Changes in outflow facility in experimental hyphema. PAUL STERNBERG, JR., RAMESH C. TRIPATHI, BRENDA J. TRIPATHI, AND ROBERT R. CHILCOTE.

To characterize the pathophysiology of hyphema clearance, we studied changes in the facility of outflow in experimental hyphema in freshly enucleated rabbit eyes. Hyphemas, with washed normal or sickled red cells (RBCs) (suspended in isotonic phosphate buffer to obtain a hematocrit value comparable to that of whole blood) and occupying 50% to 100% of the anterior chamber volume, caused a marked cell "crowding" in the chamber angle and an increase in the outflow resistance; the facility stabilized at a value 60% lower than the control (p = <0.001). No significant change in outflow facility was observed in hyphemas of either RBC type occupying 25% of the anterior chamber volume (p = N.S.). Whole blood hyphema occupying 50% of the anterior chamber volume reduced the facility of outflow by 80% of the control mock aqueous value (p = <0.001); a comparison with 50% hyphema produced by washed RBCs indicated a significant contribution by the plasma (fibrin) component (p = 0.0025) in increasing the resistance to outflow.

Hyphemas may elevate intraocular pressure, particularly when they occupy greater than one half of the volume of the anterior chamber of the eye. To our knowledge no study has examined the changes in outflow facility that may occur with the variations in the amount of hyphema and with the time course of the elevated intraocular pressure. Recently it has been suggested that sickle cell patients may be at greater risk of secondary glaucoma following hyphema, because of the slower clearance of less deformable sickle cells compared with normal RBCs.

To elucidate the pathophysiology of acute hyphema in normal and sickle cell patients and to correlate the effect of differing volumes of hyphema on the facility of aqueous outflow, we have undertaken an experimental study using the rabbit as an animal model.

Material and methods. The anterior chambers of freshly enucleated eyes of New Zealand white adult rabbits, weighing 2.5 to 3 kg, were cannulated with a 23-gauge needle and perfused at a constant pressure of 8 mm Hg (equivalent normal physiologic pressure for the enucleated eye) preset by the difference in height between the eye and the perfusate level in the reservoir. The globe was kept in a moist chamber maintaining its shape.
The rate of perfusion was monitored by changes in the weight of the perfusate reservoir hooked to a Mettler balance. A second needle in the anterior chamber connected to a Statham pressure transducer and Grass polygraph was used to constantly monitor the intraocular pressure. The coefficient of outflow was calculated from the rate of aqueous outflow divided by intraocular pressure. The values obtained were subjected to Student's t test to determine the significance of the difference in outflow facility in the experimental groups as compared with mock aqueous.

At least six eyes were studied with each of the following perfusates: (1) mock aqueous as control, (2) autologous rabbit RBCs washed and suspended in isotonic phosphate buffer, (3) normal human RBCs washed and suspended in isotonic phosphate buffer, (4) freshly drawn, whole normal human blood (without anticoagulant), and (5) sickled RBCs from a patient with hemoglobin SS disease, washed and suspended in isotonic phosphate buffer. The sickled nature of the RBCs was confirmed by examination of the blood smear. By appropriate dilution of the washed, packed RBCs, the hematocrit values of the normal and sickle cells in phosphate buffer were adjusted to that of whole blood, i.e., 42%. The volume introduced into the anterior chamber was such as to produce 25%, 50%, and 100% hyphema, after which mock aqueous perfusion was continued through the same needle connected to a three-way stopcock. At the conclusion of each experiment, the eyes were fixed intact for 24 hr in buffered isotonic glutaraldehyde-paraformaldehyde mixture, pH 7.4, and processed for histologic study.

**Results.** The mean values of the outflow facility together with standard deviations in each experimental group are given in Table I. In control eyes perfusion of the anterior chamber with mock aqueous showed a steady outflow facility just below 0.5 µl/min/mm Hg. In eyes perfused with washed RBCs from normal human or rabbit or from human sickle cell patients to achieve 50% or 100% hyphema, the outflow facility stabilized at a value of approximately 0.2 µl/min/mm Hg (Figs. 1 and 2), thus significantly compromising the coefficient of outflow as compared with the control (p = 0.0025).

Light microscopic study of the eyes perfused with normal and sickle RBCs showed aggregation of the normal and sickled erythrocytes, respectively, in the angle of the anterior chamber,

### Table I. Mean value of outflow facility (µl/min/mm Hg) and S.D. at 5, 20, and 40 min of anterior chamber perfusion with mock aqueous (control) and different blood samples.

<table>
<thead>
<tr>
<th></th>
<th>Rabbit RBCs</th>
<th>Sickle RBCs</th>
<th>Normal human RBCs</th>
<th>Whole blood, normal human, 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>Mock aqueous ant.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chamber perfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean facility</td>
<td>0.653</td>
<td>0.781</td>
<td>0.724</td>
<td>0.701</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.22</td>
<td>0.12</td>
<td>0.16</td>
<td>0.12</td>
</tr>
<tr>
<td>Mean facility</td>
<td>0.473</td>
<td>0.551</td>
<td>0.524</td>
<td>0.499</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.11</td>
<td>0.24</td>
<td>0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean facility</td>
<td>0.485</td>
<td>0.275</td>
<td>0.198</td>
<td>0.201</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.1</td>
<td>0.06</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Δ Facility, ant.</td>
<td>0.168</td>
<td>0.506</td>
<td>0.526</td>
<td>0.5</td>
</tr>
<tr>
<td>Mean facility</td>
<td>0.25</td>
<td>0.19</td>
<td>0.12</td>
<td>0.196</td>
</tr>
</tbody>
</table>

p values vs. mock aqueous

<0.001 <0.001 <0.001 <0.001 NS <0.001

NS = not significant.

*p values are calculated from the difference between the mean values at 5 and 40 min.
trabecular meshwork, and ciliary body stroma. No appreciable difference was found in the distribution of the normal vs. sickled RBCs. In the eyes perfused with whole blood, fibrinoid material was present in addition to the blood corpuscles.

Discussion. In pilot studies, we introduced washed and packed human RBCs into the anterior chambers of anesthetized rabbits. Histologic study of these eyes revealed a large amount of fibrin and other proteinaceous material in the anterior chamber at the conclusion of the experimental procedure (1 to 2 hr). Realizing the improbability of obtaining freshly enucleated human eyes, we chose to use freshly enucleated rabbit eyes for further investigation. Although morphologic differences exist in the organization of the angular region of the eyes of rabbits and primates, the mechanism of aqueous outflow is fundamentally similar. The use of enucleated eyes eliminated the pseudofacility component, and thus the rate of outflow equaled the rate of perfusion.

Our studies demonstrate a significant compromise in aqueous clearance in hyphemas occupying greater than 50% of the volume of the anterior chamber. The volume-dependent nature of the phenomenon is further apparent in that we did not find similar results with 25% hyphema. The accumulation of RBCs in the angle of the anterior chamber and also in the extracellular spaces of the structures of the angular region caused a crowding effect in the exit pathway of aqueous humor and accounts for the decreased coefficient of outflow. A further reduction in outflow facility in eyes perfused with whole blood indicates the contribution by plasma in increasing the outflow resistance. Thus our observations provide experimental evidence to the suggestion that fibrin formation in the plasma contributes to decreased outflow and that fibrinolytic agents are of value in the treatment of traumatic hyphema.

In a recent study of traumatic hyphema in patients with sickle hemoglobinopathy, it was inferred that these patients are at a greater risk of secondary glaucoma because of slow clearance of the less deformable sickled erythrocytes. Although the environment of the anterior chamber enhanced sickling of the RBCs, the sickle cells were primarily cleared through the conventional outflow pathway. Our study suggests that intact SS sickled RBCs occupying less than 50% of the anterior chamber volume of rabbit eyes do not compromise the aqueous humor clearance more than do normal RBCs. It is realized, however, that the matter of severity of secondary glaucoma in sickle cell patients cannot be answered by short-term experiments in vitro. The rate of hemolysis and the formation of blood products such as ghost cells that occur with time may be different for normal vs. sickled erythrocytes, and the tendency for red blood to may again differ from A to S groups of patients.

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REFERENCES