Influence of intraocular pressure and trabeculotomy on aqueous outflow in enucleated monkey eyes

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Quantitative aqueous perfusion was performed at a series of pressures from 5 to 50 mm Hg in enucleated monkey eyes on which an iridotomy had been performed to minimize artificial changes in depth of the anterior chamber. Total facility of outflow became less when the pressure was increased, similar to what was observed in separately reported experiments on enucleated human eyes. When the pressure was next reduced, total facility tended to increase in monkey eyes, though not in human eyes. Trabeculotomy of one quadrant from within the anterior chamber in monkey eyes made the total facility of outflow several times greater than normal and was calculated to eliminate 83 to 97 per cent of the resistance to outflow in the trabeculotomized quadrant.

Key words: Trabeculotomy, aqueous perfusion, outflow facility, trabecular meshwork, Schlemms canal.

Because the eyes of monkeys are often used in studies of dynamics of aqueous humor, it seems important to ascertain how closely the aqueous outflow characteristics of monkey eyes may resemble those of human eyes. Previous experiments that we have done have shown that in enucleated human eyes the facility of aqueous outflow is reduced when the intraocular pressure is raised without deepening the anterior chamber and that a large proportion of the resistance to aqueous outflow in human eyes is removed by trabeculotomy. For comparison we have evaluated these factors in a similar manner in enucleated monkey eyes.

Methods

Eyes were removed from rhesus monkeys (Macaca mulatta) within one to four hours after they were killed during investigation unrelated to the eyes. The monkeys were adults of both sexes, with ages estimated from two to eight years. If not perfused immediately the eyes were stored in a moist environment at 4°C until approximately one-half hour prior to perfusion. Perfusion was started at one and one-half to 46 hours after death. The same apparatus and experimental procedure was used as in a parallel study which we have performed on enucleated human eyes. This was a modification of the constant pressure perfusion

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procedure of Bárány. Perfusion solution closely similar to Bárány's was employed, consisting of a sterile phosphate-buffered balanced salt solution containing glucose, micropore filtered before use. Routinely, the center of the cornea was trephined to provide an opening 5 mm. in diameter for access to the anterior chamber. Radial iridotomy was performed prior to perfusion in all experiments in this report to prevent abnormal deepening of the anterior chamber. When the eye was to be perfused, the opening in the cornea was closed by means of a previously described stainless steel fitting with a cannula built in for infusion into the anterior chamber.4 Trabeculotomies were performed with a cystotome through the 5 mm. hole in the cornea under direct microscopic observation.

Steady-state inflow rates were determined at 5, 10, 20, 30, 40, and 50 mm. Hg, as in the experiments in the human eyes, usually allowing ten minutes to establish steady-state conditions at 5 mm. Hg, and four to five minutes at each of the other pressure levels. Subsequently, the inflow rates were measured also as the pressure was reduced stepwise to 40, 30, 20, 10, and 5 mm. Hg.

Total outflow facility (C_t) at each pressure was calculated by dividing total steady-state flow by the intraocular pressure. Also we have calculated what we have called "two-step" outflow facility values (C_s) by dividing the difference in steady-state inflow rates at two pressure levels by the corresponding difference in intraocular pressure, because this is a method that has been used frequently for determining outflow facility in eyes of living monkeys.

**Results**

The mean values for C_t and C_s obtained from 17 rhesus monkey eyes at each of the pressure levels are given in Table I. These data indicate that as intraocular pressure was raised from 5 to 50 mm. Hg the total outflow facility decreased progressively from 0.824 to 0.299 µl/min./mm. Hg. Then as pressure was reduced from 50 to 100 mm. Hg the total facility of outflow increased. However, when pressure was lowered to 5 mm. Hg the facility became less again. It is evident that the total facility value obtained at each pressure in the rising sequence of pressures differed from that obtained with a decreasing sequence of pressures. Under both conditions the rate of change of total facility of outflow in monkey eyes appears to have been greatest in the lower portion of the pressure range, particularly between 5 and 20 mm. Hg.

Comparison of C_t and C_s values at each pressure in Table I shows that different values of facility of outflow are obtained by the two methods of calculation due to the change in facility of outflow with change in pressure. This is most noteworthy because the two-step method often utilized in experiments in living animals assumes that the same value for facility of outflow should be obtained by both methods.5

Results of measurement of total facility of outflow at each of the several intraocular pressures before and after trabeculotomy had been performed in three hours of the circumference in ten rhesus monkey eyes are shown in Table II. According to these results, the outflow facility increased markedly after trabeculotomy, and the relationship of outflow to intraocular pressure changed (Fig. 1). After trabeculotomy, as intraocular pressure was raised the outflow facility increased instead of decreasing as in the intact eye. The proportion of the resistance to aqueous outflow removed by the trabeculotomy was calculated making use of a formula that we have previously utilized in studies on human eyes and
Table II. Stepwise sequence of intraocular pressures, with corresponding mean values for total outflow facility ($C_t$) before and after trabeculotomy of one quadrant in 10 rhesus monkey eyes and with calculated values for the proportion of outflow resistance eliminated in the trabeculotomized quadrant.

<table>
<thead>
<tr>
<th>Pressures (mm Hg) (read down)</th>
<th>$C_t$ ($\mu$L/min./mm. Hg)</th>
<th>% Resistance eliminated in trabeculotomized quadrant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before trabeculotomy</td>
<td>After trabeculotomy</td>
</tr>
<tr>
<td>5</td>
<td>0.820</td>
<td>1.11</td>
</tr>
<tr>
<td>10</td>
<td>0.540</td>
<td>1.22</td>
</tr>
<tr>
<td>20</td>
<td>0.310</td>
<td>1.48</td>
</tr>
<tr>
<td>30</td>
<td>0.247</td>
<td>1.66</td>
</tr>
<tr>
<td>40</td>
<td>0.225</td>
<td>1.84</td>
</tr>
<tr>
<td>50</td>
<td>0.225</td>
<td>2.03</td>
</tr>
<tr>
<td>40</td>
<td>0.248</td>
<td>1.95</td>
</tr>
<tr>
<td>30</td>
<td>0.230</td>
<td>1.85</td>
</tr>
<tr>
<td>20</td>
<td>0.315</td>
<td>1.71</td>
</tr>
<tr>
<td>10</td>
<td>0.350</td>
<td>1.53</td>
</tr>
<tr>
<td>5</td>
<td>0.240</td>
<td>1.09</td>
</tr>
</tbody>
</table>

making the same assumptions as for the human eyes that under the conditions of the experiment the flow out of the anterior chamber was all in a radial direction, with no circumferential flow in Schlemm's canal, and that the resistance to outflow before trabeculotomy was the same in all quadrants.2-4 The calculated values (Table II) indicate that trabeculotomy eliminated 83 to 97 per cent of the resistance to outflow in the trabeculotomized quadrant.

By observing enucleated monkey eyes with the operating microscope during perfusion, one could see after trabeculotomy that in the trabeculotomized quadrant the conjunctiva and subconjunctival tissue became engorged with fluid, whereas in other perilimbal areas the appearance remained as before trabeculotomy, with no observable increase of outflow in quadrants other than the trabeculotomized quadrant. This was made even more conspicuous when fluorescein was added to the perfusion fluid, demonstrating obviously greater outflow through the trabeculotomized quadrant than through the other quadrants. Whether the fluid was passing entirely through normal outflow channels or was also passing transsclerally could not be determined under these experimental conditions.

A noteworthy technical problem encountered in these experiments was that in the monkey eyes small shallow cyclodialyses, not completely detaching ciliary muscle and ciliary body from the sclera, often occurred unavoidably when the trabeculotomies were being performed. Monkey eyes seemed to be more prone to this complication than human eyes. To evaluate the influence of these inadvertent cyclodialyses, the same type of dialysis was produced intentionally without trabeculotomy in two eyes in three hours of...
the circumference. Measurements by perfusion before and after showed that this type of cyclodialysis did not appreciably affect the facility of outflow and did not cause localized increased appearance of perfusion fluid externally.

Discussion

Enucleated rhesus monkey eyes with lens-iris diaphragm in neutral position (i.e., after iridotomy) generally have a higher facility of outflow than do human eyes, but they appear to be similar to human eyes in that they respond to increase in pressure with decrease in outflow facility. In both types of eyes the facility of outflow appears to be especially sensitive to change in intraocular pressure in the range 5 to 20 mm. Hg. For some obscure reason, monkey eyes appear to differ significantly from human eyes in their behavior during decreasing pressure. In the human eye as pressure is reduced from 50 to 40 mm. Hg the outflow facility decreases, but during further reduction of pressure the facility remains essentially constant and consistently less than that during a preceding sequence of rising pressures. In the monkey eye as pressure is reduced from 50 mm. Hg there is an increase rather than a decrease in outflow facility, and through the range of 50 to 20 mm. Hg the values remain greater than in the increasing sequence. Assuming that outflow is inertia free in monkey eyes as in human eyes and that change in resistance to flow with change in pressure implies that the aqueous outflow system is not geometrically rigid or fixed in either type of eye, we conclude that the outflow system of the monkey eye reacts to decreasing pressure in a manner appreciably different from that of the human eye.

The results of our experiments on enucleated monkey eyes in which iridotomy was performed to prevent artificial deepening of the anterior chamber by fluid infused into the anterior chamber indicate that under these conditions it is not valid to assume that the outflow facility is independent of pressure anywhere within the range from 5 to 50 mm. Hg or to expect the two-step perfusion technique to provide an accurate evaluation of total outflow facility. However, in perfusion experiments in vivo iridotomy usually has not been done, and we have found previously in enucleated human eyes that when the lens-iris diaphragm is intact and prevents flow from the anterior chamber to the posterior chamber, infusion of fluid into the anterior chamber causes artificial deepening of the anterior chamber and tends to maintain a more constant facility of outflow as the pressure is raised. To ascertain whether a similar mechanism operates in monkey eyes, more particularly whether such a mechanism operates in the eyes of living monkeys, will require further investigation. Some evidence already published has supported the assumption that facility of outflow is constant within the pressure range and under the conditions of most animal aqueous perfusion studies, but other evidence has indicated that facility may decrease with increased pressure in the eyes of monkeys.

Our dissection and perfusion experiments indicate that in eyes of both rhesus monkeys and human beings most of the resistance to aqueous outflow depends upon the integrity of the trabecular meshwork and the intimately associated inner wall of Schlemm's canal. In the monkey eye an even larger proportion of the resistance is related to this tissue than in the human eye. Our results indicate 83 to 97 per cent of the outflow resistance is eliminated in the trabeculotomized portion in the monkey eye compared to approximately 75 per cent in human eyes.

Edwards and associates and Dannheim and Bárány have reported that when trabeculotomy was performed on live monkeys and measurements were made weeks later there was little or no evidence of increase in facility of outflow. Considering the immediate effects which we have found from trabeculotomy in enucleated eyes, we think most likely the reported
lack of increase in outflow facility was due to postoperative reaction and healing.

Findings from experimental cannulation of Schlemm's canal of monkey eyes and measurement of pressure differential between the anterior chamber and the canal by Perkins\textsuperscript{12} and by Sears\textsuperscript{13} have been interpreted as indicating that only a small proportion of the total resistance to outflow is located between anterior chamber and Schlemm's canal in these eyes. An explanation for the difference in conclusions arrived at by the cannulation and the trabeculotomy procedures will presumably eventually be found with better knowledge of the special properties of trabecular meshwork and the walls of Schlemm's canal. Many subtleties are still to be explored. We do not yet know the effect on outflow resistance of holding the canal open with a cannula or how easily the inner wall of the canal may be injured by a cannula, and we have not established whether there may be inadvertent but significant injury of the outer wall by our trabeculotomy technique.

REFERENCES