The permeability of the outer layers of limbus and anterior sclera. M. Bruce Shields, Michael J. Bradbury, John D. Shelburne, and Susan W. Bell.

An in vitro study suggests that flow through the scleral flap of a trabeculectomy procedure can significantly increase outflow. It also suggests that this flow may be through vessels in the flap or through the ground substance of the flap.

A possible route of external filtration following trabeculectomy is the flow of aqueous through the substance of the protective scleral flap. This paper reports an in vitro study of the permeability of the outer layers of limbus and anterior sclera that comprise this flap.

Materials and methods. Sixteen human autopsy eyes were used in this study. Each was free of known ocular disease and was used within approximately 48 hr. of death.

Six eyes were studied with a simple quantitative perfusion apparatus. The apparatus consisted of a horizontal glass column that had a volume of 1 ml. and was calibrated in 0.01 ml. divisions. The column was connected by polyethylene tubing to a methyl methacrylate fitting in a 5 mm. central corneal trephine similar to that in the technique described by Grant. The height of the column was adjusted to provide a perfusion pressure of 15 mm. Hg.

Ringer's lactate was used as the perfusion fluid. A drop of dilute red dye was placed in the distal end of the glass column, separated from the perfusion fluid by an air bubble. This protected the perfusion fluid from evaporation and aided in locating the distal end of the perfusion column.

Outflow facility was estimated by timing the movement of perfusion fluid in the column. The meniscus of the perfusion fluid was observed through a magnifying lens, and the time required for it to traverse 0.05 ml. in the column was recorded with a stopwatch. An average of three such measurements was used to calculate the outflow facility, based on a perfusion pressure of 15 mm. Hg.

A peripheral iridectomy was made in each eye before the perfusion was started. Each eye was then perfused for 1 hr. at room temperature before a baseline outflow facility was established. A trabeculectomy was then performed, with a partial-thickness 7 by 7 mm. limbus-based scleral flap and 1 by 4 mm. fistula into the anterior chamber. The flap was approximated with five sutures, and the edges were sealed with a cyanoacrylate adhesive to prevent flow around the margins of the scleral flap. As the adhesive dried, it formed a thin film over the central portion of the
Fig. 1. Early phase of perfusion with India ink showing a vessel (V) filling on the scleral flap. The margins of the scleral flap (SF) are outlined by ink beneath the adhesive. A large episcleral vessel (EpV) is noted to fill adjacent to the flap.

scleral flap. This film was carefully dissected from the central 5 by 5 mm. portion of the flap, and the outflow facility was again measured.

Following the final perfusion measurement, an incision was made across the scleral flap and adjacent sclera, and the thickness of the flap was measured relative to the adjacent sclera, by means of a photographic enlargement of the cut tissue edges.

In eight eyes, a simple qualitative perfusion apparatus was used to study the route of tracer elements through the substance of the scleral flap. This consisted of a 10 ml. reservoir connected by polyethylene tubing to a fitting in a 5 mm. central corneal trephine. The height of the reservoir was adjusted to give a perfusion pressure of approximately 20 mm. Hg.

A trabeculectomy was performed as previously described, and the edges of the scleral flaps were sealed with adhesive. Seven eyes were perfused with a dilute solution of India ink. Two additional unoperated eyes were coated with a thin layer of adhesive over one half of the sclera and perfused with the ink solution to determine the influence of the adhesive on outflow into episcleral vessels. The perfusion was observed through an operating microscope and photographed at intervals during a minimum of 30 min. Tissue was then taken from the surgical site in each eye and from limbal tissue away from the surgical sites and from the two control eyes. These specimens were stained with hematoxylin and eosin and studied by light microscopy.

One eye was perfused with a solution of ferritin. The central portion of the scleral flap was then fixed in 0.1M cacodylate-buffered 3 percent glutaraldehyde. The tissue was postfixed in 2 percent osmium tetroxide and imbedded in Epon. Thin sections were examined on a transmission electron microscope (Model 100 B; JEOL USA, Electron Optics, Medford, Mass.), both with and without uranyl acetate and lead citrate staining.

Results. Table I summarizes the observations in the quantitative perfusion study. The trabeculectomy increased the outflow facility an average of 3.42-fold over the baseline, with a range of 1.50- to 11-fold. The increase in outflow appeared

<table>
<thead>
<tr>
<th>Eye No.</th>
<th>Baseline outflow (μL/min./mm. Hg)</th>
<th>Increase in outflow after trabeculectomy (fold increase over baseline)</th>
<th>Flap thickness (fraction of scleral thickness)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.15</td>
<td>2.93</td>
<td>1/2</td>
</tr>
<tr>
<td>2</td>
<td>0.11</td>
<td>2.55</td>
<td>1/2</td>
</tr>
<tr>
<td>3</td>
<td>0.12</td>
<td>11.00</td>
<td>1/3</td>
</tr>
<tr>
<td>4</td>
<td>0.10</td>
<td>4.90</td>
<td>1/2</td>
</tr>
<tr>
<td>5</td>
<td>0.15</td>
<td>1.50</td>
<td>2/3</td>
</tr>
<tr>
<td>6</td>
<td>0.17</td>
<td>5.20</td>
<td>1/3</td>
</tr>
<tr>
<td>Avg.</td>
<td></td>
<td>3.42</td>
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</tr>
</tbody>
</table>
Fig. 2. Histologic section of an eye perfused with India ink (not the same eye as shown in Fig. 1). Ink particles fill spaces between collagen bundles in the inner layers of the scleral flap (SF) and fill one vessel (V) on the surface of the flap. (Hematoxylin and eosin; x40.)

India ink solution was observed to fill one to three vessels on the scleral flap in five of the seven eyes (Fig. 1). The ink was also observed to fill episcleral vessels at locations away from the surgical site (Fig. 1). Ink also filled episcleral vessels in the unoperated eyes, both in areas covered by adhesive and in uncovered areas. The ink solution did not actually appear to penetrate the substance of the scleral flaps, although clear fluid was observed to well up on the flaps. The histologic specimens revealed accumulation of ink particles between collagen lamellae in the inner layers of the flaps and in occasional vessels on the surface of the flaps (Fig. 2). Histologic specimens of limbal tissue away from the surgical site and from the unoperated eyes revealed ink particles in Schlemm's canal, outlet channels, and episcleral vessels.

The tissue perfused with ferritin solution revealed numerous tracer particles in the ground substance. This was seen in equal concentrations between the individual collagen fibrils and between the fibril bundles (Fig. 3).

Discussion. This study suggests that flow through the outer layers of limbus and anterior sclera in a trabeculectomy flap can significantly improve the outflow facility. It further suggests that the route of flow may be through vessels in the flap or through the collagen ground substance between individual fibrils. The clinical significance of these observations is limited by the fact that these were in vitro studies, which may not accurately depict the in vivo situation.

It is not surprising that the ink solution was observed to flow through patent vessels in the flap. It seems unlikely, however, that these small channels remain patent in life, since the cut ends of the much larger Schlemm's canal have been reported to close by scar tissue after a trabeculectomy.2-4 However, it is possible that vessels might degenerate, regenerate, and recanalize. Benedikt,5 for example, injected fluorescein into the anterior chamber of patients with successful trabeculectomies and found fluorescein-stained aqueous in “newly developed aqueous veins (trabeculectomy-veins) and lymphatic vessels.”

The observation that significant flow can occur through the ground substance of the scleral flap may be of more clinical importance. Although the ink particles appeared to be trapped by collagen lamellae in the inner layers of the scleral flap, the smaller ferritin particles were able to pass through the full thickness of the scleral flap within the ground substance. The observation that the ferritin particles were evenly distributed throughout the ground substance suggested that fluid may flow both through and around bundles of collagen fibrils.

If the above observations accurately depict the situation in living tissue, the compactness of the collagen fibrils might be a factor in resistance to aqueous flow. In a separate study, it was found that the compactness of fibrils is the same in the inner and outer layers of human limbal collagen.6 Therefore the outer tissue appears to possess no unique advantage in this regard. There was a suggestion in the present study that the resistance to flow may be related to the thickness of the...
Fig. 3. Electron microscopic section of limbal tissue perfused with ferritin. The concentration of ferritin particles in the ground substance is approximately equal in the interfibrillar spaces (IFS) and interbundle spaces (IBS). (Unstained section; x59,000.)

ciliary flap. However, the number of eyes used in that portion of the study was too small to be statistically significant.

It was not possible to determine from this study whether the increased outflow through the trabeculectomy flap was primarily through patent aqueous vessels or through the collagen ground substance. Furthermore, it is not certain what influence the adhesive may have had on resistance to flow. Even though the film was carefully removed from the central 5 by 5 mm. of the flap, we do not know what residual effect it may have had. India ink perfusion of the unoperated eyes suggested that the adhesive did not retard the flow of fluid into the episcleral vessels.

This study does not prove that aqueous flow through the substance of the scleral flap is the main route of external filtration after trabeculectomy. However, it provides some support for this theory. Another likely route of external filtration is around the margins of the scleral flap. Further study is needed to determine the relative importance of these two, and possibly other, routes of external filtration after trabeculectomy.

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