Maul received support from New Eyes for the Needy, Inc., during the tenure of his glaucoma fellowship.

Reprint requests: Dr. Eugenio Maul, Department of Ophthalmology & Visual Science, Yale University School of Medicine, 333 Cedar St., New Haven, Conn. 06510.

Key words: nitrogen mustard, irritative response, refractometry, Evans blue, axone reflex, ocular hyperemia, aqueous humor, intraocular pressure, blood-aqueous barrier.

REFERENCES


6. Harada, H., Takeuchi, M., Ito, T., et al.: Identification of a single intravitreal injection of purified egg albumin (Sigma Chemical Company, grade VI) dissolved in normal saline (with a 27-gauge needle on a 1 ml. tuberculin syringe through the pars plana); anterior paracentesis relieved the subsequent rise in intraocular pressure. The 14 remaining rabbits received a single intravitreal injection of purified egg albumin (Sigma Chemical Company, grade VI) dissolved in normal saline (5 mg. per 0.1 ml.) and an anterior paracentesis. Reflux was minimal after all injections. At 1, 3, 5, 7, 14, 21, and 28 days after injection, aqueous samples of approximately 0.2 ml. and vitreous samples of approximately 0.5 ml. were aspirated and stored in sterile tubes at 8° C. Animals were killed with sodium pentobarbital.

Aqueous and vitreous assays were performed within four days of their collection on a Coleman spectrophotometer. The lysozyme assay kit (Worthington Biochemical Corporation) containing standard lysozyme from lyophilized hen egg white and lyophilized Micrococcus lysodeikticus substrate was used in all our measurements.

After reconstitution of our standard and our substrate, we adjusted our spectrophotometer to 550 nm. and prepared a standard curve of lysozyme activity. All reactions were carried out at 26° C. The change in optical density of each unknown sample was read off the standard curve to yield the concentration. All concentrations of

Lysozyme, a basic protein with a molecular weight of 15,000, is composed of 130 amino acids. Almost 75 per cent of the total body lysozyme is concentrated within or released from polymorphonuclear leukocytes and monocytes.

Previous work by Tessler and Weinberg has confirmed the presence of lysozyme in the aqueous humor and has demonstrated an increase in the level of aqueous lysozyme in certain types of ocular inflammation. The purpose of this investigation was (1) to determine the normal aqueous and vitreous lysozyme, (2) to determine if these normal levels increase after a single intravitreal injection of a foreign protein, and (3) to determine when these levels reach their maximum.

Materials and methods. Twenty-five albino rabbits weighing approximately 2 to 3 kilograms were used in this study. Four rabbits received no treatment and served to establish normal values. Seven rabbits received a single intravitreal injection of 0.1 ml. of normal saline (with a 27-gauge needle on a 1 ml. tuberculin syringe through the pars plana); anterior paracentesis relieved the subsequent rise in intraocular pressure. The 14 remaining rabbits received a single intravitreal injection of purified egg albumin (Sigma Chemical Company, grade VI) dissolved in normal saline (5 mg. per 0.1 ml.) and an anterior paracentesis. Reflux was minimal after all injections.


We determined normal aqueous and vitreous lysozyme levels in rabbit eyes and induced experimental uveitis to record the uppermost aqueous and vitreous lysozyme levels. The normal aqueous humor of the rabbit eye contained 1.05 µg per milliliter lysozyme and the normal vitreous humor contained 0.45 µg per milliliter. After the intravitreal administration of a foreign protein, the aqueous and vitreous lysozyme levels rose within one day, reaching maximum values of 38.4 µg per milliliter and 114 µg per milliliter, respectively, at 14 days, and subsequently declining to minimal values by 28 days after injection.

Lysozyme, a basic protein with a molecular weight of 15,000, is composed of 130 amino acids. Almost 75 per cent of the total body lysozyme is concentrated within or released from polymorphonuclear leukocytes and monocytes.

Previous work by Tessler and Weinberg has confirmed the presence of lysozyme in the aqueous humor and has demonstrated an increase in the level of aqueous lysozyme in certain types of ocular inflammation. The purpose of this investigation was (1) to determine the normal aqueous and vitreous lysozyme, (2) to determine if these normal levels increase after a single intravitreal injection of a foreign protein, and (3) to determine when these levels reach their maximum.

Materials and methods. Twenty-five albino rabbits weighing approximately 2 to 3 kilograms were used in this study. Four rabbits received no treatment and served to establish normal values. Seven rabbits received a single intravitreal injection of 0.1 ml. of normal saline (with a 27-gauge needle on a 1 ml. tuberculin syringe through the pars plana); anterior paracentesis relieved the subsequent rise in intraocular pressure. The 14 remaining rabbits received a single intravitreal injection of purified egg albumin (Sigma Chemical Company, grade VI) dissolved in normal saline (5 mg. per 0.1 ml.) and an anterior paracentesis. Reflux was minimal after all injections.


We determined normal aqueous and vitreous lysozyme levels in rabbit eyes and induced experimental uveitis to record the uppermost aqueous and vitreous lysozyme levels. The normal aqueous humor of the rabbit eye contained 1.05 µg per milliliter lysozyme and the normal vitreous humor contained 0.45 µg per milliliter. After the intravitreal administration of a foreign protein, the aqueous and vitreous lysozyme levels rose within one day, reaching maximum values of 38.4 µg per milliliter and 114 µg per milliliter, respectively, at 14 days, and subsequently declining to minimal values by 28 days after injection.

Lysozyme, a basic protein with a molecular weight of 15,000, is composed of 130 amino acids. Almost 75 per cent of the total body lysozyme is concentrated within or released from polymorphonuclear leukocytes and monocytes.

Previous work by Tessler and Weinberg has confirmed the presence of lysozyme in the aqueous humor and has demonstrated an increase in the level of aqueous lysozyme in certain types of ocular inflammation. The purpose of this investigation was (1) to determine the normal aqueous and vitreous lysozyme, (2) to determine if these normal levels increase after a single intravitreal injection of a foreign protein, and (3) to determine when these levels reach their maximum.

Materials and methods. Twenty-five albino rabbits weighing approximately 2 to 3 kilograms were used in this study. Four rabbits received no treatment and served to establish normal values. Seven rabbits received a single intravitreal injection of 0.1 ml. of normal saline (with a 27-gauge needle on a 1 ml. tuberculin syringe through the pars plana); anterior paracentesis relieved the subsequent rise in intraocular pressure. The 14 remaining rabbits received a single intravitreal injection of purified egg albumin (Sigma Chemical Company, grade VI) dissolved in normal saline (5 mg. per 0.1 ml.) and an anterior paracentesis. Reflux was minimal after all injections.


We determined normal aqueous and vitreous lysozyme levels in rabbit eyes and induced experimental uveitis to record the uppermost aqueous and vitreous lysozyme levels. The normal aqueous humor of the rabbit eye contained 1.05 µg per milliliter lysozyme and the normal vitreous humor contained 0.45 µg per milliliter. After the intravitreal administration of a foreign protein, the aqueous and vitreous lysozyme levels rose within one day, reaching maximum values of 38.4 µg per milliliter and 114 µg per milliliter, respectively, at 14 days, and subsequently declining to minimal values by 28 days after injection.
AQUEOUS AND VITREOUS LYSOZYME IN
EXPERIMENTAL UVEITIS

Fig. 1. Aqueous and vitreous lysozyme levels in experimental uveitis.

samples in this study are expressed in terms of
hen egg white lysozyme. We also assayed samples
of our purified egg albumin injection solution
twice, which revealed levels of 1.0 µg per milliliter
and 1.6 µg per milliliter.

Results. Our normal aqueous and vitreous
lysozyme values are presented in Table I. Table
II summarizes and Fig. 1 illustrates the aqueous
and vitreous lysozyme levels at timed intervals.
Aqueous and vitreous lysozyme levels increased
within one day after intravitreal administration
of the purified egg albumin and reached their max-
imum values of 38.4 µg per milliliter and 114 µg
per milliliter, respectively, at 14 days. These
values slowly declined until at 28 days, the aque-
ous lysozyme value was only 4.9 µg per milliliter,
and the vitreous lysozyme value was 32.1 µg per
milliliter. On the first day after injection, the
aqueous lysozyme levels in the control rabbits
rose to 3.0 and 4.9 µg per milliliter; vitreous levels
were 1.2 and 0.9 µg per milliliter. Levels re-
mained elevated until day 5, by which time they
had returned to normal (below 2.0 µg per milli-
liter).

Discussion. Our findings confirm that both
the aqueous humor and vitreous humor contain
lysozyme. The normal aqueous level of lysozyme
was found to be 1.05 µg per milliliter, which is
probably proportional to serum values. The vitre-
ous level of lysozyme, 0.45 µg per milliliter, may
result from both posterior diffusion of this protein from the anterior chamber and turnover of vitreous hyalocytes.5

After intravitreal injection of the purified egg albumin (99 per cent electrophoretically pure), the aqueous and vitreous levels of lysozyme rose within one day in the experimental and control eyes. This initial elevation most likely results from the associated trauma of intravitreal injection and anterior paracentesis. The intravitreal injection by necessity disrupts choroidal vasculature and allows blood and protein to enter the posterior chamber. Electron microscopic studies by Raviola6 and Pedersen5 suggest marked blood reflux into the anterior chamber after simple anterior paracentesis. Despite this insult and the cellular inflammatory response to it, the aqueous and vitreous lysozyme levels in the control eyes progressively approached normal levels at five days. Aqueous and vitreous lysozyme levels persisted below 2.0 μg per milliliter throughout the 28-day test period.

Clinical uveitis and early posterior subcapsular cataracts were observed in the majority of experimental eyes as early as seven days after protein injection. The elevated aqueous and vitreous lysozyme concentrations most likely reflect the beginning of a local antibody reaction to the egg albumin deposited in the posterior chamber. Hall and Pribnow8 reported the presence of antibody to intraocularly injected foreign protein at day 7 after injection. Segawa and Smelsera believe this antibody complexes with antigen and chemotactically draws polymorphonuclear leukocytes and activated macrophages into the posterior chamber. The subsequent degranulation of polymorphonuclear leukocytes and the phagocytosis of the immune precipitates by macrophages are most likely responsible for the maximum aqueous and vitreous lysozyme levels present at 14 days.

The available antigen, i.e., egg albumin, complexes with antibody and becomes phagocytosed by the macrophages. The production of lysozyme decreases and the remaining lysozyme is soon cleared into the serum from the anterior chamber. In a parallel study, Zimmerman and Silverstein10 report a virtually normal-appearing anterior chamber and only a slight "infiltrate of large and small mononuclear cells" in the posterior chamber at 28 days. Understandably then, the lysozyme level is only 4.9 μg per milliliter in the aqueous and 32.1 μg per milliliter in the vitreous humor.

We thank Edward Cotlier, M.D., for the use of his laboratory facilities, Jeanne Miller and Ethelle Katz for their instruction in laboratory methods, Jane Lantz for her editorial assistance, and Lillian Sumoski for her typing expertise.

From the Department of Ophthalmology, University of Illinois Eye and Ear Infirmary, Chicago, Ill. 60612. Supported in part by Public Health Service grant 1107-02 and by the Lions Foundation of Illinois. Submitted for publication Aug. 26, 1975. Reprint requests: Dr. Gholam A. Peyman, University of Illinois Eye and Ear Infirmary, 1855 W. Taylor St., Chicago, Ill. 60612.

Key words: aqueous humor, vitreous humor, lysozyme, uveitis, egg albumin.

REFERENCES


**Table I. Normal values of lysozyme in aqueous and vitreous humor of rabbits**

<table>
<thead>
<tr>
<th></th>
<th>Aqueous</th>
<th>Vitreous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.D.</td>
<td>1.05 ± 0.1</td>
<td>0.45 ± 0.05</td>
</tr>
<tr>
<td>No. eyes</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Range (μg/ml)</td>
<td>0.6-14</td>
<td>0.0-0.9</td>
</tr>
</tbody>
</table>

*All values of lysozyme in this investigation are expressed in micrograms per milliliter of hen egg white lysozyme.

**Table II. Aqueous and vitreous lysozyme levels in rabbits with experimentally induced uveitis**

<table>
<thead>
<tr>
<th>Interval (days)</th>
<th>Aqueous (μg/ml)</th>
<th>Vitreous (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.1 ± 1.5*</td>
<td>6.9 ± 3.1</td>
</tr>
<tr>
<td>2</td>
<td>1.2-5.4</td>
<td>1.7-9.7</td>
</tr>
<tr>
<td>3</td>
<td>12.6±1.8</td>
<td>4.3±0.4</td>
</tr>
<tr>
<td>5</td>
<td>9.6-14.2</td>
<td>3.7-4.9</td>
</tr>
<tr>
<td>7</td>
<td>16.3±5.3</td>
<td>24±6.2</td>
</tr>
<tr>
<td>14</td>
<td>9.0-24.0</td>
<td>16.0-33.0</td>
</tr>
<tr>
<td>21</td>
<td>25.6±9.6</td>
<td>42.3±10.2</td>
</tr>
<tr>
<td>28</td>
<td>15.6-40.8</td>
<td>25.2-52.4</td>
</tr>
<tr>
<td>32</td>
<td>16.3±14.2</td>
<td>114±6.6</td>
</tr>
<tr>
<td>42</td>
<td>29.1-50.0</td>
<td>104-120</td>
</tr>
<tr>
<td>72</td>
<td>8.0±1.0</td>
<td>70.2±9.6</td>
</tr>
<tr>
<td>126</td>
<td>7.2-9.6</td>
<td>58.0-82.0</td>
</tr>
<tr>
<td>168</td>
<td>4.9±1.1</td>
<td>32.1±11.8</td>
</tr>
<tr>
<td>216</td>
<td>3.6-6.6</td>
<td>14.0-46.0</td>
</tr>
</tbody>
</table>

*Arithmetic mean and standard error of lysozyme values. All values except for those at day 1 are statistically significant at least at the 5 per cent confidence level. *Actual range of lysozyme values (four eyes).
The effect of imidazole on the disruption of the blood-aqueous barrier in the rabbit eye. Elisabeth Bengtsson.

The aqueous flare (AF) of an intact rabbit eye was measured by a photoelectric instrument and the intraocular pressure by vibration tonometry. Prior treatment with imidazole given intraperitoneally noticeably inhibited the disruption of the blood-aqueous barrier in rabbit eyes induced by topical prostaglandin E\(_2\) (PGE\(_2\)), topical arachidonic acid (AA), infrared irradiation of the iris, endotoxin of Proteus mirabilis given intravenously, and \(\alpha\)-melanocyte-stimulating hormone (\(\alpha\)-MSH) given subcutaneously. Prior treatment with imidazole given topically had no effect on the disruption of the blood-aqueous barrier caused by topical PGE\(_2\), topical AA, infrared irradiation of the iris, or endotoxin of \(P.\) mirabilis given intravenously and \(\alpha\)-melanocyte-stimulating hormone (\(\alpha\)-MSH) given subcutaneously.

Prior treatment with imidazole did not affect the histological changes of the anterior ciliary processes induced by \(\alpha\)-MSH given subcutaneously.

In a previous paper, the inhibiting effect of topical indomethacin on the aqueous flare response (AFR) in the rabbit eye was reported. Indomethacin, like aspirin, which specifically blocks the conversion of arachidonic acid (AA) to prostaglandin (PG), is able to inhibit those effects on the eye of traumatic agents, which are mediated by prostaglandin. We found that topical indomethacin inhibited the AFR to all tested agents except \(\alpha\)-MSH. This supports the belief that \(\alpha\)-MSH works differently on the eye from many other traumatic agents. Another reported inhibitor of the ocular effect of AA\(^6\) and PG\(\_2\)^\(^7\) is systemically administered imidazole. The mechanism of the imidazole effect on the eye is unknown. Zink, Podos, and Backer have shown that intraperitoneal imidazole inhibits the increase of intraocular pressure and protein concentration in aqueous humor elicited by nitrogen mustard also, although the actions of nitrogen mustard do not seem to be prostaglandin mediated. The present report deals with the ability of imidazole to affect the breakdown of the blood-aqueous barrier caused by \(\alpha\)-MSH compared to that caused by prostaglandin and prostaglandin-mediated agents. In our experiments we have used only the aqueous flare as a parameter of the breakdown of the blood-aqueous barrier and we have therefore found it important to test the correlation between the elevation of the IOP and the AFR to prostaglandin.

Materials and methods.

Animals. Adult pigmented female rabbits of mixed strains, weighing between 2.0 and 4.0 kilograms were used. They were given pellets and water ad libitum.

Chemical preparations. Imidazole (Sigma) was freshly dissolved in saline (10 to 250 mg. ml\(^{-1}\)). PGE\(_2\) (Upjohn) was dissolved in ethanol (10 mg. ml\(^{-1}\)) and saline was added giving a solution containing 1.0 mg. PGE\(_2\) per milliliter. Arachidonic acid (Sigma) was freshly dissolved in peanut oil. The resulting solution contained 2 per cent arachidonic acid (w/w). Endotoxin of \(P.\) mirabilis was dissolved in distilled water to a concentration of 5 \(\mu\)g ml\(^{-1}\) and kept at \(+4^\circ\) C. \(\alpha\)-MSH (CIBA) was freshly dissolved in saline (100 \(\mu\)g ml\(^{-1}\)).

Infrared irradiation. Infrared irradiation of the iris was performed with the same technique as that described in a previous report.

Aqueous flare response (AFR). The course of the inflammatory response was in all cases followed by quantitative measurements of the aqueous flare in the intact eye by means of a photoelectrical instrument described in a previous report. The flare was measured in arbitrary units.

Intraocular pressure (IOP). The IOP was measured by vibration tonometry. In the present case a calibration was made on enucleated rabbit eyes connected to an open manometer. The results are given in millimeters of Hg. It was possible to measure the rabbits sitting in their natural position and no local or general anesthesia was needed.

Histological procedures. Suitable pieces of tissues were obtained from the anterior segment and quenched in a liquid propane-propylene mixture, freeze-dried, fixed by exposure to formaldehyde gas for 1 hour at 80°C, embedded in