig. 7). The difference between normal and iodate-damaged RPE results was not statistically significant.

To determine the rate of protein entry from the systemic circulation, independent of vitreous protein, FITC-albumin was injected intravenously. Its concentration in serum, 2 hours after intravenous injection, was 1132.2 ± 283.9 µg/ml (n = 4). The FITC-albumin concentration in subretinal fluid was less than 0.1 µg/ml immediately and 2 hours after detachments made over normal RPE (Fig. 8). After RPE damage with sodium iodate, the concentration of FITC-albumin in the subretinal fluid rose from less than 0.1 µg/ml immediately to 29.1 µg/ml 2 hours after detachment (P < 0.05).

Discussion
Our results indicate that experimental retinal detachments filled with a saline solution show a rapid influx of albumin. When there was no damage to the RPE, the concentration of albumin increased much more quickly (P < 0.005) to 13.67 µg 2 hours after detachment.

In using the reinjection method to measure the albumin content of detachments, we simultaneously obtained a measure of the fluid volume that had been absorbed. These data show that Hanks'-filled detachments absorbed steadily after formation (Fig. 5). The volume fell to 68.5% of its initial value 4 hours after detachments made over normal RPE and to 39.6% 2 hours after detachments made over iodate-damaged RPE.

The osmolality of Hanks' solution is 284 mOsm/kg, but when we measured subretinal fluid as soon as possible (approximately 1 minute) after forming detachments, the osmolality averaged 291 mOsm/kg (Fig. 6). Osmolality stayed between 291 and 294 mOsm/kg during the next several hours, regardless of whether the RPE was normal or had been damaged with sodium iodate. Vitreous osmolality in the rabbit measured 294 mOsm/kg, but this value was not statistically different from the subretinal fluid measurements.

To determine the rate of vitreous protein entry into the subretinal space, independent of serum protein, we injected FITC-albumin into the vitreous. Its concentration 2 hours after the intravitreal injection was 257.8 ± 88.7 µg/ml (n = 5) in experiments with normal RPE and 248.4 ± 44.1 µg/ml (n = 6) in experiments with iodate-damaged RPE. The FITC-albumin concentration in subretinal fluid obtained immediately after retinal detachment was less than 0.1 µg/ml but rose to 20.3 µg/ml 2 hours after detachments made over normal RPE and to 24.8 µg/ml over iodate-damaged RPE (Fig. 7). The difference between normal and iodate-damaged RPE results was not statistically significant.

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barrier, the subretinal concentration of albumin remained low (28.7 \mu g/ml after 4 hours) relative to serum albumin (approximately 36.7 mg/ml) within the short time frame of our experiments. Our results with intravenous FITC-albumin confirm that when the RPE is intact, albumin cannot move readily through it, although it can diffuse slowly across the sensory retina.\textsuperscript{17} We found that FITC-albumin in the vitreous entered the subretinal space at a rate similar to that of autologous albumin (see analysis below), suggesting that over an intact RPE, the vitreous is the major source of subretinal protein. However, the vitreous is probably not the major source in most nonrhegmatogenous clinical detachments, insofar as the RPE is usually damaged.

Damaging the RPE barrier with sodium iodate resulted in faster entry of albumin, presumably from the choroid. In support of this interpretation, FITC-albumin in the serum was found to enter the subretinal space only when the RPE barrier was damaged with sodium iodate, and fluorescein leakage through iodate-damaged RPE has been shown previously with angiography.\textsuperscript{18} Nevertheless, the concentration of albumin still remained low relative to serum, suggesting that the actual volume of protein entry through RPE "leaks" may be smaller than intuitively imagined from fluorescein angiographic appearances.

In the experiments using FITC-albumin, we injected 500 \mu g into the vitreous, which has a volume of approximately 1 ml in the rabbit. If the albumin diffused evenly from the injection site, this diffusion would yield a concentration of approximately 500 \mu g/ml shortly after injection. Because the FITC-albumin concentration in the vitreous measured 257.8 \mu g/ml 2 hours after injection, the average concentration during this time period was approximately 379 \mu g/ml. If we correct the measured subretinal FITC-albumin injection to account for the ratio of FITC-to-native albumin in the vitreous (379:244), we obtain a corrected value of 15.1 \mu g/ml 2 hours after detachments over normal RPE. This represents 5.4% of the normal vitreal albumin concentration and has the same order of magnitude as measurements of subretinal native albumin (9.7 \mu g/ml) 2 hours after detachment, which
Figure 8. Concentration of FITC-albumin in detachments after intravenous injection of FITC-albumin. The FITC-albumin concentration in the subretinal fluid was significantly greater after sodium iodate damage to the RPE.

is 4.0% of the normal vitreal level. Therefore, our two methods of protein measurement are consistent, in support of the conclusion that vitreous is the main source of subretinal protein when the RPE is intact.

If we apply the same correction to our data with iodate-damaged RPE after intravenous injection of FITC-albumin, to account for an FITC-to-native albumin ratio of 1132:36700 in the serum, we arrive at a corrected value of 943.4 μg/ml for the subretinal FITC-albumin concentration 2 hours after detachment. This is approximately 2.6% of the normal serum albumin concentration. This value is similar to our measured value of 688.9 μg/ml (1.9%) for subretinal native albumin 2 hours after retinal detachment over iodate-damaged RPE.

Our procedure for making experimental detachments involves creating a small hole in the sensory retina. It is possible that albumin entered the subretinal space by flowing through this hole as well as by diffusion through the retina. However, we doubt that this occurred to a significant degree because these pipette holes have been shown previously to be effectively self-sealing. Furthermore, when we made multiple holes in the retina, the rate of albumin accumulation did not increase as one would expect if the holes were a major conduit for protein movement.

All of our experimental detachments showed steady fluid absorption over time. Our experiments with the reinjection method allowed us to measure the volume changes, as plotted in Figure 5. The rate of fluid absorption was greater over iodate-damaged RPE, consistent in general with previous observations. Detachments in the earlier study often absorbed within 30 minutes, but they were much smaller in size. These results reinforce the conclusion that fluid absorption across intact or damaged RPE can take place despite the concomitant entry of protein. Of course, the protein levels achieved during these acute experiments are nowhere near serum levels or levels that have been observed in subretinal fluid from rhegmatogenous detachments. However, even subretinal serum and fluid containing high-molecular-weight dextran have been shown to be absorbed from the subretinal space within hours. Thus, acute osmotic changes from protein entry or concentration within the subretinal space probably do not represent the major constraint, although they may be contributing factors for either fluid entry or fluid persistence in serous detachment.

This conclusion must be tempered by the fact that it is hard to make accurate osmotic measurements to assess the effects of subretinal protein, especially under circumstances where the proteins might be either breaking up into osmotically active units or turning viscous and altering fluid movement by mechanical means. Our measurements show that subretinal osmolality grew within 1 minute of forming detachments from the level of Hanks' solution (284 mOsm/kg) to a level near vitreous osmolality. We presume that this represents ionic and fluid equilibration with the vitreous, which suggests that osmolar effects of subretinal protein would also tend to be neutralized rapidly. We measured subretinal osmolality between 291 and 294 mOsm/kg, irrespective of RPE damage and irrespective of the duration of detachment. However, our measurements were not sufficiently accurate to discriminate shifts less than 2 or 3 mOsm/kg in magnitude. This is a problem that would need to be overcome to properly evaluate osmotic effects, because a shift of only 1 mOsm/kg is equivalent to at least 17 mm Hg of oncotic pressure, and even small osmolar effects could be significant in terms of fluid movement.

Serous retinal detachments are known to occur in a variety of chorioretinal disorders, predominantly conditions that involve choroidal ischemia. This includes central serous chorioretinopathy, in which the fluid appears to enter the subretinal space through focal defects in the RPE, although larger areas of choriocapillaris damage have been demonstrated by indocyanin green angiography. Detachments can also be produced experimentally by choriocapillaris injury. In these conditions, where the fluid entering the subretinal space comes from the choroidal extracellular fluid, we may presume that it enters with high protein content. This is a different situation from that.
in the present experiments, in which nonproteinaceous saline was injected into the subretinal space to document sources of protein diffusion. The questions of relevance to clinical attachments will relate more to mechanisms of protein and fluid removal than to mechanisms of protein entry. Nevertheless, the present data give some indication of the rates of protein movement that might be expected across the retina and across a damaged pigment epithelium. That subretinal osmolality equilibrates within minutes and that protein enters steadily from the vitreous indicate that the subretinal space is not a "closed compartment." 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 on any single source or sink.

Key Words
albumin, nonrhegmatogenous retinal detachment, osmolality, retinal pigment epithelium, subretinal fluid

References