

Reports

Systemic aspirin and indomethacin do not prevent the response of the monkey eye to trauma. MICHAEL A. KASS, ARTHUR H. NEUFELD, AND MARVIN L. SEARS.

The stability of the blood-aqueous barrier of the monkey eye was challenged by three different methods: anterior chamber paracentesis, intravitreal shigella endotoxin, and subconjunctival arachidonic acid. Systemic aspirin and indomethacin were ineffective in stabilizing the blood-aqueous barrier in all three of these systems.

Prostaglandins are implicated among the mediators of the breakdown of the blood-aqueous barrier.¹⁻³ Aspirin and indomethacin have been employed to prevent the synthesis of prostaglandins and to stabilize this barrier.^{2, 4-6} The monkey eye responds to the administration of prostaglandins with an increased aqueous humor protein concentration,⁷ elevated intraocular pressure,⁷ and miosis.⁸ However, experiments on the therapeutic use of inhibitors of the synthesis of prostaglandins have, primarily, utilized the rabbit eye. The present experiments were designed to determine the efficacy of the widely available inhibitors of prostaglandin synthesis, aspirin and indomethacin, in stabilizing the blood-aqueous barrier of the primate eye to various challenges.

Materials and methods.

Paracentesis. Rhesus monkeys, 8 to 10 kilograms, were anesthetized with intramuscular phencyclidine (2 mg. per kilogram). Four monkeys received indomethacin, 25 to 100 mg. intraperitoneally or orally. Four monkeys received aspirin, 600 to 1,800 mg. by rectal suppository. Eight monkeys served as controls. One hour after the medication, 100 μ l of aqueous humor was aspirated from both eyes with a 30-gauge needle. One hour later, a second paracentesis was performed with a 27-gauge needle. The protein concentration was determined by the method of Lowry and co-workers⁹ on primary and secondary aqueous humor samples.

Arachidonic acid. Rhesus monkeys, 8 to 10 kilograms, were anesthetized with intramuscular phencyclidine. The intraocular pressure was measured with the Mackay-Marg tonometer or with the Alcon Pneumotonograph. Six monkeys received 600 mg. of aspirin by rectal suppository; five monkeys served as controls. One hour later, all of the monkeys received 0.1 ml. of 20 per cent arachidonic acid in peanut oil, subconjunctivally to one eye. The other eyes received an equal volume of peanut oil. The intraocular pressure was measured at 15, 30, 60, and 120 minutes.

Table I. Effect of paracentesis on the protein concentration of the aqueous humor of the rhesus monkey

Treatment	No. of eyes	Aqueous humor protein concentration (mg. %) mean \pm S.E.M.
Primary aqueous humor	32	26 \pm 3
Secondary aqueous humor	16	830 \pm 220
Secondary aqueous humor (indomethacin-pretreated animal)	8	707 \pm 56
Secondary aqueous humor (aspirin-pretreated animal)	8	1,700 \pm 370

Shigella endotoxin. Vervet monkeys, 4 to 6 kilograms, were anesthetized with intramuscular phencyclidine. Six monkeys had been pretreated with intraperitoneal indomethacin, 25 mg. twice a day for two days prior to the experiment and the treatment was continued for the duration of the experiment. Five monkeys served as controls and received no indomethacin. Shigella endotoxin (Difco Laboratories, Detroit, Mich.) was diluted with saline and passed through a Millipore filter. Ten micrograms of endotoxin in a total volume of 50 μ l was injected intravitreally in both eyes. The monkeys were examined with a Haig-Streit slit lamp and an ophthalmoscope, and graded on an arbitrary scale from 0 to 4+, as to conjunctival hyperemia, anterior chamber cell and flare, pupil size and reactivity, and haziness of the optical media. Paracentesis of the anterior chamber for protein determination was performed on selected animals.

Results.

Paracentesis. There was a 30-fold increase in the aqueous humor protein concentration 60 minutes after paracentesis in the control monkeys. Pretreatment with systemic aspirin and indomethacin did not significantly inhibit this increase (Table I). There was no dose-response trend of the aqueous humor protein concentration after paracentesis to doses of systemic aspirin varying from 600 to 1,800 mg. Therefore, the results were combined and considered together. The aqueous humor protein concentration following paracentesis in the aspirin-pretreated monkeys did not differ significantly from the controls (p. greater than 0.05). The difference in the means reflects the large scatter of the data.

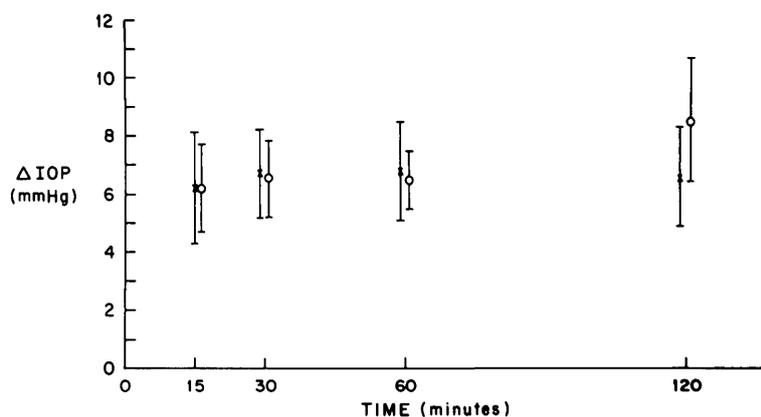


Fig. 1. Intraocular pressure after subconjunctival arachidonic acid. At time 0, 20 mg. of arachidonic acid was injected subconjunctivally to one eye, peanut oil to the other eye. The values given are the means of the differences in intraocular pressure of the treated eye minus the opposite eye \pm S.E.M. X, control monkeys ($n = 5$); O, monkeys pretreated with aspirin ($n = 6$).

Arachidonic acid. Following subconjunctival arachidonic acid, the intraocular pressure increased 6 to 9 mm. Hg over the control eyes. Pretreatment with systemic aspirin did not inhibit this rise in intraocular pressure (Fig. 1).

Shigella endotoxin. During the seven days of observation following intravitreal shigella endotoxin, a severe uveitis developed and then subsided. The indomethacin-treated monkeys differed in only one regard from the control monkeys. The degree of conjunctival hyperemia was less in the indomethacin-treated monkeys from day 1 to day 6, following injection. The other parameters of anterior chamber cell and flare, pupil size and reactivity, and opacity of the ocular media did not differ in the two groups. Paracentesis of the anterior chamber revealed no difference in the protein concentrations of the indomethacin-treated monkeys from the control monkeys.

Discussion. Aspirin and indomethacin have been reported to be effective in inhibiting the synthesis of prostaglandins¹⁰ and stabilizing the blood-aqueous barrier.^{2, 4-6} The majority of the published reports concerning inhibition of the synthesis of endogenous prostaglandins in the eye have utilized the rabbit. In the present studies, systemic aspirin and indomethacin were ineffective in stabilizing the blood-aqueous barrier of the primate eye to three different challenges: paracentesis, subconjunctival arachidonic acid, and intravitreal shigella endotoxin. In *in vitro* experiments, most ocular tissues were less sensitive to inhibitors of the synthesis of prostaglandins than other tissues, such as rabbit kidney.⁶

Systemic indomethacin was capable of reducing conjunctival hyperemia following intravitreal shigella endotoxin. This is *in vivo* confirmation of a previous report that conjunctival tissue is more

sensitive to indomethacin than other rabbit ocular tissues in an *in vitro* system.⁶

The question arises as to why aspirin and indomethacin were ineffective in stabilizing the blood-aqueous barrier in these experiments. The doses of aspirin and indomethacin employed are comparable to those previously found effective in the rabbit. It is possible the drugs did not reach the eye in adequate concentration, were actively removed from the eye, were metabolized in the eye, or were bound in an inactive form. Further studies are in progress to explore these possibilities.

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Key words: monkey, aspirin, indomethacin, prostaglandin, paracentesis, arachidonic acid, shigella endotoxin.

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Photographic measurements of retinal blood oxygen saturation: falling saturation rabbit experiments. RONALD A. LAING, ALLEN J. COHEN, AND EPHRAIM FRIEDMAN.

A noninvasive photographic method of measuring retinal blood oxygen saturation and arteriovenous oxygen difference was developed and calibrated on rabbits. The experiments reported show that the accuracy of the measurements made using the method is better than 1.0 per cent. The method is applicable to vessels located anywhere in the fundus and represents a significant improvement over previous methods.

Not long after the modern fundus camera was developed by Littman¹ the first noninvasive methods of measuring the oxygen saturation of retinal blood were reported by Hickam and Frayser.² In this and subsequent papers,³⁻⁵ a photographic method is described in which two photographs of the ocular fundus are taken each with a different color of light. The film densities of the optic disc and the retinal vessels of interest are measured using a modified microfilm reader. These values are combined with an assumed film sensitivity curve to give values of retinal blood optical density. The arterial blood optical density values were related to oxygen saturation values using the results of calibration experiments in which arterial blood samples were withdrawn at the time the retinal photographs were taken. Many interesting

results pertaining to changes in retinal arterial and venous oxygen saturations with inspired gas mixture, age, etc., were studied by Hickam and co-workers²⁻⁵ over a seven-year period. Unfortunately, many of the techniques and critical details essential for the successful application of their method, details which are often exceedingly difficult to identify, write down, and publish, were not passed on to others. As a result, nearly ten years have elapsed during which time several other groups have attempted to apply Hickam and Frayser's method without success even though considerable effort was expended to this end.

This paper summarizes an improved photographic method which was developed to non-invasively measure the oxygen saturation of retinal blood in vivo and presents the results of animal experiments performed to calibrate and determine the accuracy of the method. The details of the instrumentation and the problems involved in developing it will be presented elsewhere⁶ as will the measurements made on cats, monkeys, and humans.

Materials and methods.

Theory. A theoretical analysis was performed to serve as a foundation of the present photographic method. This analysis predicted a dependence of a measurable saturation parameter, R , upon the blood oxygen saturation, S , and served to guide the development of the photographic method along pathways having a sound mathematical foundation.

Instrumentation. In the PEO method, a suitably modified Zeiss fundus camera is used to take two simultaneous photographs of the patient's fundus using two different wavelengths of light λ_1 and λ_2 . The two wavelengths presently used are $\lambda_1 = 470$ nm. (blue) and $\lambda_2 = 515$ nm. (green), although $\lambda_1 = 650$ nm. (red) and $\lambda_2 = 805$ nm. (infrared) have also been used. The PEO camera back which was used to take these two simultaneous photographs is shown in Fig. 1. After development the negatives are scanned on a scanning microdensitometer to give the film optical density of the image of a given retinal vessel and of the "background" adjacent to the vessel.

Fig. 2, A shows a typical PEO photograph taken of a normal Dutch hooded rabbit. Fig. 2, B shows a chart recording obtained from microdensitometrically scanning a given artery-vein pair of this photograph.

Analysis. A microdensitometric scan of the sensitometric step tablet which is exposed on each roll of film enables the determination of the film characteristic curve relating film optical density to the intensity of light reflected from the fundus. Using the measured characteristic curve and the measured optical density values of the retinal vessel image and vessel "background," the optical density, $D(\lambda)$, of the actual blood con-