soon after parturition. The calcium distribution between CSF and plasma does not achieve adult values until after birth.\(^2\) Magnesium, in contrast, has an adult AH/P distribution by 137 days gestation in the goat. Adult CSF/P for magnesium is achieved by 48 days gestation in the sheep\(^3\) and by second trimester gestation in the monkey.\(^4\) The adult AH/P and CSF/P for calcium and magnesium appear independent of changing plasma concentrations.\(^5\) \(^6\) \(^7\) \(^8\)

Anterior chamber AH has a higher PCO\(_2\) than arterial blood. The pH, Po\(_2\), and HCO\(_3\)-in AH are less than arterial blood. Metabolic activity of the lens and cornea may contribute to the elevated PCO\(_2\), \([H^+]\), and reduced Po\(_2\) of AH. The lowered HCO\(_3\)-may be a consequence of buffering activity secondary to products from lens and cornea. Similar results have been reported for anterior chamber AH and CSF in man.\(^9\) There are no acid-base data available for posterior chamber AH in goat or man.

While the acid-base status of AH suggests significant modification of primal plasma filtrate, it is not incongruous with the hypothesis of an active HCO\(_3\)-secretion into the posterior chamber.\(^10\) Electrolyte composition and acid-base status of AH, particularly the posterior chamber fluid, prior to 137 days gestation in the goat warrants investigation.

The authors thank J. Evans, W. Bunnell III, N. L. Murtha, and B. Wallis for excellent assistance and Dr. C. W. Leffler for his reading of the manuscript. This research was conducted under the guiding principles in the care and use of animals approved by the Council of the American Physiological Society and the National Institutes of Health.

From the Department of Physiology, College of Medicine, University of Florida, Gainesville, Fla. 32610. Supported in part by National Institutes of Health Grant HE 10834-06 and Florida Heart 73AG17. Submitted for publication June 10, 1975. Reprint requests: Dr. S. Cassin, Department of Physiology, College of Medicine, University of Florida, Gainesville, Fla. 32610.

**Key words:** aqueous humor, electrolytes, acid-base status, fetus, neonate, Gibbs-Donnan equilbrium.

**REFERENCES**


**Aqueous and serum lysozyme values in experimental uveitis in rabbits. Howard H. Tessler, and Robert S. Weinberg.**

The purpose of this study was to determine what happens to normal aqueous lysozyme levels during experimentally induced uveitis. Horse-serum-induced uveitis increased the average rabbit aqueous lysozyme value (0.9 μg per milliliter) to 13.8 μg per milliliter, but did not elevate the average serum lysozyme value (4.0 μg per milliliter). These findings suggest that ocular inflammation alone is not sufficient to elevate the serum lysozyme level.

Hilding, in 1935,\(^1\) attempted to measure aqueous humor lysozyme in pigs' eyes and concluded that there was little or no lysozyme present. We attempted to determine (1) if normal aqueous contains lysozyme, (2) if experimentally induced uveitis results in elevated aqueous lysozyme, and (3) if elevated aqueous levels are mirrored by elevated serum levels.

**Materials and methods.** Albino male New Zealand rabbits between 2 and 4 kilograms were used in all experiments. Aqueous humor samples of 0.1 ml. were obtained by anterior chamber paracentesis with a 25-gauge needle on a 1 ml. tuberculin syringe after the rabbits had been anesthetized with intraperitoneal pentobarbital sodium. Three to 5 ml. of blood was obtained by venesection from an ear vein after the ear had been swabbed with xylol. Serum was obtained after centrifugation of whole blood. Both aqueous

\(^{(1)}\) Hilding, in 1935,\(^{1}\) attempted to measure aqueous humor lysozyme in pigs' eyes and concluded that there was little or no lysozyme present.
Table I. Normal values for aqueous and serum lysozyme in albino male rabbits

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µg/ml.)</td>
<td>4.1 ± 1.9</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td>Number</td>
<td>15 rabbits</td>
<td>30 eyes</td>
</tr>
<tr>
<td>Range</td>
<td>1.2 - 8.1</td>
<td>0.0 - 2.6</td>
</tr>
</tbody>
</table>

Table II. Aqueous lysozyme at one hour and 48 hours after paracentesis

<table>
<thead>
<tr>
<th></th>
<th>One hour</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µg/ml.)</td>
<td>3.8 ± 1.7</td>
<td>1.4 ± 0.7</td>
</tr>
<tr>
<td>Number</td>
<td>6 eyes</td>
<td>5 eyes*</td>
</tr>
<tr>
<td>Range (µg/ml.)</td>
<td>2.0 - 6.8</td>
<td>0.8 - 2.8</td>
</tr>
</tbody>
</table>

*One eye with a persistent hyphema and an aqueous lysozyme of 5.2 µg per milliliter was not included.

Table III. Lysozyme during acute immune uveitis

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse serum</td>
<td>Normal saline control</td>
<td></td>
</tr>
<tr>
<td>A. In previously nonimmune albino male rabbits:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (µg/ml.)</td>
<td>6.0 ± 2.2</td>
<td>13.8 ± 10.2</td>
</tr>
<tr>
<td>Number</td>
<td>6 rabbits</td>
<td>6 eyes</td>
</tr>
<tr>
<td>Range (µg/ml.)</td>
<td>2.9 - 9.6</td>
<td>1.2 - 28.0</td>
</tr>
<tr>
<td>B. In previously immune albino male rabbits:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>Aqueous</td>
<td></td>
</tr>
<tr>
<td>Mean (µg/ml.)</td>
<td>4.2 ± 0.9</td>
<td>12.6 ± 6.3</td>
</tr>
<tr>
<td>Number</td>
<td>5 rabbits</td>
<td>10 eyes (5 rabbits)</td>
</tr>
<tr>
<td>Range (µg/ml.)</td>
<td>3.2 - 5.6</td>
<td>1.1 - 20.2</td>
</tr>
</tbody>
</table>

*One eye with a persistent hyphema had an aqueous lysozyme of 7.2 µg per milliliter and was not included.

Humor and serum were stored at 4°C until analyzed.

Aqueous and serum lysozyme were determined with a Coleman spectrophotometer according to the method of Litwack as modified by Prockop and Davidson. The Lysozyme Assay Kit (Worthington Biochemical Corporation) was used in all measurements, with the substrate Micrococcus lysodeikticus, 10 mg per 100 ml in 0.06 M sodium phosphate buffer, pH 6.2. Reactions were run at 25°C with optical density readings taken at 30 second intervals for 3 minutes at 550 nm. A commercially available (Worthington Biochemical Corporation) egg white lysozyme preparation containing 40 µg of lysozyme per milliliter was used to prepare a standard curve. Lysozyme values were then determined by plotting changes in optical density over the three-minute test period for the test serum and comparing the result to the standard curve. Results are reported in micrograms of egg white lysozyme per milliliter of test solution.

In three rabbits, anterior chamber paracentesis was repeated bilaterally one hour after initial bilateral paracentesis and venesection. Forty-eight hours later venesection and bilateral anterior chamber paracentesis were repeated.

After anterior chamber paracentesis 0.1 ml. of horse serum (lysozyme value, 2.9 µg per milliliter) was injected intravitreally through the pars plana of the left eye of six rabbits, and 0.1 ml. of normal saline was injected intravitreally in the same manner in the right eye of the same six rabbits. These rabbits had no evidence of pre-existing antihorse serum antibodies. Twelve days later the rabbits were examined at the slit lamp, and the amount of ocular inflammation was graded according to the method of Hogan, Kimura, and Thygeson. Serum and aqueous lysozyme levels were determined by the method previously described.

Five rabbits were systemically sensitized to horse serum by an intravenous route as described by Wong, Anderson, and McMaster. Three months later 0.1 ml. of horse serum was injected intravitreally through the pars plana into each eye. The rabbits were examined at the slit lamp after 48 hours, and the amount of inflammation...
was graded. Aqueous and serum samples were then obtained.

**Results.** Normal values for serum and aqueous lysozyme are presented in Table I.

Aqueous lysozyme levels one hour and 48 hours after paracentesis in normal rabbits are presented in Table II. Mean aqueous lysozyme values one hour after paracentesis were significantly different at the $P = 0.05$ level of confidence from the normal levels. However, mean aqueous lysozyme values 48 hours after a second paracentesis were not significantly different from normal values.

The mean serum lysozyme value at 12 days during acute immune uveitis (Table III) is not significantly different than Student's $t$-distribution at $P = 0.05$ from the mean for the normal group. However, there is a statistically significant difference between the normal aqueous lysozyme level and that 12 days after intravitreal horse serum. There is no such difference between the normal aqueous lysozyme level and that 12 days after intravitreal saline when one eye which had a persistent hyphema was not included.

Rabbits systemically sensitized to horse serum showed no difference from the normal in serum lysozyme levels before or after intravitreal challenge with horse serum (Table III). Aqueous values after intravitreal challenge were markedly elevated but similar to the mean value in the non-immunized rabbit eyes that had received intravitreal horse serum.

**Discussion.** These experiments demonstrate that lysozyme is present in normal rabbit aqueous and that methods currently employed to determine serum levels may be used without modification to measure aqueous levels. Lysozyme in secondary aqueous, present one hour after paracentesis, approached the serum level. This may reflect reflux of blood and plasma proteins into the anterior chamber.

Once the presence of lysozyme in normal aqueous had been demonstrated, the question of the relationship between aqueous lysozyme and ocular inflammation arose. To prove this problem and the additional possibility of experimentally elevating aqueous lysozyme, ocular inflammation was induced by the monocular intravitreal injection of the foreign protein horse serum. As a control normal saline was injected into the opposite eye. While the serum lysozyme was not changed significantly after intravitreal injection of horse serum, the lysozyme level in the aqueous of eyes that had received horse serum was significantly higher than the normal level and also significantly higher than the control eyes. It was unchanged in the control eyes that received saline alone.

In the final experiment an intraocular active Arthus reaction was created by intravitreal injection of horse serum in rabbits systemically sensitized to horse serum. The serum lysozyme values remained normal, but aqueous values were significantly elevated after this intravitreal challenge. This elevation was similar to that produced in the first experiment and may imply a similar degree of inflammation.

The sources of serum lysozyme consist primarily of polymorphonuclear leukocytes, monocytes, and their precursors. At present, no difference in the lysozyme produced by each of these cell types has been determined. We assume that aqueous lysozyme comes from the same sources. Clinical and histologic evidence confirmed the presence of increased lysozyme in those eyes with more cellular reaction; our clinical evaluation of the severity of the anterior chamber reaction correlated well with the aqueous lysozyme levels (Fig. 1). Thus, in the aqueous, as elsewhere in the body, lysozyme appears to correlate with the extent of the inflammatory response.

The fact that an inflammatory focus in the eye did not elevate the serum lysozyme level is not surprising. The eye apparently is not a large enough source of lysozyme to elevate systemic levels.

We would infer from these findings that in human cases of uveitis in which the serum lysozyme is elevated, a systemic focus of inflammation must be responsible for the serum elevation rather than an ocular one alone.

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**Key words:** lysozyme, uveitis, inflammation, aqueous humor, serum.

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