The rate of aqueous humor formation in buphthalmic rabbit eyes

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Aqueous humor turnover (K _oat min.⁻¹) for buphthalmic rabbits was 0.003 ± 0.0015 at steady state. This is equivalent to a rate of aqueous humor formation of 2.0 µL/min., and is only 46 per cent of the value (4.2 µL/min.) obtained in normal rabbits of the same strain.

Reports of spontaneous buphthalmos in rabbits date back to Pichler¹ in 1909. Shortly thereafter, Vogt² bred two such animals and obtained three young, all of which developed the condition. These studies suggested that the disease could be genetic in origin, and further studies by Geri,³ and Hanna and associates,⁴ showed that transmission was by means of an autosomal recessive gene.

The gross ocular enlargement which characterizes buphthalmia is accompanied by fibrosis of the filtering angle⁴⁻⁶ and by a decrease in facility ("C") of aqueous humor outflow.⁶⁻⁷ Kolker and co-workers⁷ performed repeated tonographic measurements on a series of rabbits that had this hereditary disease. The animals were followed from the age of three months to one year. These authors found that the "C" values were depressed at the time of their first measurement (at 3 months) and remained depressed throughout the entire period of observation. However, the pressure of some of these enlarged eyes can be in the normal range even when the same eye has a reduced facility of outflow.⁸ Similarly, the ocular pressure of the buphthalmic rabbits studied by Kolker and associates often was not as high as one might expect for animals with such a low value of "C," assuming they had a normal rate of aqueous humor inflow. As Kolker and his colleagues mentioned, these data suggest that some other factor, perhaps a reduced inflow, could act as a compensatory pressure mechanism. Some evidence that the inflow was decreased in buphthalmia was obtained by Rochon-Duvigneaud,⁹ who found a delayed time of fluorescein appearance in the one buphthalmic eye examined.

To determine directly and quantitatively the rate of aqueous humor turnover, K _oat, in buphthalmic rabbit eyes, the studies reported below were undertaken. The inflow rate in these eyes was then compared with the rates in normal rabbit eyes which had been previously measured¹⁰ with the same technique.⁹ In order to determine a relationship between the two, a comparison of inflow rates and the eye pressure was made. The episcleral venous...
pressure was also measured in some buphthalmic animals to help localize the physiologic block to outflow.

Materials and methods

Adult New Zealand White rabbits were obtained from the National Institutes of Health Animal Production Section. The animals were anesthetized by pentobarbital sodium intravenously (25 to 50 mg per kilogram) and then snugly wrapped in a towel to inhibit movement of the limbs. Two thousand units of heparin was given intravenously and proparacaine HCl applied locally to the eye before inserting the needles into the anterior chamber. The steady state aqueous humor formation was measured by inulin 14C, as described previously. Episcleral venous pressure ("head-on") was measured manometrically with a polyethylene cannula inserted against the venous flow. Recordings of the latter pressure were considered acceptable, provided an injection of saline through the microcannula failed to show leaks, and a continuous venous backflow into the cannula occurred when the closed end of the transducer was opened.

Results

The aqueous humor turnover rate ($K_{out}$), intraocular pressure (IOP), and aqueous humor volume for each of the buphthalmic eyes appear in Table I, along

![Graph](https://iovs.arvojournals.org/data/journals/iovs/932995/)  
Fig. 1. Relationship of steady state intraocular pressure to aqueous humor formation in individual buphthalmic eyes in vivo.

with the calculated inflow ($\mu$L per minute). The average $K_{out}$ was 0.003 (range 0.001 to 0.006); the average anterior chamber volume was 701 $\mu$L (range 485 to 896); and the average IOP was 37.44 mm Hg (range 18.20 to 68.07). The average calculated inflow per minute ($K_{out}$ times AC volume) was 2.01 $\mu$L per minute, and varied from 0.69 to 3.34 $\mu$L per minute.

A plot of the rate of aqueous humor turnover vs. the ocular pressure is presented in Fig. 1. Although the inflow appears to be slightly decreased at high IOP, the scatter of points shown in this figure illustrates the lack of correlation between these variables. An attempt to demonstrate a statistically significant relationship between the two by a bivariate correlation analysis failed ($r = 0.385$).

The episcleral venous pressures were measured in 6 eyes of another series of 5 buphthalmic rabbits (Table II). The

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**Table I. Aqueous humor turnover rates in buphthalmic rabbits**

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>AC volume</th>
<th>$K_{out}$</th>
<th>Average IOP</th>
<th>Calculated inflow in $\mu$L per min.</th>
<th>$K_{out} \times AC$ volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>694</td>
<td>0.001</td>
<td>27.34</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>485</td>
<td>0.006</td>
<td>35.20</td>
<td>2.91</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>893</td>
<td>0.003</td>
<td>18.20</td>
<td>2.68</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>668</td>
<td>0.005</td>
<td>22.55</td>
<td>3.34</td>
<td></td>
</tr>
<tr>
<td>5 OS</td>
<td>827</td>
<td>0.002</td>
<td>46.14</td>
<td>1.65</td>
<td></td>
</tr>
<tr>
<td>5 OD</td>
<td>767</td>
<td>0.002</td>
<td>40.20</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>896</td>
<td>0.002</td>
<td>18.95</td>
<td>1.93</td>
<td></td>
</tr>
<tr>
<td>7 OS</td>
<td>606</td>
<td>0.002</td>
<td>62.60</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td>7 OD</td>
<td>656</td>
<td>0.004</td>
<td>46.40</td>
<td>2.62</td>
<td></td>
</tr>
<tr>
<td>8 OS</td>
<td>724</td>
<td>0.002</td>
<td>68.07</td>
<td>1.45</td>
<td></td>
</tr>
<tr>
<td>8 OD</td>
<td>520</td>
<td>0.004</td>
<td>25.95</td>
<td>2.08</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>37.44</td>
<td>2.01</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>±136</td>
<td>±0.0015</td>
<td>±17.05</td>
<td>±0.80</td>
<td></td>
</tr>
</tbody>
</table>

*AC: Anterior chamber.  
$K_{out}$: Per cent of anterior chamber fluid which leaves the AC per minute.  
IOP: Intraocular pressure in the anterior chamber.  
OS: Left eye; OD: right eye.
values varied from 7.0 to 13.5 mm. Hg and averaged 9.3 mm. Hg.

Discussion

The rate in inulin C\textsubscript{14} turnover appears reasonably accurate as an estimate of aqueous humor turnover and gives a mean value for $K_{out}$ in normal rabbit eyes of 0.018 (min\textsuperscript{−1}). This value is in agreement with that obtained by other techniques.\textsuperscript{10-14} It appears that, in normal eyes, the results obtained by this technique are not complicated by considerations of undue degrees of inulin diffusion from the anterior chamber.\textsuperscript{9} It is possible, however, that the diffusional situation in the buphthalmic rabbits could be different. We may reason then, that if the outward diffusion rate of inulin in the buphthalmic rabbit eye was less than the already small amount in the normal rabbits, $K_{out}$ could not be significantly affected; if the differential rates in both types were the same, comparisons would be ideal; should the differential rate in the buphthalmic eye be greater than in the normal eyes, then the measurement of $K_{out}$ would overestimate the actual rate of aqueous humor turnover. The values of $K_{out}$ reported here therefore represent the upper limit of aqueous humor turnover or inflow.

The ocular pressure is a function of elasticity of the coats and internal volume; the latter is influenced, among other things, by aqueous humor inflow and outflow. Any impediment to outflow would raise the ocular pressure in the absence of a corresponding decrease in inflow. Under the conditions of the experiments reported here, the average inflow rate of the buphthalmic eyes was abnormally low. The mean inflow in this series of hydrophthalmic eyes was 46 per cent of the normal (2.0 $\mu$L per minute as opposed to 4.2 $\mu$L per minute). The decreased aqueous formation may in part account for the nearly normal pressures seen in some of these eyes. At present the reason for the decreased inflow in the buphthalmic eye remains undetermined, but possible explanations include ciliary body atrophy,\textsuperscript{4, 5} other congenital abnormalities, secondary pathologic changes due to high pressure, or alternatively some regulatory mechanism which decreases the inflow as the ocular pressure increases. In regard to the latter possibility, no statistically significant correlation was obtained when individual IOPs were compared with the corresponding rate of aqueous humor formation. The general unavailability of these animals, however, precluded a greater number of observations which could possibly alter the statistics of these findings. Nevertheless, the lack of a correlation between aqueous humor turnover and pressure suggests that factors other than the facility of outflow may also be involved in the regulation of ocular pressure.

Previous studies on buphthalmic rabbit eyes\textsuperscript{4-5} have emphasized the anatomic changes at the angle and have postulated that they constitute at least one site of obstruction to aqueous humor outflow. The measurements on the episcleral venous pressure reported here (9.3 mm. Hg) support this concept. Since these pressures are in the normal range,\textsuperscript{13, 16} they indicate that one site of obstruction of aqueous humor outflow lies between the trabeculum and the episcleral veins.

REFERENCES

3. Geri, G.: Considerazioni e recerche sull’