Retinal traction in vitro
Biophysical aspects

Hanan Zauberman,* Henri de Guillebon, and Frank J. Holly

The forces required to detach the retina from the pigment epithelium and the subsequent structural changes were studied with a device that produced retinal traction at different rates over localized, equatorial areas of freshly enucleated owl monkey and albino rabbit eyes. Slow traction (0.2 to 0.8 mm. per minute) produced higher and larger retinal detachments and bigger tears in both types of experimental animals. Medium traction rates (8.5 and 21.5 mm. per minute) resulted in lower and smaller detachments and smaller retinal tears. Above these rates (42.5 to 425.0 mm. per minute), the choroid separated grossly from the sclera. The ratio of traction force and height of detachment was proportional to the logarithm of the traction speed for slow and medium traction speeds. Structural studies showed that the separation of the retina occurred mainly between the two layers, at a traction rate of 0.2 mm. per minute, and at a level of the pigment epithelium at the rate of 8.5 mm. per minute. The structural changes and the force requirements during retinal detachment by traction in vitro are explained on the basis of the rheologic properties of the cementing substance between the retina and pigment epithelium.

Key words: retinal detachment, pigment epithelium, traction, adhesion, cohesion, force

In higher vertebrates, the outer segments of the retinal photoreceptors are not anatomically attached to the villous processes of the pigment epithelium. Interposed between these two cell layers is a thin mucopolysaccharide matrix. This matrix may serve as an adhesive between retina and pigment epithelium. Indeed, a small but measurable force needed to separate the sensory retina from the pigment epithelium was first recorded by Zauberman and Berman and subsequently by other workers. Yet, a better understanding of the mechanism of retinal adhesion and of retinal detachment could be achieved by disrupting the adhesion at different rates as has been done in physical models. With an instrument devised to exert traction on the retina in vitro at low, intermediate, and high rates, we studied the structural changes produced by retinal detachment and the forces needed to detach the retina in owl monkeys and rabbits.

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Materials and methods

Instrumentation. The instrument devised to measure the force and displace the eye was modified from a previous one and consisted of a force transducer (RCA/5734) coupled to a similar transducer to minimize thermal drifts (Fig. 1). The transducers were fixed to a microscope stage mounted on a microscope stand and were linked to a paper recorder (322 Sanborn) and a digital voltmeter. A glass capillary tube (A) of 1 mm. diameter was glued to the shaft of the force transducer; another glass tube (B), bent at a 90 degree angle near its narrowed lower end, was glued to tube A. A polyethylene tube (PE 10) of 0.61 mm. outer diameter and 0.28 mm. inner diameter was inserted through tube B, and the end was pulled out to a length of 3 mm. This allowed any force exerted at the tip of the polyethylene tube to be transmitted rigidly to the force transducer. The other end of the polyethylene tube was connected to a microsyringe containing silicone oil of 500 centistokes viscosity, and the tube was filled with oil to eject the air. n-Butyl-2-cyanoacrylate adhesive, 10 μl, was then aspirated into the lower segment of the polyethylene tube. Next, a small amount of air was introduced into the polyethylene tube to avoid direct contact between the adhesive and the vitreous. The silicone oil in the system permitted controlled release of minute amounts of adhesive with the microsyringe.

Traction experiments. Twenty owl monkeys weighing between 650 and 750 grams were anesthetized with an intramuscular injection of 20 mg. of sodium pentobarbital. Two diathermy marks, 180 degrees apart, were applied 10.5 mm. posterior to the corneoscleral limbus to locate the equatorial region. Each eye was light adapted and enucleated shortly before the start of an experiment. A Flieringa ring was sutured around the eye 3 mm. posterior to the limbus. The eye was then placed in a mold consisting of two hemispheres that, fastened together, took the shape of the posterior segment of the eye.

The anterior segment of the eye (including the cornea), a 3 mm. rim of sclera posterior to the limbus, the iris, ciliary processes, and lens were removed. The plastic mold containing the eye was then placed on a motorized platform that allowed horizontal displacement of the eye at various speeds. The tip of the polyethylene tube was introduced into the eye and brought into contact with the retina with the electric motor, while the procedure was observed through a Zeiss operating microscope at ×16 magnification. Adhesive, 1 μl, was injected, producing a strong bond between the retina and the tip of the polyethylene tube. The platform supporting the mold and eye was then displaced away from the tip of the tube with resulting retinal traction.

After study of the characteristics of retinal traction in several pilot experiments, it was considered convenient to use speeds of 0.2 and 0.8 mm. per minute for low rates of traction, 8.5 and
Table I. Number of eyes and experiments at various traction rates in four rabbits and 20 owl monkeys

<table>
<thead>
<tr>
<th>Traction speed (mm./min.)</th>
<th>20 Owl monkeys</th>
<th>4 Rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of experiments</td>
<td>No. of eyes</td>
</tr>
<tr>
<td>0.2</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>0.8</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>8.5</td>
<td>57</td>
<td>17</td>
</tr>
<tr>
<td>21.5</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>42.5</td>
<td>38</td>
<td>6</td>
</tr>
<tr>
<td>85.0</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>425.0</td>
<td>18</td>
<td>3</td>
</tr>
</tbody>
</table>

21.5 mm. per minute for medium rates, and 42.5, 85.0, and 425.0 mm. per minute for high rates of traction. The force exerted to detach and tear the retina was recorded on the voltmeter at 15 second intervals and plotted by hand for the experiments at traction speeds of 0.2 and 0.8 mm. per minute. At faster traction rates, the force was automatically recorded on graph paper. Selected experiments were photographed through the objective of the operating microscope. Retinal traction experiments were also performed on freshly enucleated eyes of four albino rabbits weighing between 2.6 and 3.1 kilograms. The traction force was not recorded in these experiments, but the results served as a reference for subsequent experiments. Table I gives the number of eyes and experiments for various traction rates in both types of experimental animals.

In five experiments performed at 8.5 mm. per minute, the detached retina of the owl monkey was allowed to regain contact with the underlying tissue by reversing the movement of the platform for an equal distance before the tissue tore. Retinal traction was then exerted again at the same rate to determine whether adhesion had been re-established and whether the vitreous exerted any force to counteract retinal displacement.

The structural changes occurring after retinal detachment were studied with the light microscope. The area submitted to traction was sectioned out and fixed in four per cent glutaraldehyde, postfixed in one per cent osmic acid, dehydrated in graded alcohols, and embedded in araldite. Sections 1μ thick were cut with a LKB Ultrotome and stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope.

Results

Structural changes. Low traction rates applied to retinas from owl monkey eyes resulted in higher retinal detachments and in larger areas of detachment (Fig. 2) than did medium traction rates. A retinal tear occurred only late in the experiment, and on one occasion the glass tube reached the opposite wall of the eye without tearing the retina. Fig. 2 shows the result of an experiment at a low traction rate (0.2 mm. per minute). Progressively higher rates resulted in lower and smaller retinal detachments (Fig. 3).

At medium traction rates (8.5 and 21.5 mm. per minute), a mild chorioretinal elevation occurred before retinal detachment took place. The higher traction rates (42.5 mm. per minute and over) resulted in an obvious chorioretinal elevation. Table II summarizes data for retinal elevation, chorioretinal elevation, and the size of retinal detachment and retinal tears observed. Depigmentation of the pigment epithelium and pigment dots on the outer retinal layers were observed at intermediate speeds.

Traction experiments performed in rabbits resulted in qualitatively similar phenomena.

Histology. At a low traction rate (0.2 mm. per minute), the cleavage between the pigment epithelium and the sensory layer occurred mainly between the outer segments of the photoreceptors and the villous processes of the pigment epithelium in both owl monkey (Fig. 4) and rabbit eyes (Fig. 5). At a medium speed (8.5 mm. per minute), stretching and tearing of the villous processes of the pigment epithelium occurred in both species (Figs. 6 and 7).
Fig. 2. Right, polyethylene tube glued to retina. Left, retinal tear and detachment caused by traction at 0.2 mm. per minute. Retinal detachment extends well beyond the area covered by the traction tube.

Fig. 3. Retinal detachments caused by traction at 8.5 mm. per minute are smaller than those in Fig. 2.
Fig. 4. Cleavage between retina and pigment epithelium in owl monkey following traction at 0.2 mm. per minute. Amorphous corpuscles which may represent matrix substance are seen on the surfaces of villous processes and outer segments. (Toluidine blue. Original magnification x300.)

Table II. Observations during retinal traction in owl monkey eyes

<table>
<thead>
<tr>
<th>Traction speed (mm./min.)</th>
<th>Total retinal elevation (mm.)</th>
<th>Chorioretinal elevation</th>
<th>Size of retinal detachment in disc diameter</th>
<th>Retinal tear in disc diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>&gt;4.33 ± 1.51</td>
<td>-</td>
<td>3.85 ± 1.18</td>
<td>1 to 1½</td>
</tr>
<tr>
<td>0.8</td>
<td>&gt;1.93 ± 0.77</td>
<td>-</td>
<td>2.45 ± 0.79</td>
<td>1 to 1½</td>
</tr>
<tr>
<td>8.5</td>
<td>1.74 ± 1.07</td>
<td>±</td>
<td>1.40 ± 0.60</td>
<td>¼ to 1½</td>
</tr>
<tr>
<td>21.5</td>
<td>1.40 ± 0.61</td>
<td>±</td>
<td>0.64 ± 0.31</td>
<td>1/4 to 1½</td>
</tr>
<tr>
<td>42.5</td>
<td>1.50 ± 0.79</td>
<td>+</td>
<td>0.56 ± 0.30</td>
<td>1/4 to 1½</td>
</tr>
<tr>
<td>85.0</td>
<td>4.11 ± 1.38</td>
<td>+</td>
<td>0.83 ± 0.23</td>
<td>1/4 to 1½</td>
</tr>
<tr>
<td>425.0</td>
<td>2.56 ± 1.1</td>
<td>+</td>
<td>0.97 ± 0.29</td>
<td>1/4 to 1½</td>
</tr>
</tbody>
</table>

Force recordings. The total traction force (Table II) recorded is the sum of the forces employed to disrupt the chorioretinal adhesion and to stretch and tear the retina.

The recording of an experiment at a traction speed of 8.5 mm. per minute is shown in Fig. 8. The base-line segment between the arrows is related to the height of detachment; the peak of the record, when the retina tore, corresponds to the total traction force. It was not possible to measure only the force necessary to detach a given area of retina; therefore, we evaluated the force needed to elevate the retina. A linear relation was observed between the height of detachment and the total traction force. Slow experiments (0.2 and 0.8 mm. per minute) gave linear recordings only when the height of the retinal detachment did not exceed 1.5 mm. Experiments at medium and high traction rates were always linear. Within the limits of linearity, the ratio of traction force and height of detachment was calculated for all traction experiments and the
The disruption by traction of the adhesion between the retina and pigment epithelium was observed and measured continuously for each experiment. At low traction rates (0.2 and 0.8 mm. per minute), relatively large heights and areas of detachment could be achieved in both the owl monkey and the rabbit eyes, because the small force applied did not exceed the tensile strength of the retina. In this case, the initial detachment was followed by a low-angle peeling and stretching of the retina. Fluid accumulated in the subretinal space, possibly through normal channels in the retina or through microtears and tears in this tissue.

In both types of experimental animals, medium traction speeds of 8.5 and 21.5
mm. per minute resulted in detachments of much lesser height and area, since the stronger force required for these rates rapidly exceeded the tensile strength of the retina.

For high traction rates (42.5 mm. per minute and over), the force apparently exceeded the shear strength of the retina. Retinal tearing was accompanied by a detachment of the weakly adherent choroid to the sclera.

Although the experiments were performed in open eyes, intraocular pressure was not considered to be an important factor in opposing retinal detachment. Most of the intraocular pressure is contained by the sclera, since the flow conductivity of the retina is greater and its elasticity lower than the same parameters for the sclera.

Vitreous resistance to retinal detachment was negligible. In the five experiments in which the retina was reapposed to the pigment epithelium after detachment, no force was recorded during the reattachment of the retina. Also, no drag by the fluid vitreous resisting retinal elevation was registered.

At a low traction rate (0.2 mm. per minute), cleavage occurred mainly between the retina and the pigment epithelium, indicating failure of the cement substance (Figs. 4 and 5); whereas at a rate of 8.5 mm. per minute, the pigment epithelium was stretched and partly torn, indicating a cohesive failure of both the pigment epithelium and the cement substance (Figs. 6 and 7). Keeping in mind that the real situation is certainly more complex, we can attempt to interpret these
Fig. 7. At traction rate of 8.5 mm. per minute, tearing and stretching of villous processes takes place. Note torn inner faces of pigment epithelial cells attached to outer segments of albino rabbit eye. (Original magnification x5,900.)

Results as representing the detachment of two layers which adhere to each other by means of a viscous adhesive.

It has been shown in a simple model that the product of force and time needed to separate two solids by a given distance depends on the mechanical properties of the adhesive, such as viscosity or viscoelasticity, and on the geometry of the system. When traction is applied to the system, stresses are produced at various cross-sections of the specimen. The behavior of the components of the system will determine the result. For the initial stage of pulling, the most significant in the process, the following equation has been derived to describe tackiness:

$$ Ft = \frac{3\pi a^4}{4} \left( \frac{1}{h_i} - \frac{1}{h_f} \right) $$

where $F$ is the force applied, $t$ is the duration of its action, $\eta$ is the viscosity of the adhesive liquid, $a$ the radius of the specimen, and $h_i$ and $h_f$ are the initial and final thicknesses, respectively, of the adhesive. This equation demonstrates that there is no absolute force opposing the breaking of the adhesion. The resistance to separation depends on the rate of separation. As $h$ is increased by a force tending to separate the surfaces, a viscous flow of the adhesive toward the center of the
detachment will take place. The separation will occur within the adhesive if the force applied is small and of enough duration to allow for such viscous flow. This type of failure is cohesive rather than adhesive, because it eventually takes place within the bulk of the adhesive instead of at the interface between the adherend and the adhesive. The phenomenon is rheologic in nature and similar to the one observed in our experiments at low traction rates (Figs. 4 and 5).

If the force applied to separate the surfaces is great, the cohesive failure may take place in either of the adherends. This is due to the fact that the force necessary to induce a fast viscous flow of the adhesive is greater than the shear or tensile strength of the adherends. Such a phenomenon was observed during retinal traction at medium (Figs. 6 and 7) and high rates. In the case of good adhesive joints, the failure almost never occurs at the adhesive-adherend interface. For this reason, the force measured during the retinal detachment yields no information on the strength of the attractive molecular forces across the interfaces.

The sensory retina and the pigment epithelium relate to each other through a matrix that has been histochemically shown to consist of mucopolysaccharides. Chemically, half sulfated chondroitin sulfates and sialoglycans have been extracted from the retina. These substances could serve as an adhesive between retina and pigment epithelium and explain in part the normal adhesion of the retina, since they meet the requirements of complete wetting and viscosity.

The force applied to detach the retina in the owl monkey eye appeared to be related to the logarithm of the rate of traction, except for experiments in which the traction rate was faster than 21.5 mm. per minute when a choroidal detachment took place. A similar relationship between force and rate of retinal detachment was also obtained for retinal peeling experiments and is consistent with adhesive phenomena studied in physical models. Conceivably, this behavior of the adhesive joint between the retina–pigment epithelium protects the retina against considerable traction of short duration. An auxiliary mechanism of adhesion in vivo consisting of a hydrostatic pressure difference across the retina has been postulated and is presently being studied in our laboratory.

Physiologic or abnormal vitreous adhesions to the retina can, under certain conditions, exert continuous traction and play a major part in the development of retinal tears and detachment. It is possible that some of the clinical effects of vitreous traction on the retina are related to our experimental observations. The clinical
counterpart of slow traction, for instance, could be found in the late stages of the retinopathy of prematurity and diabetic retinopathy, resulting in large detachments without retinal tears. Relatively faster traction phenomena may result in localized tears. Conceivably, very fast traction, as may result from contusion injury, may act on the choroid causing hemorrhage around a vortex vein where the choroid is anchored by the vessel to the sclera.

It is also possible that in the presence of a weakened retinal adhesion, such as in areas of “white without pressure,” retinal traction will result in a break and a fast-developing retinal detachment.

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REFERENCES


