Functional and electron microscopic changes in the trabecular meshwork remaining after trabeculectomy in cynomolgus monkeys

E. Lütjen-Drecoll and E. H. Bárány

Electron microscopic morphometry of untouched trabecular meshwork was done in nine eyes of six young cynomolgus monkeys trabeculectomized 329 to 447 days earlier, in two unoperated control eyes and one eye where a piece of ciliary muscle was excised. Outflow facility before and after pilocarpine had been repeatedly measured in all eyes. Correlations were computed between perfusion results and morphometric measurements and between different morphometric measurements. All operated eyes but not control eyes showed deposits beneath the inner wall endothelium of Schlemm's canal, previously found only in very old human and monkey eyes and in chronic simple glaucoma. In the uveal and corneoscleral trabeculae of operated eyes there was marked thickening of the sheaths covering intratrabecular elastic fibers. Thicker elastic fiber sheaths were seen in eyes with lower facilities and lower pilocarpine effects, both early and late after operation. A direct causal connection is improbable, however. Subendothelial deposits seemed to be more plentiful in eyes which had shown a high facility shortly (three months) after trabeculectomy. The amount did not correlate with facility at the time of enucleation. This suggests that the subendothelial deposits are due to underperfusion of the unoperated meshwork caused by a temporary bypass for the aqueous. The nature of the deposits remains to be established.

Key words: *Macaca fascicularis*, trabecular meshwork, trabeculectomy, aqueous outflow facility, eye, quantitative electron microscopy, morphometry, chamber angle, glaucoma operations.

The influence of fistulizing glaucoma operations on the physiology and morphology of the nonoperated portion of the trabecular meshwork has not been sufficiently studied. The clinical experience that the intraocular pressure and the outflow resistance can rise after closure of a traumatic or surgical fistula may indicate that the trabecular meshwork gradually loses its normal function after fistulization of the anterior chamber. In trabeculotomized cynomolgus monkey eyes, Dannheim and Bárány observed a decrease in rest-
Material and methods

Twelve eyes of six cynomolgus monkeys (Macaca fascicularis, previously Macaca irus) were used. The eyes of these animals had been perfused before trabeculectomy, and were perfused repeatedly after surgery for up to 447 days. They were fixed in vivo with glutaraldehyde during the final perfusion, as previously described. These animals belong to a larger group for which the light microscopic and perfusion data have been previously reported. The selection of animals for electron microscopic studies had no relation to the perfusion data or the results of surgery. Animals Nos. 103, 104, 111, 114, 115, and 119 were utilized (cf. Table I in Barany and co-workers). The trabeculectomy was performed as described by Linner. The postoperative reaction was mild and disappeared within a month.

No surgery had been performed in eyes Nos. 115R and 119R, while in No. 103L, it seemed from the histology that the canal had not been opened, so that in a certain sense this case can be considered as sham-operated. In case No. 114L, only the posterior half of the canal was removed. In case No. 119L, an open connection between the anterior chamber and the canal was still patent at the last perfusion. In case No. 114R, an unusual vascular structure seemingly acted as a new drainage channel. The histology of these eyes has been previously described.

Anterior chamber perfusions. The mechanics of the perfusions were conventional, but the protocol was new, taking account of nonlinearity of the outflow resistance. The perfusions were performed under clean, but not aseptic, conditions.

Postperfusion anterior chamber reactions under these conditions last, at most, for a few weeks and are mild (E. B., unpublished). The eyes were examined biomicroscopically immediately prior to each perfusion and were always free from inflammation.

Material and methods

Twelve eyes of six cynomolgus monkeys (Macaca fascicularis, previously Macaca irus) were used. The eyes of these animals had been perfused before trabeculectomy, and were perfused repeatedly after surgery for up to 447 days. They were fixed in vivo with glutaraldehyde during the final perfusion, as previously described. These animals belong to a larger group for which the light microscopic and perfusion data have been previously reported. The selection of animals for electron microscopic studies had no relation to the perfusion data or the results of surgery. Animals Nos. 103, 104, 111, 114, 115, and 119 were utilized (cf. Table I in Barany and co-workers). The trabeculectomy was performed as described by Linner. The postoperative reaction was mild and disappeared within a month.

No surgery had been performed in eyes Nos. 115R and 119R, while in No. 103L, it seemed from the histology that the canal had not been opened, so that in a certain sense this case can be considered as sham-operated. In case No. 114L, only the posterior half of the canal was removed. In case No. 119L, an open connection between the anterior chamber and the canal was still patent at the last perfusion. In case No. 114R, an unusual vascular structure seemingly acted as a new drainage channel. The histology of these eyes has been previously described.

Anterior chamber perfusions. The mechanics of the perfusions were conventional, but the protocol was new, taking account of nonlinearity of the outflow resistance. The perfusions were performed under clean, but not aseptic, conditions.

Postperfusion anterior chamber reactions under these conditions last, at most, for a few weeks and are mild (E. B., unpublished). The eyes were examined biomicroscopically immediately prior to each perfusion and were always free from inflammation.

Material and methods

Twelve eyes of six cynomolgus monkeys (Macaca fascicularis, previously Macaca irus) were used. The eyes of these animals had been perfused before trabeculectomy, and were perfused repeatedly after surgery for up to 447 days. They were fixed in vivo with glutaraldehyde during the final perfusion, as previously described. These animals belong to a larger group for which the light microscopic and perfusion data have been previously reported. The selection of animals for electron microscopic studies had no relation to the perfusion data or the results of surgery. Animals Nos. 103, 104, 111, 114, 115, and 119 were utilized (cf. Table I in Barany and co-workers). The trabeculectomy was performed as described by Linner. The postoperative reaction was mild and disappeared within a month.

No surgery had been performed in eyes Nos. 115R and 119R, while in No. 103L, it seemed from the histology that the canal had not been opened, so that in a certain sense this case can be considered as sham-operated. In case No. 114L, only the posterior half of the canal was removed. In case No. 119L, an open connection between the anterior chamber and the canal was still patent at the last perfusion. In case No. 114R, an unusual vascular structure seemingly acted as a new drainage channel. The histology of these eyes has been previously described.

Anterior chamber perfusions. The mechanics of the perfusions were conventional, but the protocol was new, taking account of nonlinearity of the outflow resistance. The perfusions were performed under clean, but not aseptic, conditions.

Postperfusion anterior chamber reactions under these conditions last, at most, for a few weeks and are mild (E. B., unpublished). The eyes were examined biomicroscopically immediately prior to each perfusion and were always free from inflammation.

Material and methods

Twelve eyes of six cynomolgus monkeys (Macaca fascicularis, previously Macaca irus) were used. The eyes of these animals had been perfused before trabeculectomy, and were perfused repeatedly after surgery for up to 447 days. They were fixed in vivo with glutaraldehyde during the final perfusion, as previously described. These animals belong to a larger group for which the light microscopic and perfusion data have been previously reported. The selection of animals for electron microscopic studies had no relation to the perfusion data or the results of surgery. Animals Nos. 103, 104, 111, 114, 115, and 119 were utilized (cf. Table I in Barany and co-workers). The trabeculectomy was performed as described by Linner. The postoperative reaction was mild and disappeared within a month.

No surgery had been performed in eyes Nos. 115R and 119R, while in No. 103L, it seemed from the histology that the canal had not been opened, so that in a certain sense this case can be considered as sham-operated. In case No. 114L, only the posterior half of the canal was removed. In case No. 119L, an open connection between the anterior chamber and the canal was still patent at the last perfusion. In case No. 114R, an unusual vascular structure seemingly acted as a new drainage channel. The histology of these eyes has been previously described.

Anterior chamber perfusions. The mechanics of the perfusions were conventional, but the protocol was new, taking account of nonlinearity of the outflow resistance. The perfusions were performed under clean, but not aseptic, conditions.

Postperfusion anterior chamber reactions under these conditions last, at most, for a few weeks and are mild (E. B., unpublished). The eyes were examined biomicroscopically immediately prior to each perfusion and were always free from inflammation.

Material and methods

Twelve eyes of six cynomolgus monkeys (Macaca fascicularis, previously Macaca irus) were used. The eyes of these animals had been perfused before trabeculectomy, and were perfused repeatedly after surgery for up to 447 days. They were fixed in vivo with glutaraldehyde during the final perfusion, as previously described. These animals belong to a larger group for which the light microscopic and perfusion data have been previously reported. The selection of animals for electron microscopic studies had no relation to the perfusion data or the results of surgery. Animals Nos. 103, 104, 111, 114, 115, and 119 were utilized (cf. Table I in Barany and co-workers). The trabeculectomy was performed as described by Linner. The postoperative reaction was mild and disappeared within a month.

No surgery had been performed in eyes Nos. 115R and 119R, while in No. 103L, it seemed from the histology that the canal had not been opened, so that in a certain sense this case can be considered as sham-operated. In case No. 114L, only the posterior half of the canal was removed. In case No. 119L, an open connection between the anterior chamber and the canal was still patent at the last perfusion. In case No. 114R, an unusual vascular structure seemingly acted as a new drainage channel. The histology of these eyes has been previously described.

Anterior chamber perfusions. The mechanics of the perfusions were conventional, but the protocol was new, taking account of nonlinearity of the outflow resistance. The perfusions were performed under clean, but not aseptic, conditions.

Postperfusion anterior chamber reactions under these conditions last, at most, for a few weeks and are mild (E. B., unpublished). The eyes were examined biomicroscopically immediately prior to each perfusion and were always free from inflammation.
 Electron microscopy and morphometry. Sectors adjoining the operated area and comprising approximately \( \frac{1}{2} \) of the circumference of the chamber angle were studied in all eyes. Moreover, specimens of meshwork previously removed at trabeculectomy and embedded for electron microscopy were used as controls. Sagittal sections were used throughout. The preparation of the specimens for histology (semithin sections) and electron microscopy has been described. The criteria for selection of sections to be evaluated morphometrically and the procedure for randomized selection of areas and lengths to be measured were as before as regards Factors I and II, related to the inner wall.

For technical reasons, no acceptable section of inner wall was obtained in case No. 114L. Besides measurements near the inner-wall endothelium, certain measurements on three inner uveal trabeculae and three corneoscleral trabeculae from the center of the trabecular meshwork were made with a Vernier caliper on a composite picture at a magnification of \( >10,000 \). Special care was taken to use only trabeculae cut exactly sagittally. This was done by noting the orientation of the collagenous fiber bundles located in the trabecular core. The measurements concerned structural characteristics (Factors III through VIII) that already, on simple inspection, appeared different from the normal picture. The factors were chosen for study without regard to the perfusion results, however.

The following parameters were quantitatively evaluated (Table I):

I. Subendothelial deposits. The area of lightly to moderately electron-dense material of unknown nature lying directly beneath the inner-wall endothelium (Fig. 2). This material is homogeneous at lower magnification but granular at \( \times10,000 \). The accumulations are sometimes limited by adjacent cells but in other places simply stop without being bound by any other structure.

II. Empty spaces. The area of the optically empty regions beneath the inner-wall endothelium, which are limited by adjacent cells, fibers, or subendothelial deposits (shown in Fig. 3, A of ref. 5).

III. Elastic fiber sheaths. The width of the light homogenous sheath around the dark zone of the exactly cross-sectioned elastic fibers within the trabeculae (Figs. 3 and 5). Table I shows the mean values of 10 measurements on each of three inner uveal trabeculae.

IV. Basement membrane. The width of the basement membranes at their widest point, if this width was evenly present for a length of at least 3 \( \mu m \) (Fig. 3). Only sections of the basement membrane not containing curly or lattice collagen were considered. Table I shows the mean value of measurements carried out on three inner uveal trabeculae.

Table II. Significant rank correlates (Spearman) between the various morphologic data

<table>
<thead>
<tr>
<th>Factor</th>
<th>Elastic fiber sheaths (uveal)</th>
<th>Elastic fiber sheaths (corneo-scleral)</th>
<th>Basement membrane (uveal)</th>
<th>Basement membrane with curly collagen (corneo-scleral)</th>
<th>Basement membrane with curly or lattice collagen (corneo-scleral)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor I</td>
<td>0.54*</td>
<td>0.68†</td>
<td>0.80†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor III</td>
<td>+ 0.54*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VII</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VIII</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*0.05 < p < 0.1.
10.01 < p < 0.05.
10.001 < p < 0.01.

V. Basement membrane with curly or lattice collagen. The width of the basement membrane including curly or lattice collagen (long-spacing fibers) was measured at those points where the collagen was present continuously over a length of at least 3 \( \mu m \) (Fig. 4). If this occurred at several points of the trabecula, the highest value was used. A mean value of measurements on three inner uveal trabeculae was taken for Table I.

VI, VII, and VIII. Measurements corresponding to III, IV, and V were also carried out on outer corneoscleral trabeculae.

Statistics. Spearman rank correlations were used throughout.

Results and discussion

1. Morphometry. Table I shows the results of the nine trabeculectomized eyes, the sham-operated case (No. 103L), and the two control eyes (Nos. 115R and 119R). Subendothelial deposits (Factor I) were completely absent in the control eyes which had been repeatedly perfused, but not operated on, while the operated opposite eyes from the same animals had moderate amounts. The sham-operated eye
Fig. 1. Survey of perfusion data. Abscissae: time in months, one division = 4 months. Ordinates: facility of aqueous outflow, one division = 0.5 ml × min⁻¹ × mm. Hg⁻¹. The lower heavier line represents resting facility (C₀), the upper thinner line the post-pilocarpine maximum facility (Cₚp). Arrow-heads = time of operation. (Partly redrawn from Fig. 5 of the preceding paper.)
Fig. 2. Electron micrograph of the inner wall of Schlemm's canal (SC) in Case No. 104L, nonoperated segment of the trabecular meshwork, 446 days after trabeculectomy. Sagittal section, composite picture. Note the "subendothelial deposits" (Factor I, arrows) adjacent to the inner wall endothelial lining (E).
Fig. 3. A, electron micrograph of an inner uveal trabecular lamella in Case No. 103R, unoperated trabecular meshwork 447 days after trabeculectomy, sagittal section. B, higher magnification corresponding to the rectangle in A. Note the light sheath material (arrows) of the elastic fibers which are mostly cross-sectioned. BM = basement membrane.

(No. 103L), which had been opened and subjected to excision of part of the ciliary muscle, had more subendothelial deposits than any of the others. In studies of numerous other macaque eyes, similar material has been seen only in eyes from very old rhesus monkeys. It would thus seem, that in our young monkeys, this material arose as a consequence not of repeated perfusions but of trabecular and other intraocular surgery. We shall return to this matter presently.

Factor II is the area of empty spaces next to the inner-wall endothelium. There is no evident difference between the operated and nonoperated eyes.

Factor III is the width of the elastic fiber sheaths. This structure was much smaller or absent before operation, e.g., in the excised specimens and in the two control eyes. This is true also for the same structure in the corneoscleral trabeculae (Factor VI). Factors IV and VII represent the width of trabecular basement mem-
Fig. 4. Electron micrograph of an inner uveal trabecular lamella 442 days after trabeculectomy (No. 115L). Considerable widening of the basement membrane (BM) with much curly or lattice collagen (arrows).
branes. They were not clearly different in the control and experimental eyes. The width of the structure in the sham-operated eye was comparable to that in the other eyes. Lattice or curly collagen (Factor V) was present in all the uveal trabeculae except in control eye No. 115R; it greatly affected the width of the basement membrane where it occurred (Fig. 4). In the corneoscleral trabeculae (Factor VII) it was present in only two eyes (Nos. 114R and L).

2. Morphologic correlations. Table II shows the correlations between the morphologic findings which are significant at a 90 per cent or greater confidence level. The significant correlations are all positive. The thickness of the elastic fiber sheath material in the uveal part of the meshwork (Factor III) is strongly correlated with the width of the uveal basement membrane (Factor IV), but this is not so for the corresponding structures in the corneoscleral meshwork. The correlation between the thickness of the uveal sheath material (Factor III) and the amount of corneo-

Fig. 5. Comparative electron micrographs of inner uveal trabecular lamellae in Case No. 119L before and after trabeculectomy. A, part of the excised specimen, B, unoperated segment of the meshwork of the same eye 431 days after operation. Note the change in the elastic fiber sheath material. In A, the elastic fibers (arrows) show no sheath material at this magnification, while in B, there are lightly stained sheaths (arrows) surrounding the strongly osmiophilic core of the elastic fibers.
scleral lattice or curly collagen (Factor VIII) is based only on the two eyes of one animal (No. 114).

3. Case No. 111L. The histology of this case, which had quite different perfusion results (see Fig. 1) than the others, was not described in the previous paper. In semi-thin sections, the trabecular meshwork of this eye, especially its outer regions near Schlemm’s canal, appeared markedly collapsed (Fig. 6) and the lamellae were not spread, although the ciliary muscle was maximally contracted after pilocarpine treatment. Several plasma cells were evident with the intertrabecular spaces. Beneath the inner-wall endothelium there were areas containing a homogeneous material, which stained slightly metachromatic with Richardson’s method.

In electron micrographs, great quantities of “subendothelial deposits” were found, which probably are identical with the metachromatic material observed in light microscopy. The subendothelial layers of the cribriform meshwork contained many activated cells which showed a well-developed rough-surfaced endoplasmic reticulum with greatly enlarged cisterns and many free ribosomes within the cytoplasm.

The eye had been quiet biomicroscopically and gonioscopically one month after trabeculectomy and prior to each perfusion. Nonetheless, it is highly probable that a pathologic reaction of unknown origin before or during the experiment is responsible for the smallness of the post-pilocarpine facility in this case. Correlations between morphologic and perfusion data were, therefore, computed both with and without this eye.

4. Correlations between morphology and perfusion results. The perfusion data are summarized in Fig. 1. Starting facility \( C_0 \) and facility after a supramaximal dose of pilocarpine \( (20 \mu g) \) had been given into the anterior chamber \( (C_{pp}) \) are shown. There were considerable changes with time in both the starting facilities and post-pilocarpine facilities obtained after operation. To correlate the morphologic findings with the perfusion results, the data of the repeated perfusions were

Fig. 6. Semi-thin sagittal section through Schlemm’s canal (SC) and the trabecular meshwork (TM) in Case No. 111L (Richardson’s stain). The trabecular meshwork, especially in its outer portion near the inner-wall endothelium, appears collapsed and filled with a metachromatic material (arrows). Plasma cells \( (X) \) between the trabecular lamellae.
grouped as follows: (1) all preoperative results = "before op" (n = 10, with the nonoperated eyes Nos. 115R and 119R excluded); (2) results obtained at the perfusion done 95 to 106 days after the operation = "3 months after" (same eyes as group 1, n = 10); (3) perfusion done 199 to 216 days postoperatively = "7 months after" (Nos. 103L, 104L, 111L, 114L, 115L, and 119L, n = 6); and (4) final perfusion and death of the animals, last measurement, "LM" 328 to 447 days postoperatively = "14 months after" (Nos. 103R and L; 104R and L; 111R and L; 114L and 115L, n = 8). Nos. 114R and 119L were excluded because No. 119L had a histologically verified open connection between the canal and the anterior chamber and No. 114R a special vascular structure (see under Materials and methods). The perfusion results in these cases are thus not properly comparable to the others. The cases were not excluded in the other groups formed because the histology at these other points in time was unknown for all the eyes. Another grouping was tried, counting time backward from the final perfusion "LM": (5) perfusion done 225 to 235 days prior to final perfusion = "7 months before LM" (same as Group 1, n = 10); (6) perfusion done 318 to 348 days prior to final perfusion = "10 months before LM" (same as Group 3, n = 6; however, when correlating with Factor I, No. 114L could not be used [see under Materials and methods] and n = 5).

In all these groups C₀, Cₚₚ, Cₚₚ/C₀, and Cₚₚ/C₀ were tested for correlations with all the morphometric measurements. The differences or ratios between C₀ and Cₚₚ of the preoperative group and the various postoperative groups were also compared. Table III shows the correlations with p < 0.05.

**Factor I, subendothelial deposits.** In view of the strategic location of the deposits adjacent to the inner wall of Schlemm's canal, one might have expected the quantity of this material to influence the perfusion results. However, no correlation
with final perfusion results existed (Table III and Fig. 7). This might be due to the fact that there was an insufficient quantity of material to influence the filtering area decisively, but it could also be that the deposits did not cover the places that really matter or had been washed away from those places or that new pathways had been opened circumventing the blocked ones.

If, instead of final perfusion results, one correlates the amount of subendothelial material with early perfusion results, a different picture is suggested. In Fig. 8, starting and post-pilocarpine facilities three months after operation have been plotted against the amount of subendothelial deposits. If the left-most point, representing eye No. 103R is disregarded, $C_o$ increases significantly ($r = 0.89^{**}$) with increasing amounts of subendothelial deposits. The relationship is not statistically significant if eye No. 103R is included and is, therefore, not listed in Table III. Although there is no apparent reason for discarding the data from No. 103R, the relationship in the other eyes is so striking that one must suspect it is, in fact, real.

How could one explain greater amounts of obstructive material in eyes which showed higher facilities about a year earlier? Subendothelial deposits should not cause increased facility. Can the converse hold?

As mentioned in the introduction, Goldmann¹ has pointed out that an under-worked trabecular meshwork tends to become impermeable. It could be that the subendothelial deposits are due to the period of excess filtration, with aqueous bypassing the meshwork caused by the operation, and that the large early facilities are the results of the operation. At later stages, the subendothelial deposits remain, but the functional effects of the operation have disappeared in most cases. We have no evidence for a similar mechanism at work in eye No. 103L where only ciliary muscle was excised. The possibility of a period of fistulization or of damage to the meshwork could be investigated in further studies.

Fig. 8. Same as Fig. 7, but facility measurements three months after trabeculectomy. From left to right: Nos. 103R, 119L, 115L, 114R, 104R, 104L, 111R, and 103L.
Table III. Correlations (Spearman's rank) between morphologic and physiologic data

<table>
<thead>
<tr>
<th>Factor</th>
<th>Three months after operation</th>
<th>Seven months after</th>
<th>Fourteen months after = LM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C_o</td>
<td>C_pp</td>
<td>C_pp-C_o</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>-0.76*</td>
<td>-0.63*</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>-0.71*</td>
<td>-0.89*</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>-0.78†</td>
<td>-0.71*</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When two values are given, the one below is with eye No. 111L excluded. Only correlations where at least one of the two values means before operation. Eyes Nos. 114R and 119L were excluded from the “14 months after operation = LM” groups for *p < 0.05; †p < 0.01; ‡p < 0.001.

meshwork cannot be excluded, however.

Table III also shows a strong negative correlation between relative pilocarpine effect \( (C_{pp}/C_o) \) 10 months before the last measurement and amount of subendothelial deposits. The pilocarpine effect in C-units \( (C_{pp}-C_o) \) shows no consistent change with increasing subendothelial deposits but, since \( C_o \) increases, the relative pilocarpine effect decreases as the amount of subendothelial deposits increases.

**Factor II, empty spaces.** There were no significant correlations between the empty spaces and perfusion results. This could be characteristic for the monkey species used in these experiments; e.g., the main resistance in this species might be located in deeper layers than in the stumptail monkey, in which spaces and resistance are correlated. It is possible, however, that the region investigated here, being not far from the operation site, is not representative with respect to the empty spaces.

**Factors III and VI, sheaths of the elastic fibers.** It is very hard to see how the presence of this structure inside the trabeculae could, in any direct way, affect the flow of aqueous humor. There are, however, clear-cut correlations between perfusion data from different time periods and the width of this sheath in uveal and especially in corneoscleral trabeculae (Table III). These correlations always indicate that low facility, especially after pilocarpine, or a low pilocarpine effect, is associated with more sheath material. Since there was no correlation with the preoperative perfusion data, the explanation of the observed association must be that one or several factors in connection with the operation caused decreased post-pilocarpine facilities as well as increased sheath material.

**Factors IV and VII, basement membranes.** The thickness of the uveal basement membranes (Factor IV) correlates negatively with \( C_o \) seven months postoperatively and with \( C_{pp} \) three months after operation, while the thickness of the corneoscleral basement membranes (Factor VII) has only one functional correlation which disappears when case No. 111L is excluded (Table III). Since the thickness is only a fraction of a micron in either case, it seems extremely improbable that the thickness of the basement membrane directly exerts a functionally important effect on outflow facility. More probably, what has been said about the sheaths is also true for the basement membranes.

**Concluding remarks**

1. **Statistics.** If a great number of correlations are calculated, some will be significant and nonetheless spurious. We have no way to decide for which of ours this
is the case. Due to the small number of available eyes, correlations which would have been significant with more extensive data may have remained insignificant in our material. Thus, both the presence and the absence of a significant correlation must be interpreted cautiously.

2. The subendothelial deposits. Structureless material immediately adjacent to the endothelium of the inner wall of Schlemm's canal has been observed by all workers doing electron microscopy of this region. It has been described as ground substance or as homogeneous amorphous material. The subendothelial deposits observed by us in trabeculectomized cynomolgus eyes distinctly differ from this material in being much denser and more sharply demarcated. Material of similar appearance has as yet been found only in very old human and monkey eyes and in eyes with chronic simple glaucoma where it is abundant in the same location. The nature of this material is unknown. In our cases it seemed not to be caused by repeated perfusions but to arise as a consequence of the surgical procedure. It increased (probably) in amount with increasing early postoperative facility, possibly due to a shunting away of aqueous humor from the meshwork. Thus, the material may arise locally, perhaps because of underperfusion of the bypassed meshwork. Similar deposits have also been observed in human eyes excised 3 to 5 days after experimental goniotomy or trabeculectomy. These deposits were especially marked in detached parts of the trabecular meshwork across which no pressure differential could exist, and which were, therefore, unperfused. In these eyes, the deposits were absent in most of the rest of the circumference.

It is interesting to speculate that a similar process could operate in chronic simple glaucoma: the subendothelial deposits would not be the cause, but the results of local underperfusion of the juxtaendothelial meshwork, perhaps caused by a patchy disease process in the deeper layer of the meshwork.

3. Changes in the trabeculae. Intratrabecular changes were only poorly correlated with subendothelial deposits (Table II). In an unpublished study (E. L-D.) on eyes of Cercopithecus ethiops monkeys, subendothelial deposits were observed as early as eight weeks after trabeculectomy, while the intratrabecular changes (thickening of the elastic fiber sheets or basement membrane) were hardly visible at 16 weeks. These findings in monkeys speak against a proximate common cause for the deposits and the trabecular changes. On the other hand, similar subendothelial deposits and intratrabecular changes have been found in cases of chronic simple glaucoma. Furthermore, in a series of 20 such cases where trabeculectomy pieces were studied by the method employed here (E. L-D., unpublished data) there was a marked correlation between the amount of subendothelial deposits and the intratrabecular changes. Thus, there seems to be a contradiction between the findings in monkeys and those in glaucoma cases. The matter is of interest since, if there is a proximate common cause as suggested by the existence of a correlation in human eyes, it cannot very well be underperfusion; it is hard to see how this could affect uveal trabeculae. However, the contradiction may be more apparent than real:

<table>
<thead>
<tr>
<th>Ten months before LM</th>
<th>C_0</th>
<th>C_0 - C_0</th>
<th>C_0 - C_0</th>
<th>C_0 - C_0</th>
</tr>
</thead>
<tbody>
<tr>
<td>LM</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>+0.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.81</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.67</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.84*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.58</td>
<td></td>
<td></td>
<td>-0.71*</td>
<td>-0.79*</td>
</tr>
</tbody>
</table>

is significant are listed. LM means last measurement. "before" reasons explained in the text.
the monkeys represent a homogeneous and more or less disease-free population, while the human glaucoma patients represent different ages and different durations and severity of disease. This variability between the patients introduces a variable factor in the human material which is common for the changes in any one eye and will, therefore, cause a correlation between changes. Thus, while the underperfusion hypothesis for the subendothelial changes is not proved, it is not disproved by the existence of correlations with trabecular changes in glaucoma patients.

The authors thank Mrs. Ingalill Wersäll who performed all the perfusions, Mrs. Karin Schulze for her untiring help in electron microscopy and morphometry, and Mr. P. Zoefel of the Computer Center of Marburg for help with computer runs.

REFERENCES