age was 0.09, and of flicker sensitivity with age was 0.00, both of which are well below statistical significance.

A second contaminating variable in studies of visual sensitivity is the size of the pupil. Under our experimental conditions the normal pupil had a size of about 4 mm in diameter. Pupil size determines the level of retinal illumination, which in turn is linearly related to high-frequency flicker sensitivity.[7] To obtain the observed range of a factor of 10 in flicker sensitivity, retinal illumination would have to vary by about a factor of 50, and pupil diameter would have to change by the square root of 50, about a factor of 7 (from, say, 1–7 mm). Such a variation should affect central and peripheral sensitivities equally, and also have a similar effect at 40 and 25 Hz.

Our observers did not show such noticeable variations in pupil size, and the obtained product-moment correlations in central vision show significant differences between 25 and 40 Hz that cannot be explained by luminance variations. Even taking into account the total number of comparisons among all correlations (66), the difference of 0.631 between the right temporal 25 Hz correlation and the right central 40 Hz correlation is significant[5] at $P < 0.05$. Since this difference is incompatible with a pupil size hypothesis, we conclude that pupil size does not determine the relation between IOP and flicker sensitivity.

It is always possible that there is a further variable that might exert control over both IOP and flicker sensitivity. Nevertheless, the presence of a significant correlation is of interest in identifying IOP as a potential risk factor for the neural deficit. The possibility of a direct effect of IOP on neural function (as represented by flicker sensitivity) merits further study.

Key words: intraocular pressure, flicker, visual sensitivity, retina

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PMN Accumulation in Aqueous Humor and Iris-Ciliary Body during Intraocular Inflammation

Richard N. Williams and Christopher A. Paterson

The accumulation of polymorphonuclear leukocytes (PMNs) was determined in the aqueous humor and iris-ciliary body following an intravitreal injection of endotoxin in the albino rabbit. PMN accumulation in the iris-ciliary body was quantified by measuring myeloperoxidase (MPO) activity in homogenates of this tissue. Leukocyte appearance in the aqueous humor was determined by counting the number of PMNs in diluted aspirates of aqueous humor and also by measuring MPO activity in the same aspirates.

Twenty-four hours following an intravitreal injection of endotoxin, there was marked vasodilatation in the iris, breakdown of the blood-aqueous barrier, and infiltration of PMNs into the aqueous humor. There was, however, no correlation between MPO activity in the iris-ciliary body and the number of PMNs or the MPO activity in the aqueous humor. Furthermore, the number of PMNs in the aqueous humor did not increase with increasing amounts of intravitreally injected endotoxin, whereas MPO activity in the iris-ciliary body increased in a dose-dependent manner.

The results of this study suggest that quantification of leukocytes in the aqueous humor does not represent a meaningful index of intraocular inflammation. Invest Ophthalmol Vis Sci 25:105–108, 1984

Acute inflammatory responses in the eye are composed of a number of components, namely the accumulation of polymorphonuclear leukocytes (PMNs) in the ocular tissues and fluids, dilatation of blood vessels, and an increase in vascular permeability.[1] In

Table 1. Protein concentrations in the aqueous humor 24 hours after intravitreal injection of bacterial endotoxin.

Intravitreal endotoxin (μg)	*n†*	*Aqueous humor protein (mg/ml) mean ± SEM*
0.0*	3	0.9 ± 0.1
2.5	4	46.4 ± 2.2
5.0	4	50.1 ± 6.3
10.0	4	38.9 ± 3.3

* Sterile isotonic saline (10 μl) was injected into eyes not receiving endotoxin.
† n = number of eyes.

experimental models of intraocular inflammation, the increase in vascular permeability is frequently determined by measuring the concentration of protein in the aqueous humor, whereas vasodilatation usually requires a subjective evaluation. Unfortunately, quantification of the major acute inflammatory response in the eye, ie, the infiltration and accumulation of PMNs in ocular tissues, is fraught with problems. Probably the most common method is to directly count the number of PMNs in diluted aspirates of aqueous humor, although more elaborate techniques have been used in an attempt to render aqueous humor aspirates suitable for PMN measurements.[2] However, since PMNs cannot move directionally in a fluid medium, other than falling under gravitational force,[3] it is possible that PMN appearance in aqueous humor largely represents an overflow from the surrounding intraocular tissues. Such an overflow phenomenon might not be an accurate index of an intraocular inflammatory response.

Recently, an assay has been described to quantify the accumulation of PMNs in ocular tissues by measuring myeloperoxidase activity.[4] Myeloperoxidase (MPO) is found within the azurophilic granules of PMNs in high concentrations, and, as such, the enzyme has been used to quantify the accumulation of these cells in ocular and other tissues.[5,6] Therefore, in this study, we have examined the correlation between PMN accumulation in the aqueous humor and the iris-ciliary body during an intraocular inflammatory response to endotoxin.

Materials and Methods. Albino rabbits of either sex, weighing 2–3 kg, were used in all the experiments.

Intravitreal injection of endotoxin. Endotoxin (Lipopolysaccharide, E. Coli: 055:B5, Sigma) was dissolved in sterile isotonic saline. To induce an intraocular inflammatory response, the eyes were injected intravitreally with given amounts of endotoxin (2.5, 5.0, or 10 μg) in a constant volume of 10 μl, using a 30-gauge needle attached to a Hamilton syringe. Control eyes were injected with 10 μl sterile isotonic saline.

Measurement of PMN accumulation in the aqueous humor and iris-ciliary body. Twenty-four hours after an intravitreal injection of endotoxin, the animals were killed with a rapid overdose of sodium pentobarbitone. Aqueous humor was immediately aspirated into a 1 ml syringe and then diluted with heparinized saline. The number of PMNs in the diluted aqueous humor were counted under the microscope using an improved Neubauer chamber. MPO activity of the cells in the same aqueous humor samples was determined using a modification of the method described previously.[4] The aqueous humor of the rabbit contains high con-

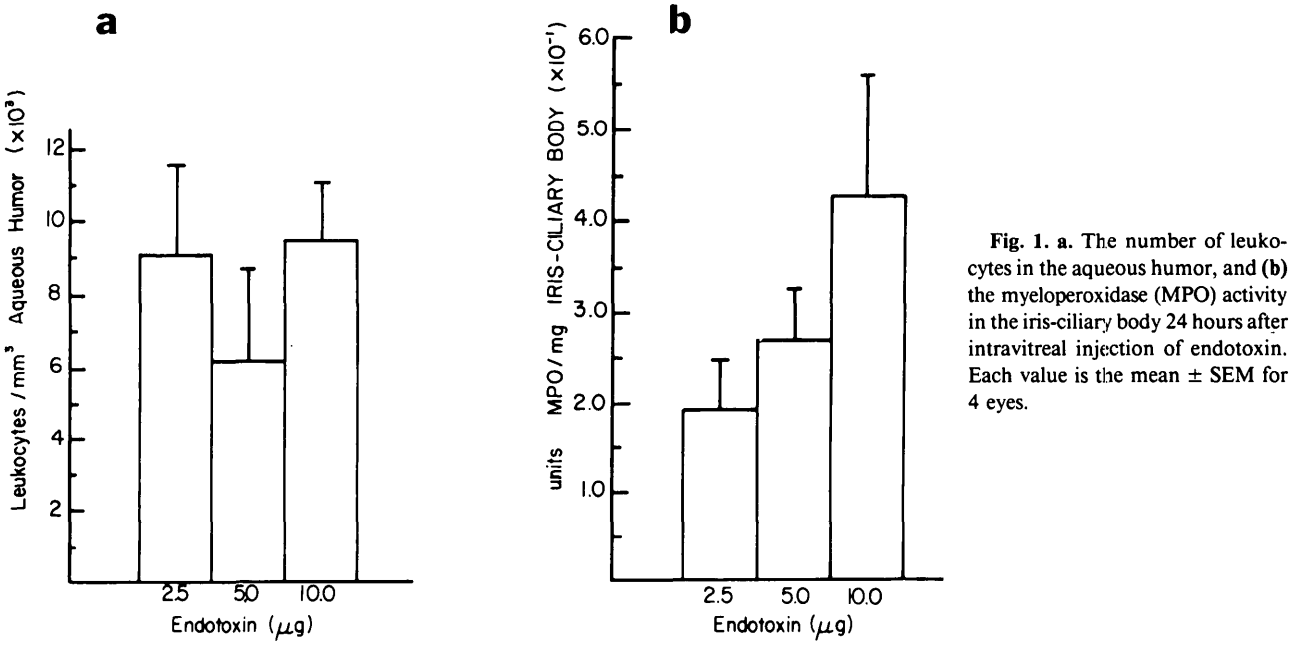

Fig. 1. a. The number of leukocytes in the aqueous humor, and **(b)** the myeloperoxidase (MPO) activity in the iris-ciliary body 24 hours after intravitreal injection of endotoxin. Each value is the mean ± SEM for 4 eyes.

centrations of ascorbic acid which interferes with the MPO assay. For this reason, the diluted aqueous humor samples were centrifuged (1,000 g for 20 min), and the MPO activity of the leukocyte pellet assayed in the presence of 0.001% hydrogen peroxide. The protein content of the diluted aqueous humor samples were determined by the method of Lowry, Rosebrough, Farr, and Randall.[7]

The accumulation of PMNs in the iris-ciliary bodies was quantitated by measuring MPO activity in this tissue as previously described.[4]

Results. Twenty-four hours after intravitreal injection of endotoxin, pronounced dilatation of conjunctival and iridial blood vessels was observed. This was accompanied by extravasation of protein into the aqueous aqueous humor (Table 1). Furthermore, as the dose of endotoxin indreased from 2.5 to 10 μg, these clinical signs of inflammation increased in severity.

PMNs were present in the aqueous humor in considerable numbers 24 hours after the intravitreal injection of endotoxin. However, the number of PMNs appearing in the aqueous humor after 24 hours did not increase with the amount of endotoxin injected (Fig. 1a). In contrast, MPO activity in the iris-ciliary body was found to increase in a dose-dependent manner with the amount of endotoxin injected (Fig 1b). In saline-injected eyes, PMNs were not found in the aqueous humor, and no MPO activity was detected in the iris-ciliary body.

There was no evident correlation (correlation coefficient = 0.337, n = 12) between MPO activity in the iris-ciliary body and the number of PMNs in the aqueous humor (Fig. 2a). However, there was a good correlation (correlation coefficient = 0.877, n = 12) between PMN numbers and MPO activity in the aqueous humor (Fig. 2b).

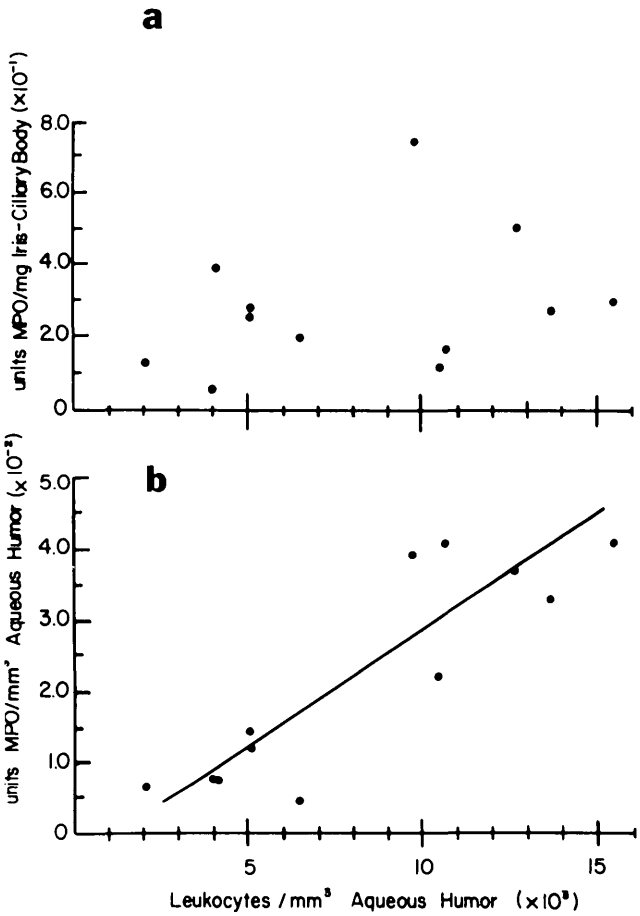

Fig. 2. a. The correlation between myeloperoxidase (MPO) activity in the iris-ciliary body and the number of leukocytes in the aqueous humor 24 hours after intravitreal injection of endotoxin (correlation coefficient = 0.337, n = 12). **b,** The correlation between the number of leukocytes in the aqueous humor and the MPO activity in the same samples 24 hours after intravitreal injection of endotoxin (correlation coefficient = 0.887, n = 12). Each point represents the value obtained from individual eyes taken from animals injected intravitreally with 2.5 to 10 μg of endotoxin.

Discussion. Counting PMN populations in aspirates of aqueous humor or inflammatory exudates is a popular approach to quantifying inflammatory reactions and also evaluating the anti-inflammatory activity of various pharmacological agents.[8-10] However, this approach would seem only to measure the overflow of PMNs from the tissues, as leukocytes are unable to move in a fluid medium except under the influence of gravity.[3] Therefore, the number of cells present in the aqueous humor or an inflammatory exudate probably does not represent a true inflammatory index at any particular given time. The present study suggests that this is the case in the inflammatory response in the rabbit eye, as there was no correlation between MPO activity in the iris-ciliary body and the number of PMNs in the aqueous humor following (24 hours) the intravitreal injection of endotoxin.

Yet another disadvantage of using the aqueous humor cell population to assess an inflammatory reaction in the eye is encountered when attempting to obtain a representative sample. First, the high protein concentrations in the aqueous humor of the inflamed eye can make aspiration difficult; and second, because leukocytes are unable to swim, falling under gravity in a fluid, they will not be distributed equally throughout the medium. The sampling error may explain why, in the present study, the number of PMNs in the aqueous humor did not increase with the dose of endotoxin injected, whereas, in contrast, the MPO activity in the iris-ciliary body was found to increase dose-dependently.

We conclude that counting the number of PMNs in the aqueous humor may not provide a true measure of the degree of ocular inflammation. Nevertheless, from a pharmacological viewpoint, a compound which

inhibits the appearance of PMNs in the aqueous humor undoubtedly inhibits inflammatory responses in the eye.[11] However, because of the difficulty in obtaining representative samples of aqueous humor, it is conceivable that less potent but potentially useful anti-inflammatory compounds, which are evaluated in this way, could be overlooked.

Key words: inflammation, endotoxin, eye, polymorphonuclear leukocytes, myeloperoxidase, aqueous humor, iris-ciliary body

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Protective Barrier Effect of the Posterior Lens Capsule in Exogenous Bacterial Endophthalmitis—An Experimental Primate Study

Todd L. Beyer, George Vogler, Dian Sharma, and Francis E. O'Donnell, Jr.

Ten eyes of five Rhesus monkeys underwent extracapsular lens extraction. The right eye of each monkey was allowed to retain an intact posterior capsule. The left eye of each monkey had a wide primary capsulectomy with minimal anterior vitrectomy. In order to exclude operative contamination, we waited 2 to 3 weeks later to challenge the eyes with bacteria. Seventy-two hours after anterior chamber injection of equal numbers of *Staphylococcus aureus,* diagnostic cultures were obtained from the anterior chamber and vitreous and correlated with the clinical findings. Injection of 10,000 *S. aureus* produced culture-positive endophthalmitis in eyes that had undergone posterior capsulectomy, but it failed to produce endophthalmitis in fellow eyes with intact posterior capsules. This suggest that a significant barrier effect against the development of bacterial endophthalmitis exists in the eyes with intact posterior capsules. Invest Ophthalmol Vis Sci 25:108–112, 1984

Exogenous, bacterial endophthalmitis is a disastrous complication of intraocular surgery. Visual prognosis in this disease remains relatively poor even with aggressive therapeutic intervention using intracameral antibiotics and early vitrectomy.[1–3] The best treatment remains prevention. Recently there has been renewed interest in extracapsular cataract extraction techniques, primarily because of the popularity of posterior chamber intraocular implants. It is the purpose of this investigation to evaluate the role that the primate posterior capsule may play in inhibiting the development of endophthalmitis by confining the infected material to the anterior chamber. A barrier effect of the posterior capsule in the rabbit model has been previously suggested by Katz and Forster in an unpublished communication.

Materials and Methods. Ten eyes of five monkeys underwent extracapsular cataract extraction. On the day prior to the operative procedures, each monkey was examined, while under ketamine sedation, for lens, retinal, or vitreous abnormalities with the direct and