Aqueous Humor Inflow in Normal and Glaucomatous Avian Eyes*

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A new fluorometric method has been employed to measure inflow of aqueous humor (F) in normal and glaucomatous avian eyes. Most inflow determinations in previous studies have involved severe disturbance to the eye, or else F has been derived indirectly, as a function of outflow facility (C) or intraocular pressure (IOP). The procedure used in this study is readily accomplished, even with a large series of subjects, and F measurement involves no insult to the eye or extrapolations from parameters whose relationship to F is not fully understood. After inflow measurements had been completed, the same experimental animals were subjected to independent determinations of volume of the aqueous space, IOP, C, and several dimensional parameters, thus permitting a comprehensive assessment of aqueous fluid dynamics during eye development.

Aqueous flow in normal adult domestic chickens was found to be 12.9 ± 1.93 µl/min; IOP in the same eyes was 11.1 ± 0.4 mmHg, C was 2.03 ± 0.24 µl/min/mm, and volume of the aqueous space was 104.1 ± 4.5 mm³. The same parameters were measured on abnormal eyes at several stages during the pathogenesis of avian glaucoma. The aqueous space was greatly reduced, to about one-third normal volume, in these glaucomatous eyes. Aqueous flow was less than half the normal adult value. In confirmation of previous findings, C was reduced, IOP was elevated, and the glaucomatous eyes were greatly enlarged. The pattern of development of the several lesions associated with avian glaucoma suggests a puzzling question: how can one explain eye enlargement which takes place in the absence of elevated IOP, and at a time when F is low and C is impaired? A search for the primary lesion in avian glaucoma continues.

1. Introduction

The physiological and morphological status of the anterior segment of the eye depends on intraocular fluids, and is of critical importance in assuring the integrity of the cornea, hence the visual capability of the eye. Of the several parameters associated with ocular fluid dynamics, aqueous flow has been the most difficult to measure except by indirect assessments based on values obtained for intraocular pressure and/or outflow facility. This poses an embarrassing problem, for the relationship of these parameters to one another is not clearly understood, although it seems probable that they are variables linked by a delicate homeostatic control mechanism. Thus there has been a need for methods of independently assessing the several aspects of ocular fluid dynamics, and in ways which would involve little or no disturbance to normal homeostatic controls.

We here report measurements of aqueous inflow obtained by use of a new fluorometric technique (Lauber, Boyd and Boyd, 1969). Independent determinations of intraocular pressure, outflow facility, volume of the aqueous space, and several other dimensional parameters were conducted on the same subjects.

The experimental subjects were domestic chickens; the avian eye is an especially favorable system for investigations of aqueous fluid dynamics. As well, chicks reared

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under continuous light develop a glaucoma-like condition characterized by eye enlargement, elevated intraocular pressure, impaired outflow facility, reduced corneal curvature, and eventual blindness (Jensen and Matson, 1957; Lauber, McGinnis and Boyd, 1966; Lauber and McGinnis, 1966; Lauber, Boyd and Boyd, 1970). We have investigated aqueous inflow at several critical stages during genesis of this condition, in an effort to elucidate the etiology of this light-induced avian glaucoma.

2. Materials and Methods

White Rock male chicks were reared from hatching under a diurnal photoperiod of 14 hr light, 10 hr darkness/day (14L/10D), or under continuous light (24L/0D). Food (commercial chick starter) and water were available at all times, and heat was provided during the early weeks by electric brooders which emitted no light.

Under Nembutal general anesthesia, and topical Ophthaine, the eyelids were retracted and the pupil brought into the field of a fiber optic probe device designed to both illuminate the anterior chamber and pick up light from it. Sodium fluorescein was then injected via a previously placed wing vein cannula. The initial dose of 1 cm$^3$/kg body weight of 10% aqueous solution was supplemented by infusion of the dye at the rate of 6.0 cm$^3$/hr. in order to assure a continuing plasma to aqueous concentration gradient.

As the dye entered the aqueous space, it was illuminated at 365 nm, the excitation wavelength of fluorescein, by ultraviolet light from a high pressure mercury arc lamp. The induced green fluorescence was monitored via optic fiber probe on an ISCO spectroradiometer, set to record energy of light received in the spectral bandwidth of 535±15 nm. The increasing level of fluorescein in the aqueous humor with time was used to calculate aqueous inflow rate ($F$), according to a method previously described (Lauber, Boyd and Boyd, 1969). Paired experimental setups made it possible, in most cases, to determine inflow simultaneously in both eyes.

The duration of an aqueous inflow determination was approximately 15 min from the time of intravenous injection of the dye. The eyes remained undisturbed during this period, except for retraction of the eyelids. The head of the subject was shielded by a black plastic cover to protect the eyes from extremes of illumination.

When inflow determinations had been completed, the subject was prepared for measurement of intraocular pressure (IOP) and determination of outflow facility ($C$). With the head immobilized in a stereotaxic device, each eye was cannulated into the anterior chamber, and IOP was recorded via a closed manometer system involving a pressure transducer and a Beckman Dynograph recorder, as previously described (Lauber, Boyd and Boyd, 1970). When IOP had stabilized, usually within 45-60 min after cannulation, constant rate infusion of saline was initiated and continued until a new pressure plateau was achieved. The change in pressure induced by a given infusion rate was used to calculate $C$. Three separate infusions, at different low rates, yielded an average $C$ value for each eye.

At the end of an experiment, the cannulae were removed and the subject was allowed to reform the anterior chamber during the ensuing 24 hr. The animal was then killed by cervical dislocation. The eyes were enucleated, trimmed of fat and extraocular muscles, weighed, and photographed in front and lateral views.

The eye photographs, which incorporated a millimeter scale, were used to measure ocular dimensions. Calculation of the volume of the aqueous space was based on the assumption that the cornea bounds a segment of a perfect sphere. Measured corneal dimensions were introduced into the formula,

$$V = \frac{1}{6} \pi h (k^2 + 3r^2),$$

where $h$ is corneal height (mm), $r$ is corneal radius (mm) and $V$ is volume of the aqueous space (mm$^3$). This figure is in turn needed for calculation of aqueous inflow by the fluorometric method used here (Lauber, Boyd and Boyd, 1969).
3. Results

*Aqueous fluid dynamics during development in normal and glaucomatous eyes*

The avian eye has a substantially higher rate of aqueous flowthrough than has been reported for several mammals (Table I). At 6 weeks of age, aqueous inflow ($F$) was $11.2\pm1.0\ \mu l/min$ for the normal eye (Table II). At the same age, IOP was $10.6\pm0.3\ \text{mmHg}$ and $C$ was $1.47\pm0.19\ \mu l/min/mmHg$. Aqueous inflow increased steadily during the growing period, to a maximum of $16.6\pm1.7$ recorded at 12 weeks, and

**Table I**

*Aqueous inflow rate in several species*

<table>
<thead>
<tr>
<th>Experimental subject</th>
<th>Inflow $\mu l/min$</th>
<th>Method</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>2.0–4.0</td>
<td>Fluorometric</td>
<td>Goldman (1950)</td>
</tr>
<tr>
<td>Monkey</td>
<td>1.2–2.0</td>
<td>$^{131}I$ in blood after infusion of $^{131}I$ into eye</td>
<td>Holm and Kraka (1968)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>2.1–3.5</td>
<td>$F = (P_o - P_e)C$</td>
<td>Bill and Bárany (1966)</td>
</tr>
<tr>
<td>Cat</td>
<td>13.2</td>
<td>$^{14}C$ inulin dilution</td>
<td>Bill (1967)</td>
</tr>
<tr>
<td>Chicken</td>
<td>10–15</td>
<td>Fluorescence in aqueous</td>
<td>Macri (1967)</td>
</tr>
</tbody>
</table>

**Table II**

*Aqueous fluid dynamics and dimensional parameters in developing avian eyes*

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>$F$ (µl/min)</th>
<th>IOP (mmHg)</th>
<th>$C$ (µl/min/mm)</th>
<th>Aqueous volume (mm$^3$)</th>
<th>Eye wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>11.2±1.0</td>
<td>10.6±0.3</td>
<td>1.47±0.19</td>
<td>41.1±1.2</td>
<td>1.8±0.0</td>
</tr>
<tr>
<td>8</td>
<td>9.9±1.3</td>
<td>10.8±0.5</td>
<td>1.29±0.10</td>
<td>57.0±2.8</td>
<td>2.3±0.0</td>
</tr>
<tr>
<td>10</td>
<td>13.3±2.0</td>
<td>12.0±0.6</td>
<td>1.79±0.28</td>
<td>67.2±2.7</td>
<td>2.3±0.1</td>
</tr>
<tr>
<td>12</td>
<td>16.6±1.7</td>
<td>10.8±0.4</td>
<td>2.05±0.21</td>
<td>85.4±3.6</td>
<td>2.7±0.1</td>
</tr>
<tr>
<td>20</td>
<td>12.9±1.9</td>
<td>11.1±0.4</td>
<td>2.03±0.24</td>
<td>104.1±4.5</td>
<td>3.4±0.1</td>
</tr>
</tbody>
</table>

Note: Each value represents the mean±s.e.m. for the number of experimental subjects indicated in parentheses. Methods employed to derive the several parameters are described in the text; $F$ is aqueous inflow, IOP is intraocular pressure, $C$ is the coefficient of outflow facility, Aq. vol. is volume of the aqueous space.
remained in that range thereafter. At this age, White Rock chickens have almost reached mature body size, and are close to sexual maturity. Intraocular pressure at 12 weeks was 10.8±0.4 mmHg, and C was 2.05±0.21 µl. These mean values for IOP and C in 14L/10D eyes of this series are within the normal range for these ages, as previously established (Lauber, Boyd and Boyd, 1970).

Aqueous inflow was reduced in glaucomatous eyes: at 20 weeks of age, the mean F value was 5.3±1.3 µl, less than half the normal inflow rate. The IOP in these adult subjects was 21.1±1.9 mmHg, almost twice the normal value and C at the same age was 0.80±0.12 µl, as compared with 2.03±0.24 for control eyes (Fig. 1). In eyes tested during the “pre-glaucoma” period, at 10 weeks of age, F was already reduced and C was impaired, but IOP, the traditional diagnostic characteristic of glaucoma, was normal.

**Eye dimensions**

During the period of rapid body growth, eye weight of normal (14L/10D) birds increased from 1.8±0.0 g at 6 weeks of age, to 3.4±0.1 g at 20 weeks. Volume of the aqueous space, as determined by the geometric technique, was 41.1±1.2 mm³ at 6 weeks, and increased steadily to 104.1±4.5 mm³ at 20 weeks of age (Table II).

Birds reared under 24L/0D developed pathological eye enlargement discernible during the first few weeks after hatching, as reported for a previous series of similar chicks (Lauber and McGinnis, 1966). At 10 weeks, 24L/0D eye weight was 3.3±0.1 g, as compared to 2.3±0.1 g for normal birds. By 20 weeks, 24L/0D eyes weighed 5.4±0.1 g, almost 60% more than normal eyes. The previously described reduction in corneal curvature and shallow anterior chamber angle (Lauber and McGinnis, 1966; Frankelson, Lauber and Boyd, 1969) were again evident in this series of glaucomatous eyes. Volume of the aqueous space in 24L/0D eyes was 26.9±1.9 mm³ at 10 weeks, and remained small at 20 weeks: the mean value of 34.8±3.3 mm³ represents approximately one-third normal size (Fig. 1).

**Fig. 1.** Aqueous fluid dynamics and dimensional parameters in glaucomatous (20 week old) and pre-glaucomatous (10 week) avian eyes. The control value in each case is represented by an unshaded bar, the vertical lines indicating one S.E. For each pair of bars, values and units are indicated on the ordinate immediately to the left of the pair, and are on the same scale at 20 weeks as at 10 weeks. Abbreviations employed, and units of measurement are the same as in Table II.
4. Discussion

The vegetative physiology of the vertebrate eye has been incompletely understood, largely because the investigator has been obliged to depend on indirect and mutually interdependent measurements of aqueous flow and intraocular pressure. Rational treatment of the functional disorders of pressure and flow has thus, of necessity, been empirical. Much of what is known about these parameters, however, supports the view that the eye is maintained by a delicate homeostatic mechanism(s). Experimental intervention involving even a slight insult to any aspect of the pressure-flow relationship might be expected to cause a compensatory response, so that the investigator may in fact be measuring artifacts of unknown proportions rather than true physiological entities.

**Method for aqueous inflow determinations**

The fluorometric method we have used to measure aqueous inflow appears to be capable of resolving some of the difficulties of earlier attempts to understand this aspect of ocular fluid dynamics. Dilution methods have been used by others to measure aqueous flow, but the instrumentation we have employed is unique, and permits greater sensitivity with an ease of measurement that has not previously been available. This present method remains an indirect one, but our inflow determinations are at least independent of intraocular pressure and outflow facility measurements. In this study we have routinely determined inflow during a 15-min period, in eyes completely undisturbed except for retraction of the lids. The method is readily transferred for use on humans, although, in our preliminary experience, a longer time is required for a determination because the inflow rate of humans is slower than for the avian eye.

**Volume of the aqueous space**

Aqueous flow may be derived from the changing concentration of dye or other substance in the aqueous humor only if the size of the fluid-containing compartment is known. The geometric method used here to estimate volume of the aqueous space has been compared with a dye dilution method: volumes obtained on the same eyes by the 2 methods agreed reasonably well, usually being within 10% of one another (Lauber, Boyd and Boyd, 1969). These volume figures, which presumably represent something more than anterior chamber volume, are nevertheless of the same order of magnitude as anterior chamber volumes reported for several mammalian eyes: chicken, 110–115 mm$^3$ (this report); rabbit, 250 mm$^3$ (Langham and Rosenthal, 1966); cat, 800 mm$^3$ (Langham, 1960); man, 194 mm$^3$ (Heim, 1941).

**Aqueous flow rate in the avian eye**

The rate of aqueous humor inflow of the normal adult chicken eye, as reported here, is approximately 10% of the total aqueous volume/min. This value is several times greater than the 1.7–2%/min rate for leghorn hens reported by Bárány (1951), who used para-amino-hippuric acid as a test substance. The fluorometric technique used for our inflow measurements leaves the eye undisturbed, whereas Bárány’s method involved taking aqueous samples at 60-min intervals by corneal puncture. Our experience, with cannulation of the anterior chamber for IOP determinations, has been that most eyes require 45–60 min to stabilize IOP after cannulation. This procedural difference may explain the discrepancy between the 2 reports.
A rather rapid exchange of fluid in the anterior segment of the avian eye is implied by the high outflow facility value. We have not measured episcleral venous pressure (EVP) in the chicken eye, but if it is assumed that EVP is 1.0 mmHg less than IOP, then net flow calculated from outflow facility values would agree well with the mean aqueous inflow rates reported here.

Avian glaucoma

With this report we add several more parameters to the characterization of light-induced avian glaucoma. However, the pathogenesis of this condition is still puzzling, nor can we yet identify the primary lesion. Eye enlargement precedes by many weeks the pathological rise in IOP. An increase in eye weight can be detected several weeks before outflow facility is impaired. Reduction in corneal curvature results in a shallow chamber angle, but these changes do not precede eye enlargement. Iridectomy did not prevent the development of light-induced avian glaucoma, as one would expect if pupillary block had contributed to iris bombé (Frankelson, Lauber and Boyd, 1969). Thus it seems clear that light-induced avian glaucoma is not due to an angle closure mechanism, although the angle is often physically blocked late in the course of the disease. We have searched for but found no evidence of hyperplasia which might explain the early enlargement of the globe.

The earliest 24L/OD inflow data reported here are for chicks 10 weeks of age. At this time, F was greatly reduced, and remained low at 20 weeks, when glaucomatous birds were again tested. At 10 weeks, C was also low, but IOP was not elevated. This suggests that reduced flowthrough is an aspect of the early pathogenesis of light-induced avian glaucoma. However, we still have no explanation for the early eye enlargement, nor is it clear why IOP should rise only rather late in the course of the disease process.

It was not possible, with the equipment used in this study, to obtain reliable flow measurements on very young chicks. Further inflow studies are underway, utilizing redesigned apparatus and a more sensitive spectroradiometer than was previously available to us. We are hopeful that reliable flow measurements during the early weeks may provide further clues about the etiology of light-induced avian glaucoma.

REFERENCES