The rate and route of fluid resorption from the subretinal space of the rabbit

Donald A. Frambach and Michael F. Marmor

Small nonrhegmatogenous detachments were formed in living rabbit eyes by injecting a few microliters of fluid into the subretinal space. The change in volume of these detachments was followed by sequential photogrammetric measurements. Detachments of 1 to 3 mm diameter filled with a balanced salt and glucose solution (Ames' solution) resorbed at a constant rate over approximately 80 min of observation and appeared to be totally resorbed within 2 to 6 hr. The rate at which fluid resorbed per unit area of pigment epithelium was consistent with the rate of resorption in other transporting epithelia as well as with clinical observations in humans. Detachments filled with isotonic sucrose resorbed much more slowly than those filled with saline solutions, suggesting that fluid resorption requires the passage of ions through cell membranes. Hypoxia markedly and reversibly reduced the rate of resorption, suggesting that active transport is involved in the removal of the saline solutions from the subretinal space. Anatomical and physiological evidence indicate that the site of active transport is the pigment epithelium. Active resorption of subretinal fluid is probably a factor in the maintenance of normal retinal adhesion. (INVEST OPHTHALMOL VIS SCI 22:292-302, 1982.)

Key words: pigment epithelium, active transport, subretinal fluid, retina, detachment, adhesion, photogrammetry

Fluid can resorb rapidly from the subretinal space, as evidenced by the frequent disappearance of subretinal fluid within 24 hr after a nondrainage operation for retinal detachment. Our laboratory has been interested in the physiology of this process for some time and recently reported a technique to study it by injecting fluid into the subretinal space of living rabbits. In these initial experiments, we produced small experimental detachments (blebs) with various solutions but were unable to measure their volume. We monitored the time required for complete resorption of blebs, but this was a difficult endpoint to determine.

We have since developed a photogrammetric technique to sequentially measure the volume of experimental detachments and thus determine the rate of fluid resorption from the subretinal space. The present report describes baseline studies with this technique and subsequent experiments which suggest that fluid is resorbed from the subretinal space by active transport across the retinal pigment epithelium (RPE). We speculate that this active transport system may contribute to the maintenance of retinal adhesion.

Methods

All experiments were performed on black Dutch rabbits (both male and female) weighing approxi-
mately 1.5 kg, selected for deep and even pigmentation of the RPE. The animals were anesthetized with sodium pentobarbital (Diamond Laboratories), 36 mg/kg subcutaneously, followed 15 min later by ketamine (Parke, Davis & Co.), 100 mg/kg intramuscularly. The pupils were dilated with 1 drop each of 1% cyclopentolate and 10% phenylephrine. Additional 50 mg doses of ketamine were administered intramuscularly as needed. Blood gas determination showed that animals were neither hypoxic nor acidic under this anesthesia. All experiments were performed under dim room light illumination. Retinas were exposed to higher illumination only during formation of the blebs and briefly at the time of each photograph.

Formation of in vivo detachments. Local detachments (blebs) were formed by injecting a few microliters of fluid into the subretinal space through a glass micropipette as described previously. Before pulling the micropipette tips, the tubing was flushed with 10 ml of a concentrated detergent solution (Haemosol), rinsed carefully with tap water, and then flushed with at least 200 ml of distilled water. This procedure was followed for all experiments reported here because it seemed to eliminate premature shriveling of the blebs, which we had observed about 10% of the time in preliminary experiments. Micropipette tips were broken manually to an outer diameter of 20 to 30 μm. To force fluid out of the tip at the appropriate rate, air pressure of 10 to 20 lb/in² was applied. The micropipettes were introduced into the eye through a hole made approximately 3 mm posterior to the limbus, and a slow flow out of the tip was maintained during passage through the vitreous to avoid clogging. The pipette was advanced with a micromanipulator until the retina was gently penetrated and a detachment began to form. Blebs of 1 to 3 mm diameter formed in 10 to 30 sec and retained their shape after the micropipette was withdrawn. The experiment was discarded if the retina or RPE were visibly damaged beyond the formation of a minute hole by the pipette tip.

Photogrammetry. The image projection system for photogrammetry, illustrated in Fig. 1, was mounted on the microscope housing at a fixed angle of approximately 15° from the microscope viewing system. A glass ronchi screen (80 lines/inch) was focused upon the retina through a 75 mm photographic enlarging lens (Soligor) mounted backwards. A diaphragm was placed near the screen to adjust the size of the light cone and minimize flare. Illumination was provided by a fiber optic light source for viewing and by a flashlamp (Nikon 200 watt-second slit-lamp photoflash) for photography. A moveable mirror was placed behind the flashlamp during photography to maximize the effective flash output. A face shield above the apparatus protected against the possibility of flashlamp explosion.

The fundus was viewed and photographed at 10 to 15 min intervals through a Goldmann contact lens and a Zeiss OpMi I operating microscope with
PHOTOGRAPH

DETACHMENT

VOLUME CALCULATIONS

Assume $\theta$ is small
Let $n = \text{number of sections}$

1. $h \approx \frac{A_i}{\sin \theta}$
2. sectional area $= \frac{A_i}{\sin \theta}$
3. volume $\approx \frac{d \sum A_i}{\sin \theta}$

Fig. 3. Photogrammetric calculation of bleb volume. The apparent height of a bleb, $x$, as seen in one of our photographs, can be used to calculate the true height of the bleb, $h$, by dividing by the sine of the angle $\theta$ between the viewing and projecting systems. The measured area in the photographs, $A_i$, is used to calculate the true cross-sectional area of the bleb in the same fashion. Bleb volume is approximated by summing the true cross-sectional areas and multiplying by the distance, $d$, between them.

Fig. 2. The contact lens was buttressed against a rigid support to minimize movement. We used Kodak 2495 specification 417 RAR instrumentation film (high-speed orthochromatic) and processed it with Kodak D-76 developer for 1.8 times the recommended time. Orthochromatic (red-insensitive) film was used so that the images would not be degraded by the diffuse red reflection off the choroid.

The black and white negatives of each photograph were mounted in glass slides (to ensure flatness) and projected onto a light table. Photographs taken through the microscope of a printed grid confirmed that no distortion was induced by the photographic or projection systems. Magnification and the angle between the viewing and projecting systems were determined empirically by photographing ball bearings of known size (painted white) placed within the eye at the same location as our experimental detachments.

We measured only the anterior surface of the blebs, but this was sufficient to determine changes in bleb volume because both bleb diameter (see Results) and posterior curvature (determined by the sclera) remained constant during the experiments. Our photogrammetric method of analysis (Fig. 3) was similar to that reported previously. The apparent cross-sectional area of each "slice," as seen in the photographs, was measured with an electronic planimeter (Model 1224; Numonics Corp.) and was converted to a true cross-sectional area by trigonometry. To eliminate the possibility of bias, slides were measured without reference to their labels. Variability and error in the system were such that measurement of photographs taken in rapid succession showed an average difference of 5% in area and 10% in volume.

Histology. Within 30 sec of enucleation, eyes for scanning electron microscopy were bisected at the limbus, the vitreous was gently removed, and the posterior pole was placed into Ames' solution with 3.5% glutaraldehyde (pH 7.2 to 7.4). From this point, the tissue was processed in the standard fashion. To define the locus of detachment, blebs were formed with a red-pigmented silicone suspension (Canton Biomedical Products) injected through a slightly enlarged micropipette tip (40 $\mu$m OD). One hour after injection, the eye was enucleated, placed in 10% formalin, and processed for light microscopy. Hypoxia experiments. A tracheostomy was performed under anesthesia, and after paralysis with
Subretinal fluid resorption

Table I. Resorption rates for blebs formed with Ames’ solution

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Inner bleb area (mm²)</th>
<th>Resorption rate (μl/hr)</th>
<th>Resorption rate/inner area (μl/mm²/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.46</td>
<td>1.79</td>
<td>0.52</td>
</tr>
<tr>
<td>2</td>
<td>1.83</td>
<td>0.68</td>
<td>0.37</td>
</tr>
<tr>
<td>3</td>
<td>1.78</td>
<td>0.49</td>
<td>0.28</td>
</tr>
<tr>
<td>4</td>
<td>1.82</td>
<td>0.47</td>
<td>0.26</td>
</tr>
<tr>
<td>5</td>
<td>1.75</td>
<td>0.47</td>
<td>0.26</td>
</tr>
<tr>
<td>6</td>
<td>2.33</td>
<td>0.52</td>
<td>0.22</td>
</tr>
<tr>
<td>7</td>
<td>1.18</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>8</td>
<td>1.92</td>
<td>0.49</td>
<td>0.26</td>
</tr>
<tr>
<td>9</td>
<td>1.29</td>
<td>0.29</td>
<td>0.23</td>
</tr>
<tr>
<td>10</td>
<td>4.22</td>
<td>1.38</td>
<td>0.33</td>
</tr>
<tr>
<td>11</td>
<td>2.14</td>
<td>0.63</td>
<td>0.29</td>
</tr>
<tr>
<td>12</td>
<td>1.04</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>13</td>
<td>2.43</td>
<td>0.76</td>
<td>0.31</td>
</tr>
<tr>
<td>14</td>
<td>1.69</td>
<td>0.59</td>
<td>0.35</td>
</tr>
<tr>
<td>15</td>
<td>0.98</td>
<td>0.22</td>
<td>0.23</td>
</tr>
<tr>
<td>16</td>
<td>2.04</td>
<td>1.08</td>
<td>0.53</td>
</tr>
<tr>
<td>17</td>
<td>1.62</td>
<td>0.93</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Note that bleb size is a major determinant of the raw resorption rates, but is only a minor factor when the rates are expressed as a function of inner area (see text).
and area vs. time, for the first 80 min of resorption of a 2.3 mm diameter detachment made with Ames' solution. Each point represents the average measurement of two photographs taken in rapid succession. Note that bleb volume decreased in a linear fashion within the period of observation, whereas the basal area of the bleb remained constant. Beyond 90 to 120 min, the small blebs had lost most their volume, and the slope of the volume line usually tailed off. Linear resorption could be documented for longer periods from larger blebs. We shall report data from only the initial linear portion of the resorptive process. The slope of the line through these data points (a minimum of five per experiment) was determined by the method of least squares and provides the rate of fluid resorption.

To study the rate of fluid resorption, 18 Ames blebs of varying size and location were formed within eight eyes (Table I). We found that detachments of approximately the same size, formed in different eyes, could have resorption rates that varied by as much as a factor of 2. Furthermore, more fluid per unit time was resorbed from large blebs than from small ones, which is to be expected since they have a larger surface area available to resorb fluid (Fig. 5, left panel).

To measure the rate of resorption per unit area, it was necessary to allow for the fact that photographs recorded the vitreal surface of the retina whereas only the inner area of a bleb (i.e., area less retinal thickness) could be resorptive. We observed that in most of our photographs, two concentric circles could be seen at the margin of the detachment (arrows in Fig. 2). The outer circle represented the change in curvature at the retinal surface, and the inner surface corresponded to the internal boundary of the bleb. This was confirmed by the observation that blebs filled with fluorescein showed dye only up to the inner circle (see Fig. 7, B). The average distance between these circles in 10 blebs was $0.11 \pm 0.02$ mm (S.D.), and did not correlate with bleb size. Because the outer circle was invariably visible in our photographs but the inner circle was not, we determined the basal interior area of all blebs by measuring the area of the outer circle and subtracting an area corresponding to a 0.11 mm ring.

Using this method, we found that the rate of resorption per unit area (Fig. 5, right panel) was relatively but not entirely inde-
Fig. 6. Scanning electron micrographs of inner bleb surfaces. A, Low-power view of the RPE beneath a bleb. Arrows depict the bleb margin. B, Higher-power view of RPE within a bleb. C, Photoreceptor surface overlying the same bleb. (Magnification bars: A, 100 µm; B and C, 1 µm.)

Fig. 6. Scanning electron micrographs of inner bleb surfaces. A, Low-power view of the RPE beneath a bleb. Arrows depict the bleb margin. B, Higher-power view of RPE within a bleb. C, Photoreceptor surface overlying the same bleb. (Magnification bars: A, 100 µm; B and C, 1 µm.)

dependent of bleb size (specific rates remained slightly greater for larger detachments). For the 18 control blebs in Table I, the average rate of fluid resorption was 0.32 ± 0.11 µl/mm²/h.

**Histology.** To verify that our injections filled the subretinal space and did not penetrate within the retina or under the RPE, three blebs were raised by using a pigmented silicone suspension instead of a saline solution. Examinations of these blebs by light microscopy showed silicone pigment only in the subretinal space.

Eyes containing blebs were also examined with scanning electron microscopy, which showed that the retina was clearly separated from the RPE with a minimum of damage to either surface (Fig. 6). Evidence of contact between the tip of the micropipette and the RPE was only present in about 20% of eyes examined by scanning microscopy and never showed more than the loss of a few cells at the point of contact.

**Route of fluid movement.** As each bleb was formed, the micropipette made a tiny hole in the neurosensory retina. To determine whether leakage through these holes might account for the resolution of blebs, we introduced fluorescein or fluorescein-dextran into formed blebs by a route other than the original hole. This was accomplished by making a second bleb nearby, using a fluorescein-stained saline solution (Fig. 7, A), and allowing the second bleb to merge with the first one (Fig. 7, B). Although the first bleb always lit up rapidly with green dye, fluorescein or
Fig. 7. Retention of fluorescein within blebs. A, Right-hand bleb (open arrow) was formed first, with normal saline; the white spot is a light-reflex. The left-hand bleb (closed arrow) is shown being formed with a fluorescein-containing solution. B, The two blebs have merged, so that dye now fills the interior of the original bleb. Note that dye only extends to the inner ring (see Fig. 2).

Fluorescein-dextran was never observed to flow out the original hole into the vitreous.

To further investigate the route of resorption, we formed paired detachments in three eyes, one detachment filled with normal saline and the other with an isotonic (0.262M) sucrose solution. Sucrose has been used as an extracellular marker because it cannot cross cell membranes without first being broken down by a disaccharidase. During the initial 80 to 90 min of observation, the resorption rates for blebs containing sucrose (Fig. 8) were only about one tenth of the rates for blebs containing saline. However, we observed that the total time required for resorption of the sucrose-filled blebs was less than twice (as opposed to 10 times) that which was required for a similar size bleb filled with Ames' solution or normal saline.

Evidence for active transport. In five experiments blebs were raised with Ames' solution while the animal was artificially respired with room air to maintain normal levels of PO₂, PCO₂, HCO₃, and pH. After approximately 40 min, the inspired O₂ was abruptly decreased to 6% to 9% and maintained at that level. Blood gases taken during this phase revealed that the animals quickly became profoundly hypoxic (PO₂ less than 25). They also developed a metabolic acidosis (PCO₂ less than 40 and pH as low as 6.95), which was corrected in two of the experiments by infusing sodium bicarbonate during the period of hypoxia. After approximately 1 hr of hypoxia, the animals were given room air, which promptly restored the PO₂ to normal (although the metabolic acidosis persisted for quite some time). Blebs were photographed at 5 min intervals through all phases of these experiments. In every case (Fig. 9), regardless of whether acidosis was present or absent, the rate of fluid resorption slowed markedly during the hypoxic phase and promptly accelerated when room air was restored.

Discussion

We have shown previously that small experimental detachments can be made in the living eye by injecting fluid under the retina through a glass micropipette. Such small detachments resorbed within a few hours, and the time required was affected by the composition of the fluid injected. However, these
initial observations suffered because the time at which the retina became reattached was difficult to determine. We have now developed a technique to measure the change in bleb volume as fluid resorption occurs, i.e., the on-going rate of resorption. This allows for more accurate examination of the factors that affect the resorption of fluid.

Interpretation of our results depends on the route by which fluid leaves the interior of the blebs. In theory, this could occur across the RPE, across the retinal substance, through the hole made by the micropipette, or by spread into the subretinal space. The last-named is very unlikely because bleb diameter did not change as the fluid resorbed. Our experiments with fluorescein and sucrose give evidence that a net loss of fluid is unlikely to occur through the retina or the retinal hole. First of all, we never observed a flow of dye out of the hole of blebs filled with fluorescein, although realistically, a tiny amount might not have been visible against the bright background of the bleb itself. Second, blebs filled with isotonic sucrose resorbed only one-tenth as fast as did blebs filled with normal saline over the initial 80 min of observation. Sucrose is a disaccharide, which does not ordinarily cross cell membranes, but it should diffuse easily through the open extracellular pathways that bridge the retina. The fact that sucrose-filled blebs resorbed so slowly suggests that the net loss of fluid does not occur through the retina, the micropipette hole, or through an area of RPE damaged by the micropipette; in contrast, the loss seems to occur primarily by a pathway across membranes through which saline but not sucrose can pass. Further evidence against leakage out the micropipette hole or damaged RPE is provided by Table 1 (and Fig. 5). Correcting for inner bleb surface area would have no effect if the rate of resorption were determined by fluid flow through a retinal hole or a small region of damaged RPE.

Alternative interpretations of the sucrose experiment must be considered. First isotonic sucrose is 30% more viscous than normal saline and will flow through retinal or other extracellular channels more slowly.

However, viscosity alone would not account for the nearly 1000% difference in resorption rate between the two substances. Second, as soon as a sucrose bleb is formed, sucrose will begin to diffuse out toward the vitreous, and extracellular ions (predominantly sodium and chloride) will begin to diffuse in. Sodium chloride diffuses more rapidly than sucrose in free solution. If more extracellular ions diffuse into a bleb than sucrose diffuses out, and water is obligated to move along with the solutes, there could be a net fluid gain that would slow the measured rate of resorption. However, it seems unlikely that this mechanism would fully neutralize resorption out of the blebs. The mobilities of sucrose and sodium chloride (which are unknown in rabbit retina) vary among tissues, and in some (e.g., rat nerve) sucrose even appears to be more mobile than sodium. The observation that our sucrose-filled blebs ultimately resorbed within twice the resorption time for saline-
filled blebs (which is much less than a 10-fold prolongation of the resorption time) may be readily explained. Our measurements concerned only the initial period of resorption, whereas over a longer period of time sufficient sodium and chloride will diffuse into (and sufficient sucrose out of) the blebs to allow more rapid clearance of the fluid. The fact that bleb content is most uniform immediately after bleb formation supports our choice of the initial resorption rate as a key parameter.

Another reason to doubt that blebs resorb into or across the retina is that there is no obvious force to drive fluid in the retinal direction. Intraocular and oncotic pressures act normally to move fluid in the opposite direction toward the sclera.\(^\text{[11]}\) The weight of detached retina might in theory serve to collapse the blebs, but it would be exceedingly small within a fluid environment, and we have demonstrated that blebs still resorbed with normal rapidity when the animals were turned over so that gravity acted to enlarge the detachments. Elasticity of the stretched retina might act to collapse our blebs. However, the elastic force would be greatest shortly after bleb formation and diminish as the blebs resorb, whereas the resorption of fluid we observed was linear for more than 1 hr.

The conclusion that fluids are resorbed from the subretinal space across cell membranes is supported by our hypoxia experiments which indicate that oxygen, and presumably energy, is required for the removal of fluid. The magnitude and prompt reversibility of the hypoxia effect strongly suggest that an active transport system is involved. These data also suggest that passive transport systems contribute very little to the resorption of fluid under our experimental conditions.

Two alternative possibilities should be mentioned. First, hypoxia might alter the permeability of the membrane barrier, through cellular swelling or contraction, and thus modify a passive transport system. However, there must be a driving force for the removal of fluid in a passive system. Hydrostatic pressure may be ruled out because the eyes are hypotonous in our experiments and we have already dealt with gravity and elastic forces. Bill\(^\text{[11]}\) has reported that the oncotic pressure of the rabbit choroid is on the order of 10 mm Hg, but osmotic flow seems unlikely to account for bleb resorption. A bleb cannot resorb unless there is a net loss of osmotically active particles. However, free water lost from blebs by osmotic flow will be replaced immediately by diffusion from the vitreous, and we doubt that small ionic concentration changes at the unstirred RPE boundary could account for enough ion movement to evacuate the blebs within the time frame of these experiments. Second, hypoxia might lead to alterations in the blood supply to the choroid. Fluid that is resorbed from our blebs is probably taken up by the choroidal circulation in our preparation, since there is no hydrostatic pressure to drive it across the sclera. A diminution of the circulation might slow resorption, but marked changes are unlikely in the choroid, and vascular compromise (if it occurs at all) would simply contribute to the energy deprivation of the RPE and thus reinforce the argument for an active transport system.

The transport mechanisms by which subretinal fluid is resorbed remain unknown, although they are almost certainly contained within the RPE. The RPE is the only structure adjacent to the subretinal space in the rabbit that possesses tight junctions and demonstrates the high electrical resistance, numerous mitochondria, and convoluted surfaces which one would expect to see in an epithelium modified for active transport.\(^\text{[12]}\) In primates and man the retinal capillaries also contain a junctional barrier, but there is no intrinsic retinal vasculature in the rabbit near the site of our blebs.

To the extent that the RPE removes the subretinal fluid, the net rate of resorption from blebs should be a function of the surface area of the bleb floor. However, the resorption rate per unit area of available RPE should be independent of bleb size. We found this true to a good approximation, but large blebs still showed a slightly higher rate of resorption per unit area than small ones. Although the width of the concentric ring vis-
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From the data in Table I, the average specific rate of fluid resorption in the rabbit was 0.32 µl/mm²/hr, a value consistent with other transporting epithelia. Although experimental measurements in the rabbit should not be directly applied to humans, it is interesting to compare this value with a representative clinical situation. This same rate would suffice to remove 2 ml of fluid in 24 hr from under a retinal detachment covering one third of the fundus of a human eye. Some human detachments clear more slowly, but transport across the RPE may well be compromised in eyes that have suffered a detachment and had retinal surgery.

We have tried to minimize trauma to the eye or pigment epithelium during these experiments, but damage to the pigment epithelium must be considered as a possible source of error. We noted occasional damage to some pigment epithelial cells by our micropipette tips. However, the rate of resorption showed no clear relationship to the presence or absence of such damage, and the relative constancy of the resorption rate per unit area would not have been observed if the rate were determined by passive flow through a few damaged cells. Prolonged hypotony has been reported to alter the integrity of pigment epithelium and the blood-retinal barrier. Changes under the short-term conditions of our experiment have not been documented, and we are currently investigating this question. Even if blood-retinal barriers have been partially altered, the results with sucrose and hypoxia would still indicate the existence of active transport across the pigment epithelium, although our estimates of the transport rate may have to be adjusted.

The active transport mechanisms that remove fluid from the subretinal space may also contribute to the process of maintaining adhesion between the retina and the RPE. Several lines of evidence show that life-dependent factors, for which the transport mechanism would be a good candidate, play a significant role in maintaining the strength of adhesion. An ongoing removal of fluid would serve to narrow the subretinal space and potentiate mechanisms of adhesion that require close approximation of the photoreceptors and the RPE, such as electrical attraction between proteins or other substances, or the viscosity of the intercellular matrix.

The fact that subretinal fluid is removed by active transport may help to explain certain types of retinal detachment. For example, in the peripheral retina, the choroidal circulation is relatively sparse and the RPE is thin and has reduced membrane infoldings. These factors will presumably reduce the rate of subretinal fluid resorption and may account for the relative weakness of adhesion at the retinal periphery. In the aging eye, where vascular disease compromises the circulation and lipofuscin clogs the RPE, one may speculate that inefficient transport systems contribute to retinal detachment or to the accumulation of subretinal fluid in senile maculopathies. We do not minimize the importance of other factors such as vitreous changes or subretinal neovascularization to the pathophysiology of the aging eye. We suggest that inadequate transport systems may be an additional factor that can make eyes susceptible to damage.

Transport processes can be modified by pharmacologic agents. In the future it may be possible to enhance resorption and adhesion therapeutically under conditions where the macular or peripheral retina is separated or is likely to become separated. Conversely, we may also learn that some agents weaken these processes and are contraindicated for patients who have, or are at risk to have, a detachment.

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REFERENCES


