The effect of metabolic inhibitors on retinal adhesion and subretinal fluid resorption

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Retinal adhesion and the resorption of subretinal fluid were studied in Dutch rabbits, with two experimental techniques. In the first, small local detachments were made in vivo by injecting a small amount of fluid under the retina with a micropipette, and the time for resorption of the detachment was monitored. In the second, strips of eyecup were maintained in a physiologic bathing medium, and the force required to peel retina from the pigment epithelium was measured. Using these techniques, we have found that both the mechanisms for removing subretinal fluid and for maintaining retinal adhesion in vitro are enhanced by exposure to ouabain, are inhibited by exposure to cyanide, and are inhibited by the replacement of the physiologic medium with normal saline. These data suggest that metabolic systems may simultaneously hydrate and dehydrate the subretinal space and that some factors which are important to the maintenance of normal adhesion are missing from normal saline.

Key words: retina, retinal pigment epithelium (RPE), adhesion, detachment, fluid resorption, ouabain, sodium pump, cyanide, subretinal fluid

The mechanisms which keep the normal retina closely apposed to the pigment epithelium (RPE) have not been established. The subretinal space of the mature animal has minimal thickness, but anatomic junctions between the photoreceptors and the RPE have never been demonstrated in mammals. There is a viscous intercellular matrix which offers resistance to separation of the layers, but not enough to account for in vivo adhesion. Hydrostatic and osmotic pressures within the eye induce a flow of water from the interior of the eye toward the sclera, which acts to compress the retina against the wall of the eye, but these forces alone are too small to explain retinal adhesion. The tight-enclosing villous sheaths of the RPE about the photoreceptor outer segments may, by mechanical forces, make it difficult to extract the outer segments. Also, metabolic factors may play a role in maintaining the adhesive force, since retina is difficult to separate cleanly from the RPE in life or immediately after enucleation but can be separated easily within a few minutes after death. Zauberman also observed that adhesive force was markedly reduced 12 hr after an injection of ouabain into the vitreous cavity.

We have been interested in defining metabolic factors which may contribute to the adhesive force, or at least to the maintenance of a tight subretinal space. To minimize postmortem effects we have sought experimental techniques that either use the intact eye or keep tissue viable in a supportive medium. In this report we describe a method for measuring the resorption of small sub-
retinal blebs within the living eye and a method for measuring the force required to peel retina from RPE within a physiologic culture medium. We have studied the effects of several metabolic inhibitors with these techniques.

Methods

All experiments were performed on Dutch rabbits (both male and female) weighing approximately 1.5 kg. For either acute experimentation or enucleation, the animals were initially sedated with 12 mg/kg thorazine intramuscularly followed by 25 mg/kg pentobarbital intravenously which was augmented as needed. All experiments were performed under ordinary room illumination. For experiments requiring observation of the fundus, the pupil was dilated with 1% cyclopentolate and 10% Neo-Synephrine eyedrops.

For the physiologic medium, either Ames' solution10 (buffered with 95% O₂, 5% CO₂ to maintain pH and dissolve the salts) or Hanks' solution (Grand Island Biological 402S) were used, and the results were compared with experiments run in commercial normal saline (Abbott, 0.9%). The following drugs were used: ouabain (Sigma), 2, 4-dinitrophenol (DNP) (Eastman Kodak), sodium cyanide (Matheson), acetazolamide (Sigma).

In vivo local detachments. Local detachments were produced by injecting a small volume of solution into the subretinal space through a glass micropipette. Pipettes were pulled from 1.2 mm glass tubing, and the tips were mechanically broken under microscopic control to yield diameters of 15 to 25 μm. With this tip diameter, moderate air pressure will force fluid through the tip at a slow rate. Filtered air was controlled by a needle valve, and pressures at the valve of 5 to 10 lb/in.² gave satisfactory flow out of the tip.

After a segment of conjunctiva was removed, the sclera was punctured 3 to 4 mm behind the limbus with a 22-gauge needle, and the micropipette tip carefully guided through the hole. A self-retaining planoconcave contact lens (Skia) was placed on the eye, and the micropipette was advanced slowly under direct observation with a Zeiss OpMi I operating microscope. A small flow of fluid out of the tip was maintained during advancement to prevent obstruction of the tip by vitreous gel. Under fine control the tip was brought into contact with the retina and advanced slowly until a bleb began to form (Fig. 1), indicating that the tip had penetrated into the subretinal space. Blebs required 2 to 6 sec to form, depending on their volumes, and they retained their shape after the micropipette was withdrawn (see Results). If the RPE was damaged or the retina torn during formation of the bleb, the results were not used. Several blebs could be made within a single eye (typically six), and both experimental and control blebs were raised in the same eye to facilitate comparison. The size of the blebs was measured with a microscope eyepiece grid, and the longest and shortest diameter were noted if the blebs were elliptic in outline. No satisfactory method for measuring bleb height was devised. The resorption time was estimated by directly observing the blebs at 5 to 10 min intervals and noting when the pigment epithelial pattern became sharply visible.

In vitro peeling experiments. Eyecup fragments were maintained in a physiologic medium while measuring the force required to peel retina from the RPE. First, an eye was rapidly enucleated and sectioned behind the pars plana; the vitreous was...
Metabolic inhibitors, retinal adhesions, subretinal fluid

Fig. 3. Diagram of the experimental setup for measuring the force required to peel retina from RPE.

Fig. 4. Retina being peeled from the RPE under saline.

removed, and the posterior segment immediately placed in a Petri dish containing oxygenated Ames' solution at 37° C. The tissue was cut into strips 5.0 mm wide with a razor blade template; usually four strips were cut as shown in Fig. 2. Under the Ames' solution, each 5 mm strip in sequence was fixed with insect pins to a small aluminum plate coated with a layer of encapsulating resin (Sylgard, Dow Corning). This plate was attached at a 23-degree angle to a long pole (to allow submersion into a beaker) which was connected to a multispeed syringe drive (SAGE Model 351) as shown in Fig. 3. The angle of 23-degrees was chosen to conform with earlier reports\(^2\) and to avoid the tissue drag which would occur if the ideal peeling angle of 0-degree was used. The retinal surface at the inferior end of the strip was glued with a minimal amount of cyanoacrylate adhesive (Permanbond 180) to the flattened edge of a small nailhead (see Fig. 4) which was suspended by micropipette...
Fig. 7. Relative speed of resorption for blebs filled with different solutions. Data from Table I expressed as the percentage of additional time required by the slower of each pair of solutions.

Table I. Relative resorption times for blebs within the same eye, filled with different solutions

<table>
<thead>
<tr>
<th>Number of eyes</th>
<th>Solution A</th>
<th>Solution B</th>
<th>Ratio of resorption times (A:B ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Ames' solution*</td>
<td>Normal saline</td>
<td>1:1.54 ± 0.07</td>
</tr>
<tr>
<td>2</td>
<td>Ames' solution</td>
<td>Oxygenated normal saline*</td>
<td>1:1.15 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td>Ames' solution</td>
<td>Ames' solution plus 2 × 10^{-4}M cyanide</td>
<td>1:1.29 ± 0.09</td>
</tr>
<tr>
<td>8</td>
<td>Oxygenated normal saline plus Ca^{++}, K^{+}, glucose†</td>
<td>Oxygenated normal saline</td>
<td>1:1.07 ± 0.05</td>
</tr>
<tr>
<td>8</td>
<td>Oxygenated normal saline</td>
<td>Normal saline</td>
<td>1:1.12 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>Hanks' solution</td>
<td>Normal saline</td>
<td>1:1.03 ± 0.03</td>
</tr>
<tr>
<td>3</td>
<td>Hanks' solution plus 5 × 10^{-4}M ouabain</td>
<td>Hanks' solution</td>
<td>1:1.24 ± 0.10</td>
</tr>
<tr>
<td>4</td>
<td>Hanks' solution at pH 5.8</td>
<td>Hanks' solution</td>
<td>1:1.02 ± 0.04</td>
</tr>
</tbody>
</table>

*Bubbled with 95% O_{2}/5% CO_{2}.
†1.15 mM CaCl_{2}, 3.6 mM KCl, 10 mM glucose.

The peeling force was measured by lowering the tissue at a slow steady rate (see Results) and simultaneously recording the tension upon the retina as it peeled from the pigment epithelial surface (Fig. 4). All peeling rates were within the range shown previously to separate the retina without fracturing of the RPE or photoreceptors. Each experiment was watched to ensure that the retina peeled evenly and did not fragment or tear; if it did, the experiment was discarded. The peeling force was DC amplified and displayed on a paper recorder (Brush 2000 series, Gould Instruments); Fig. 5 shows a typical tracing. There is an initial high transient as separation begins, then a fairly stable plateau as the length of the strip is peeled, and finally a fall of tension to zero (tissue weight was negligible under water) when the strip of retina separates completely from the RPE. The peeling force was defined as the average value within the plateau region, with the final baseline used as zero.

Electron microscopy. Tissues were fixed for at least 24 hr in cold 1.5% or 2.0% glutaraldehyde with 0.1M cacodylate buffer, pH 7.4. They were dehydrated in absolute alcohol and either critical-point dried for examination with a Coates and Welter field emission scanning electron microscope or embedded in Epon 812 for sectioning and examination with a Siemens 1A transmission electron microscope.
Results

In vivo local detachments. The goal of these experiments was to establish an in vivo model of retinal detachment and reattachment which would allow the testing of metabolic inhibitors. An initial requirement was to confirm that subretinal bleb formation and resorption were reproducible phenomena which could be quantified. When blebs of at least 1 mm diameter were formed with either Hanks' or Ames' solutions, they persisted for more than 1 hr. Resorption was gradual, first occurring at the edges to leave crescent-shaped forms which shrank steadily until the RPE pattern was again sharply visible. In theory, the time required for a bleb resorption should be a function of bleb volume and of the available pigment epithelial surface underlying the blebs. However, we noted that blebs were usually not spherical segments but were often flattened on top; and there was, in fact, no obvious relationship between bleb area and resorption time (Fig. 6). Analysis of these data by linear regression shows a correlation coefficient of only 0.128 which is not significant (p > 0.1). We also observed that blebs from any given eye, regardless of their size, tended to resorb in about the same period of time; variations in resorption times between different eyes account for most of the vertical scattering in Fig. 6. Thus control and experimental blebs are best compared within, rather than between, eyes.

To study metabolic inhibitors, 2–5 blebs with and without inhibitor were raised within each eye studied, and the ratio of the average resorption time with and without inhibitor was calculated. These ratios were averaged among all the eyes studied to provide a quantitative estimate of the inhibitor's effect. Table I lists the results of experiments which compared the effects of ouabain, cyanide, and various changes in the saline medium used for raising blebs. The interrelationship of these various conditions is shown graphically in Fig. 7. The addition of cyanide to Ames' solution slowed down bleb resorption by 29%, but the addition of ouabain to Hanks' solution speeded up resorption. Blebs filled with Hanks' solution resorbed 24% more slowly than blebs filled with Hanks' plus ouabain. These results were consistent from eye to eye; there were no eyes in which cyanide blebs resorbed faster or ouabain blebs resorbed slower than controls. Replacement of Ames' solution (which is oxygenated) with normal saline (unoxgenated) slowed resorption by a remarkable 54%, although a comparison of Ames' solution to oxygenated normal saline, and then oxygenated to unoxgenated normal saline, only showed a cumulative change of 29%. Supplementing oxygenated saline with calcium, potassium, and glucose had little effect. There was also little difference between Hanks' solution, Hanks' solution acidified to the pH 5.8 of normal saline, and normal saline (all unoxgenated).

To study the long-term effects of ouabain, 0.05 ml of 10⁻⁴M ouabain was injected into the midvitreous of two eyes. After 12 hrs, an attempt was made to raise blebs with pipettes filled with Hanks' solution. However, fluid entering the subretinal space diffused freely under the retina (as if the attachment force was nil) and blebs could not be formed.

In vitro peeling experiments. The first priority for this set of experiments was to standardize the measurements of peeling force and to eliminate artifactual forces. It would have been easiest to measure the peel-
ing force in air, but surface tension interfered. For example, an average force of 125 ± 18 mg was required to peel five 6.5 mm strips of retina in air at 8.7 mm/min. However, if the retina were allowed to fall back upon the surface of the RPE, the force required to peel again was still 59 ± 12 mg. Since normal retina has a closer apposition to the RPE than retina which has been peeled and reapplied, surface tension probably accounted for most of the apparent separation force in air. For comparison this same experiment was performed with the tissues submerged in Ames' solution at 37°. Under fluid the initial peeling force was approximately 25 mg, and the force required to peel a second time was too small to measure. Separation under fluid eliminated the nonspecific effects of surface tension and would appear to mimic more closely the situation inside the eye, where the retina is in a fluid environment. Scanning electron micrographs confirmed that the separation in these experiments occurred between the photoreceptors and RPE. There was no difference in the average peeling force between right or left eyes and no significant difference between the four regions of the retina shown in Fig. 2. Nonetheless, in experiments utilizing several eyes, care was taken to rotate the order in which the retinal segments were used.

Another factor which influences adhesive forces in vitro is the time after death, since adhesion can fall markedly within minutes. With the methods described, retinas were placed into warm oxygenated Ames' medium within 15 sec of enucleation and were maintained in that solution except for approximately 15 sec, during which they were transferred to the measuring bath. The earliest we were able to begin peeling was at 4 min, and Fig. 8 shows the force required to peel 5 mm strips of retina at 24 mm/min, as a function of time after death. Slow deterioration in the adhesive force is evident, from about 25 mg at 5 min to 12 mg after 20 min. Electron micrographs confirmed that the separation was occurring at the subretinal space.

To study the immediate effect of poisons on the peeling force, a syringe was mounted adjacent to the tissue in the measuring bath so that the poison could be injected in a gentle stream directly over the retina as it was being peeled. Injections were begun after peeling had proceeded halfway, to see whether the drug caused any immediate changes in the peeling force. Neither ouabain (5 × 10⁻⁴M), sodium cyanide (3 × 10⁻³M), DNP (5 × 10⁻⁴M), or acetazolamide suspension (1 × 10⁻³M) had any effect upon the force required to peel the retina.

To determine the effect of somewhat longer exposures, the metabolic poisons were added to the solutions in which the tissue was prepared and peeled. Thus enucleated eyecups were placed immediately either into oxygenated Ames' solution containing 5 × 10⁻⁴ M ouabain or 3 × 10⁻³M sodium cyanide or, for comparison, into normal saline (unoxgenated). Each eyecup was cut as shown in Fig. 2, and strips were peeled at 5 min intervals after enucleation (3.5 min intervals in the
case of saline). For controls, the same procedure was performed on the fellow eye of each animal, with oxygenated Ames' solution used without drugs. Eight animals were used for each condition, with the results shown in Fig. 9. Note that the effects of saline or the inhibitors are compared with a baseline which falls with time. Normal saline was notably less effective than Ames' solution in maintaining adhesion, and the peeling force required in saline was 10 to 15 mg lower than the controls at all times studied. Tissue exposed to ouabain or cyanide showed much smaller differences. Tissue maintained in cyanide solution required about 5 mg less peeling force than the controls throughout the period studied. Interestingly, tissue maintained in ouabain solution required slightly greater forces than the controls at all times after enucleation.

Long-term effects of inhibitors on the peeling force were also studied. In three rabbits, 0.05 ml of Ames' solution containing 10^{-3}M ouabain, 6 \times 10^{-3}M cyanide, or 10^{-3}M DNP was injected into the midvitreous of the right eye, and 0.05 ml of Ames' solution alone was injected into the left. Twelve hours later, the fundus of the eyes receiving cyanide and DNP appeared normal to contact lens examination, but the retina of the eye injected with ouabain was only loosely adherent and appeared grey and opacified. The force required to peel the retina was then measured for all of the eyes, as described above. The peeling forces for the eyes injected with cyanide and DNP were equal to those of their respective controls. In contrast, the peeling force for the eye injected with ouabain was virtually zero, but electron micrographs (Fig. 10) showed that the outer segments were severely swollen and fragmented and that separation of the retina had occurred mostly through the outer segments (leaving the tips adherent to the RPE) rather than at the subretinal space.

Experiments were also performed to see
Fig. 11. Strips of retina which have been gently peeled from the RPE after 1 hr in (A) Ames' solution or (B) Ames' solution plus $5 \times 10^{-4}$ M ouabain.

whether exposure to inhibitors without traction or peeling would have visible effect upon the retina. Fragments of eyecup were placed in Petri dishes containing either Ames' solution at $37^\circ C$ or Ames' solution plus $5 \times 10^{-4}$ M ouabain, $5 \times 10^{-4}$ M DNP, $10^{-3}$ M acetazolamide, or $3 \times 10^{-3}$ M sodium cyanide. During 1 hr of observation (with an operating microscope) there was no difference between control fragments which remained transparent and fragments exposed to DNP, acetazolamide, or cyanide. However, fragments exposed to ouabain became cloudy after 30 min and opaque after 1 hr. The opacified retina was actually more adherent throughout this experiment than retina exposed only to Ames' solution. Retinas maintained in Ames' solution could be peeled cleanly from the RPE within 10 min of enucleation, whereas retinas maintained in Ames' plus ouabain peeled with difficulty and retained extensive pigment, even after 60 min in vitro (Fig. 11). Scanning electron micrographs confirmed that separation in control tissues was at the subretinal space, but that separation in ouabain-treated tissues occurred, in large part, through the rupture of RPE cells or outer segments.

Discussion

We have described two experimental techniques which relate to the apposition and adhesion between retina and RPE. One measures the time required for resorption of fluid under a small detachment and depends upon systems which remove fluid from the subretinal space. The other measures the force required to peel retina from the RPE and depends upon the firmness of attachment. We recognize that these techniques do not measure the same thing: subretinal fluid resorption is not adhesion. However, mechanisms which affect bleb resorption may be reasonably assumed to enhance adhesion by keeping the subretinal space at minimum thickness.

Some potential sources of errors in the bleb-resorption model require comment. Of necessity, the micropipette made a tiny retinal hole as each bleb was formed. However, if resorption depended upon leakage of fluid through this hole, then the time for resorption should have been a function of bleb volume (instead of being independent of it), and no difference in time would have been observed in blebs raised in the presence of metabolic inhibitors. Making a pars plana hole for the micropipette caused the intraocular pressure to fall to near zero, and the rate at which intraocular pressure was regained could in theory influence the bleb resorption time. However, data from other experiments suggest that these pressure changes are probably of little consequence to adhesion and that in
any event, pressure effects would be the same for all substances tested. Bleb height could not be accurately measured, leaving uncertainty about the volume of fluid being resorbed per unit time. However, blebs generally had a low convex shape, so that bleb height did not appear to vary greatly with diameter; furthermore, our data showed that resorption time was largely independent of bleb size. These findings suggest that the RPE in the floor of each bleb was absorbing fluid at a uniform rate per unit area.

The peeling experiments also have potential sources of error and must be put in some historical perspective. Previous investigators have studied peeling as an index of retinal adhesion, but the experiments were difficult to interpret because the time after death was not strictly controlled, the tissues were not supported in a nutrient physiologic medium, and in some cases the peeling was performed in air. The present study was designed specifically with these problems in mind and has sought to eliminate as many of them as possible. For example, fragments of eyecup are kept in a warm physiologic medium (which supports the electrical activity of the retina) from 15 sec after enucleation (except for the few seconds required to transfer the strips to the peeling chamber). Peeling is performed under fluid to eliminate surface tension artifacts. Even with these precautions, we noted a steady drop in the peeling force from about 25 mg to 15 mg over the first 20 min after enucleation. Investigators who work with isolated retinas will be aware that retina is difficult to peel from RPE immediately after enucleating an eye but that it separates cleanly after a few minutes in saline and may float off spontaneously after 20 to 30 min. We cannot judge how much the resistance to peeling has been weakened during the time (approximately 4 min) between enucleation and our earliest measurements. However, the fact that a distinct resistance to peeling is present and can be modified by time or metabolic inhibitors suggests that we are measuring at least a part of the normal adhesivity of the retina. Our data reinforce the concern that in vitro data on retinal adhesion must be viewed with great caution unless the effects of death and the time after death are accounted for.

We believe that the force required to peel retina from the RPE depends upon factors which normally keep the retina adherent, even though the retina is not ordinarily subject to stress at the acute angle of our experiments. Pulling (as opposed to peeling) patches of retina from the RPE might have been a more realistic model of retinal tears, but pulling experiments are harder to control because the measurement consists of only a brief surge of force which will be inaccurate unless the entire surface separates at the same instant. In peeling experiments, the initial peak of force is ignored, and the separation continues steadily for a period of time (see Fig. 5), during which any irregularity or tearing of the tissue can be noted. Also, in contrast to a pulling experiment in which the entire retinal surface must be attached to the transducer, peeling measurements are made well away from the areas of retina exposed to cyanoacrylate glue. We found that the peeling force was typically in the range of 25 mg for a 5 mm strip peeled at 24 mm/sec; this value is less than that obtained by others but differences in technique prevent a close comparison. Although this force may seem small, it represents a considerable adhesiveness when one considers that peeling only measures the force along a thin line of separation, whereas retina is normally adherent over an area of RPE.

One may argue that these experiments are not physiologic because the subretinal space is exposed to fluid at the margin of each strip. On the other hand, these conditions may be relevant to the process of rhegmatogenous detachment in which a torn margin of retina is exposed to fluid vitreous. In our experiments, this exposure may contribute to the weakening of adhesion over time, but during the actual measurements the retina is probably being pulled away faster than fluid can seep in. Furthermore, the fact that inhibitors alter the peeling force indicates that separa-
tion is not solely determined by an entry of fluid at the strip margin. In fact, these experiments may be used as evidence that passive hydrostatic or osmotic pressures within the intact eye cannot fully account for the normal apposition of the retina, for if they did, one would expect retinal separation as soon as the eyecup were sectioned.

Zauberman has reported that intravitreal ouabain weakens adhesion. Thus we were surprised initially to find that ouabain increased the force required to peel retina in vitro and sped up the resorption of subretinal fluid. However, upon reflection, these effects are consistent with the action of ouabain, which is to block the Na-K exchange pump. This pump normally moves Na, and thereby water, out of the cell interior and is involved in secretory functions such as the production of aqueous humor. Miller and Steinberg have shown in the frog that a ouabain-sensitive Na pump is present only in the apical membrane of the RPE, so that pump activity transports fluid into the subretinal space. Thus inhibition of the Na pump with ouabain will reduce fluid movement toward the retina and thereby enhance the resorption of fluid out of the subretinal space. The resulting dehydration of the subretinal space should tighten the apposition of retina and RPE and thereby increase the force required to peel retina from the RPE.

Ouabain may also have other effects which strengthen the attachment between the outer segments and the RPE, since adhesion seems to be remarkably strong after exposure to ouabain. For example, after 1 hr in vitro, ouabain-treated retina still fails to separate cleanly from the RPE, and after intravitreal ouabain, the photoreceptors fragment rather than separate from the RPE. The latter observation accounts for Zauberman’s findings; the ouabain-poisoned retina separates easily by schisis even though the adhesive forces are actually strengthened. Another mechanism by which ouabain might strengthen adhesion is by causing cellular edema, which in theory could tighten the ensheathment of RPE microvilli about the outer segments like a ring about an injured finger. However, cyanide and exposure to saline in vitro also cause edema but do not strengthen adhesion. Perhaps ouabain has some specific action upon the shape or motility of the RPE microvilli or upon electrical and molecular forces which affect the subretinal space.

If blocking the Na pump enhances bleb resorption and increases the peeling force, why do cyanide and postmortem changes produce the opposite effects? Presumably, the latter block not only the Na pump but also the active mechanisms which pump fluid out of the subretinal space. These reciprocal pumps must normally be biased in favor of fluid movement out of the subretinal space, since retina is normally adherent. Thus the net effect of blocking all metabolic systems will be to inhibit the resorption of fluid, which would secondarily loosen the interdigitation between outer segments and RPE microvilli. Mention should be made of the fact that cyanide as well as ouabain, DNP, and acetazolamide, had no effect on the peeling force when injected directly over retina as it was being peeled. Only 15 to 30 sec remained in each peel when these injections were begun, and this may have been an insufficient time for measurable effects to develop.

We were surprised to find that normal saline replacing Ames’ solution in either blebs or the peeling chamber was at least as effective as cyanide in weakening the apparent mechanisms of resorption and adhesion. Bleb resorption time was nearly doubled, and the peeling force was nearly halved, from the earliest measurement (4 to 5 min) to the latest (15 min). Normal saline lacks the glucose and ionic diversity of Ames’ solution, has a lower pH, and was not routinely oxygenated in our experiments. We attempted to sort out these factors by using different saline combinations in the bleb resorption experiments (see Fig. 7). However, these initial results are hardly conclusive. Oxygenation and the restoration of glucose and salts speeded up resorption of the blebs, but not enough to match the effect of replacing Ames’ solution with saline. Further experiments are planned to define the critical factors for bleb resorption more precisely and to determine
whether these same factors are important to adhesiveness (peeling force).

Acetazolamide was found to have no effect when injected directly over the retina as it was peeled and during the in vitro observations of eyecup fragments, but cyanide also had no effect under these two conditions. Miller and Steinberg have shown that bicarbonate and chloride flux across the frog RPE is blocked by acetazolamide, and thus acetazolamide may be expected to affect systems which move fluid in and out of the subretinal space. Experiments are in progress to determine whether acetazolamide alters the speed of bleb resorption or the force required to peel the retina.

This study allows some general conclusions to be drawn about the mechanisms of retinal adhesion. First, it is of interest that the effects cyanide, ouabain, and normal saline in enhancing or hindering bleb resorption were matched by their effects in strengthening or weakening the force required to peel retina. Thus there appears to be a link between mechanisms for subretinal fluid resorption and the maintenance of physical adhesion. Second, the facts that a peeling force was measurable in excised tissue and that both peeling force and bleb resorption time were influenced by metabolic inhibitors give evidence that passive hydrostatic and osmotic pressures cannot be solely responsible for normal adhesion. Third, since normal saline was equally if not more effective than cyanide in inhibiting both bleb resorption and peeling force, there appears to be some critical process, metabolic or otherwise, which is controlled by a factor missing in normal saline. Finally, the fact that ouabain enhances adhesion suggests that antagonistic "pumps" may normally act upon the subretinal space and that there may be other mechanical factors of importance in the maintenance of adhesion.

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REFERENCES