The regression line \( Y = 3.885 + 0.741 \) (Run No.) covering this limited part of the data has a better fit \((r = 0.989)\) than that of all mean values. After run 9 no mean flow value differs from the mean flow at 9 by more than \( \pm 1 \) standard error unit.

**Discussion.** The randomized constant-pressure infusion technique generates discrete observations upon factors affecting measured inflow. The advantages of using this procedure in combination with a wide pressure range are three-fold: (1) the data are more amenable to statistical analysis than are observations derived from the two-level technique because statistical tests are based on the premise of a random data base; (2) the technique can isolate several factors affecting flow without having the experiment specifically designed for that one factor in the study of a given population; and (3) the technique minimizes the potential for experimental error by stressing the infused eye over such a wide range of pressures and for a sufficiently long time to ensure that observations are coherent and are not the product of a changing function in the slope of the measured inflow-pressure curve. Our data show that this curve is linear over the range from 15 to 40 mm Hg, but that the inflow-pressure curves for pentobarbital alone and phenacyclidine plus pentobarbital are significantly different from each other. As suggested by Bárány,\(^2\) the length of time that the eye has been infused with fluid also has a significant effect on flow. Investigations on the fluid dynamics of the in vivo eye have been concentrated on the collection of \(4\)e values obtained at moderate infusion pressures and the association of morphological configurations of the meshwork and canal with fluid infusion at different pressures.\(^2\) Neither the in vivo nor the in vitro studies have provided a statistical analysis of measured inflow results, so it is not possible to compare those data to ours. However, the results presented here indicate that flow does not decrease with increasing pressure and thus that the anatomical changes caused by different infusion pressures may not be quantitative expressions of flow rates.

We would like to acknowledge the capable assistance of Mr. Lawrence Snyder.

**Key words:** constant-pressure infusion, rhesus monkey.

**REFERENCES**


**Immunofluorescent studies on the trabecular meshwork in open-angle glaucoma.**

M. BRUCE SHIELDS, RALPH C. MCCOY, AND JOHN D. SHELBURNE.

The trabecular meshwork of eyes with open-angle glaucoma has been demonstrated to have an increase in gamma globulin and plasma cells, raising the question of an immunogenic mechanism in this disorder. In the present study, however, immunofluorescence assays on the trabecular meshwork of eyes with open-angle glaucoma were negative for specific immunoglobulins and for complement components that would result specifically from an antigen-antibody reaction. The study fails to provide any evidence in support of an immunogenic mechanism in open-angle glaucoma.

Over a decade ago, Becker and co-workers raised the question of an immunogenic mechanism in open-angle glaucoma by demonstrating an increase in gamma globulin and plasma cells in the trabecular meshwork of eyes with this disorder.\(^1\)\(^2\) Despite growing evidence for immunogenic mechanisms in many ocular disorders, additional evi-
Study in which immunofluorescence techniques were used to assay for the presence of immunoglobulins and complement components C3 (0.4 mg/ml), C4 (0.03 mg/ml), and Cq (0.02 mg/ml) (Hyland Laboratories, Costa Mesa, Calif.). Monospecificity of the antisera was tested by immunoelectrophoresis and Ouchterlony double immunodiffusion.

Results. All assays were negative except for the presence of the complement component Cq. This was positive in the trabecular meshwork of glaucoma cases 1 and 3b (Fig. 1) and was trace-positive in glaucoma cases 11 to 14. However, Cq also localized in the trabecular meshwork of control cases 2 and 3 (Fig. 2) and was trace-positive in control case 4.

Discussion. Becker and co-workers1,2 used im-

Table I. Open-angle glaucoma cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Race</th>
<th>Sex</th>
<th>Preop med.</th>
<th>Duration of med.</th>
<th>Prior surgery</th>
<th>Surgical procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>B</td>
<td>M</td>
<td>P, E, A</td>
<td>1 yr.</td>
<td>—</td>
<td>PLS</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>B</td>
<td>M</td>
<td>El, E, A</td>
<td>4 mos.</td>
<td>—</td>
<td>PLS</td>
</tr>
<tr>
<td>3a (OS)*</td>
<td>52</td>
<td>B</td>
<td>M</td>
<td>El, E, A</td>
<td>6 mos.</td>
<td>Trabec.</td>
<td>PLS</td>
</tr>
<tr>
<td>3b (OD)</td>
<td>52</td>
<td>B</td>
<td>M</td>
<td>El, E, A</td>
<td>1 yr.</td>
<td>Trabec.</td>
<td>PLS</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>W</td>
<td>M</td>
<td>P, E, A</td>
<td>4½ mos.</td>
<td>—</td>
<td>PLS</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>B</td>
<td>M</td>
<td>El, E, A</td>
<td>4 yrs.</td>
<td>Trabec.</td>
<td>PLS</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>B</td>
<td>M</td>
<td>El, E, A</td>
<td>3 yrs.</td>
<td>—</td>
<td>PLS</td>
</tr>
<tr>
<td>8</td>
<td>66</td>
<td>B</td>
<td>M</td>
<td>El, E, A</td>
<td>2 yrs.</td>
<td>PLS</td>
<td>PLS</td>
</tr>
<tr>
<td>9</td>
<td>49</td>
<td>B</td>
<td>M</td>
<td>El, E, A</td>
<td>?</td>
<td>Iriden.</td>
<td>PLS</td>
</tr>
<tr>
<td>10a (OS)*</td>
<td>75</td>
<td>B</td>
<td>M</td>
<td>P, E, A</td>
<td>?</td>
<td>—</td>
<td>PLS</td>
</tr>
<tr>
<td>10b (OD)</td>
<td>75</td>
<td>B</td>
<td>M</td>
<td>P, E, A</td>
<td>?</td>
<td>—</td>
<td>PLS</td>
</tr>
<tr>
<td>11</td>
<td>59</td>
<td>B</td>
<td>M</td>
<td>P, E, A</td>
<td>3 mos.</td>
<td>—</td>
<td>PLS</td>
</tr>
<tr>
<td>12</td>
<td>25</td>
<td>B</td>
<td>M</td>
<td>P, E, A</td>
<td>6 mos.</td>
<td>—</td>
<td>PLS</td>
</tr>
<tr>
<td>13</td>
<td>58</td>
<td>B</td>
<td>M</td>
<td>P, E, A</td>
<td>4 yrs.</td>
<td>—</td>
<td>PLS</td>
</tr>
<tr>
<td>14</td>
<td>53</td>
<td>B</td>
<td>M</td>
<td>P, E, A</td>
<td>4½ yrs.</td>
<td>Trabec.</td>
<td>PLS</td>
</tr>
</tbody>
</table>

*Specimens were obtained from both eyes of cases 3 and 10.

Medication key: P, pilocarpine; E, epinephrine; El, echothiophate iodide; A, acetazolamide.

Surgery key: PLS, posterior lip sclerectomy; Trabec., trabeculectomy; Iriden., iridencleisis.

dence for such a mechanism in open-angle glaucoma has been sparse.3,4 This paper reports a study in which immunofluorescence techniques were used to assay for the presence of immunoglobulins and complement in the trabecular meshwork of eyes with open-angle glaucoma and eyes without glaucoma.

Materials and methods. Portions of the trabecular meshwork from 16 eyes of 14 patients with open-angle glaucoma were obtained at the time of filtering surgery. The diagnosis of open-angle glaucoma was based on characteristic field and/or disc changes, the gonioscopic appearance of the angle, and the exclusion of any other apparent cause for the pressure elevation (with the possible exception of uveitis in case 9). Prior to surgery, each patient had an intraocular pressure that was uncontrolled on maximum medical therapy. Further data on these patients are shown in Table I.

Portions of trabecular meshwork were also obtained from five eyes enucleated for malignant melanoma of the choroid. In each case, the anterior segment and intraocular pressure were normal. Each eye was subjected to fluorescein angiography and 32P uptake prior to enucleation. Further data on these patients are shown in Table II. The specimen was excised immediately after enucleation, with the same technique as for the posterior lip sclerectomies. These specimens were used as nonglaucomatous controls.

Each specimen was placed on gauze, moistened with phosphate-buffered saline (PBS), and incubated for 30 minutes with fluorescein-labeled goat or rabbit antisera to human IgG (0.1 mg per milliliter), IgM (0.3 mg/ml.), and IgA (0.1 mg/ml.) and complement components C3 (0.4 mg/ml.), C4 (0.03 mg/ml.), and Cq (0.02 mg/ml.) (Hyland Laboratories, Costa Mesa, Calif.). Monospecificity of the antisera was tested by immunoelectrophoresis and Ouchterlony double immunodiffusion.

Results. All assays were negative except for the presence of the complement component Cq. This was positive in the trabecular meshwork of glaucoma cases 1 and 3b (Fig. 1) and was trace-positive in glaucoma cases 11 to 14. However, Cq also localized in the trabecular meshwork of control cases 2 and 3 (Fig. 2) and was trace-positive in control case 4.

Discussion. Becker and co-workers1,2 used im-
Fig. 1. Trabecular meshwork from open-angle glaucoma case 3b, showing immunofluorescent staining for complement component C3.

Immunofluorescence techniques were used to assay for gamma globulin in the trabecular meshwork of eyes with primary open-angle glaucoma. Their tissue was obtained from trephine buttons and surgical and autopsy eyes. Approximately three fourths of these specimens were positive for gamma globulin, compared with approximately 14 per cent of routine autopsy eyes. Approximately 80 per cent of the glaucoma specimens revealed the presence of plasma cells, compared with 20 per cent of the routine autopsy eyes.

These findings raised the question of immunologic activity in the trabecular meshwork of eyes with open-angle glaucoma. However, as the authors pointed out, neither gamma globulin nor plasma cells are proof of the presence of immunoglobulins (antibodies) or of antigen-antibody reactions. The results of the present study provide no evidence for the presence of immunoglobulins in the trabecular meshwork of eyes with open-angle glaucoma.

The assay for components of complement was performed to look for evidence of previous antigen-antibody reactions in the trabecular meshwork. Components C9 and C3 were selected because they occur only in the classic pathway, which is initiated by antigen-antibody complexes.

C3 was selected because it occurs in both the classic pathway and alternate pathways, which may be initiated by nonimmunologic processes such as general tissue damage. The presence of C3 alone in this study, therefore, suggests, but does not prove, that the complement activation resulted from a nonimmunologic process. Furthermore, this does not appear to be specific to glaucoma, since it was present in an even higher percentage of the nonglaucomatous melanoma eyes. It would be helpful to know if C3 occurs in eyes other than those with glaucoma or malignant melanoma.

Allansmith and co-workers have reported immunofluorescence assays for immunoglobulins in normal autopsy eyes. Although most ocular structures, including sclera, contained immunoglobulins, the trabecular meshwork was peculiarly devoid of immunoglobulin deposition, almost as if it had been "washed clean" of serum proteins. The present study suggests that eyes with open-angle glaucoma or choroidal melanoma do not differ from normal eyes in this respect. The paper by Allansmith and associates did not report an assay for complement.

Henley and co-workers also failed to find evidence for an immunologic abnormality in open-
angle glaucoma. They observed cell-mediated immunity, as indicated by leukocyte migration inhibition in only three of 10 patients with open-angle glaucoma and no other ocular diseases. Evidence which may link open-angle glaucoma to immunologic abnormalities include the observation that such patients have a higher incidence of positive antinuclear antibody reaction (44 per cent), compared with nonglaucomatous controls (7.5 per cent) and gg topical steroid responders (7 per cent). Also, certain HL-A antigens appear in a higher percentage of open-angle glaucoma patients than in the normal population. These studies differ, however, as to which of the HL-A antigens are abnormally increased. Cooperative studies are currently underway to more clearly define this question and to establish its significance in open-angle glaucoma.

Appreciation is expressed to Ms. Linda Cleveland for excellent technical assistance.

From the Duke University Eye Center and the Departments of Pathology, Duke University Medical Center and Veterans Administration Hospital, Durham, N. C. This work was supported in part by the Electron Microscopy Laboratory, Durham Veterans Administration Hospital, and a grant from the National Society for the Prevention of Blindness, New York, New York. Submitted for publication July 12, 1976. Reprint requests: Dr. Bruce Shields, Duke University Eye Center, Durham, N. C. 27710.

Key words: immunofluorescence, immunoglobulins, antibodies, complement, trabecular meshwork, open-angle glaucoma, choroidal melanoma.

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