Isoproterenol was applied topically to one eye of each of 20 normal human volunteers. The rate of aqueous humor flow was measured with fluorophotometry. Intraocular pressure fell slightly in the treated eye, but the rate of aqueous humor flow was unaltered. Even when applied to the cornea in high concentrations, this beta-adrenergic agonist appeared to have no measurable effect on the flow of aqueous humor through the anterior chamber in the human eye. Invest Ophthalmol Vis Sci 25:357-359, 1984

Isoproterenol is an adrenergic agonist that was tested many years ago as a therapeutic agent for glaucoma. It is known that isoproterenol lowers intraocular pressure, but its clinical usefulness has been limited by a rather consistent production of tachycardia in the elderly patient and lack of long-term control of intraocular pressure. Despite its clinical disadvantages, isoproterenol is an interesting adrenergic agent because it is a beta-adrenergic agonist.

The studies of Ross and Drance, Eakins, and Gaasterland et al suggest that isoproterenol reduces the rate of aqueous formation. However, the studies of Bill suggest that the drug increases the rate of aqueous humor formation. Two other beta-adrenergic agonists, salbutimol and metaproterenol, have been found to increase aqueous formation. This study was undertaken to measure what effect, if any, isoproterenol has in the human eye on the rate of disappearance of fluorescein from the anterior chamber, which is a more direct indicator of aqueous humor flow.

Materials and Methods. Twenty normal human volunteers were studied. Informed consent was obtained from each subject prior to the study. Isoproterenol HCl 2% or 4% racemic mixture, IND 8774, was instilled into one eye and a placebo vehicle instilled into the fellow eye (random assignment, double-masked). The drug and the vehicle were formulated, packaged, and labeled at the National Institutes of Health; the test procedures were carried out at the Mayo Clinic. The stability of the solutions was checked using high pressure liquid chromatography.

On the first morning of testing, the intraocular pressure was measured, and isoproterenol/placebo instilled into the assigned eyes. Five minutes later, drop instillation was repeated. Iontophoresis was carried out 1 hr later. Tonometry was repeated immediately after iontophoresis. The sequence of drop instillation was repeated 3 hr and 6 hr after initial instillation to achieve a steady level of drug action. Prior to every instillation of isoproterenol, proparacaine was instilled to improve ocular penetration, to mask the burning sensation of the drug, and to prolong the contact time of the drug with the eye. The pulse of each subject was checked after each instillation to determine whether systemic absorption had produced tachycardia. If the pulse rate were greater than 133% of the baseline, the second drop of that dose period was not instilled. (This situation occurred in only one subject.)

On the first day of testing, iontophoretically applied fluorescein was used to measure aqueous humor flow and endothelial permeability by method 2 of Nagataki and Brubaker. On the second day of testing (a week or more after the first day of testing), the fasting subjects underwent baseline measurement of fluorescent intensity in the cornea and anterior chamber, and hourly measurements followed the oral administration of fluorescein in capsules, 7 mg/kg body weight. Venipunctures were performed 45 min and 2 hr after dosing. These specimens were used to determine the unbound plasma concentration of fluorescein. The unbound plasma concentration was determined from the combined measurements of fluorescent intensity and fluorescence polarization. The drug and placebo drops were given in the same way and at the same times as on the first day of testing. Fluorescence intensity measurements were made in the anterior chamber at hourly intervals for 5 hr. At the end of the fluorescein testing, specular microscopy was repeated (all patients underwent prestudy specular microscopy) to measure corneal thickness and endothelial cell density.

Results. The initial intraocular pressure (8:00 AM, predrug) was found to be 15.6 ± 2.7 mmHg (mean ± SD) in the group of eyes that was to receive the medication and 15.5 ± 3.1 mmHg in the group of eyes that was assigned to receive the vehicle. This small difference was not statistically significant. One hour...
after drug instillation, the intraocular pressure in the treated group of eyes was 13.4 ± 3.2 mm Hg and in the control eyes 13.9 ± 3.0 mm Hg. This small difference was statistically significant (P = 0.025). At the end of the 8-hr test period, the intraocular pressure in the treated eyes was 11.9 ± 3.0 mm Hg and in the control eyes 13.2 ± 3.5 mm Hg. The average difference between the two eyes was 1.4 ± 1.9. This difference was small, but was statistically significant (P < 0.005).

The volume of the anterior chamber of the eyes that received the medication was 186 ± 50 μl and of the eyes that received the vehicle 187 ± 47 μl. Isoproterenol had no clinically observable effect on the size of the pupil or the depth of the anterior chamber.

The anterior chamber loss coefficient kₐ was 1.9 × 10⁻² ± 0.5 × 10⁻² min⁻¹ treated eye and 1.8 × 10⁻² ± 0.4 × 10⁻² min⁻¹ in the control eye. This difference was not statistically significant. The diffusional exchange coefficient between plasma and the anterior chamber was 1.7 × 10⁻² ± 2.3 × 10⁻³ in the treated eye and 1.3 × 10⁻² ± 1.3 × 10⁻³ min⁻¹ in the untreated eye. This difference was not statistically significant with a t test but was significant using the Wilcoxon test for paired samples (P < 0.05). Whether statistical significance is accepted or not, the absolute difference of kₐ in the treated and the untreated eyes was small.

The rate of flow of aqueous humor as calculated for the treated eyes was 3.2 ± 0.9 μl/min and in the untreated eye 3.1 ± 0.9 μl/min. This small difference was not statistically significant.

No statistically significant difference was found when the endothelial cell size of the two groups of eyes before treatment was compared with cell size after treatment. The thickness of the cornea was 0.55 ± 0.03 mm before treatment and 0.54 ± 0.03 mm in the two groups after the study. This difference was not significant. The endothelial permeability to fluorescein was 2.04 × 10⁻⁴ ± 0.96 × 10⁻⁴ cm min⁻¹ in the treated group of eyes and 2.09 × 10⁻⁴ ± 0.68 × 10⁻⁴ cm min⁻¹ in the control group. This difference was not significant. Thus, no corneal effects of the drug were found.

The responses in eyes receiving 4% isoproterenol HCl did not differ from those in eyes receiving the 2% concentration.

**Discussion.** The only effect of isoproterenol in the normal human eye that we could demonstrate in this study was a lowering of intraocular pressure quite comparable to that observed in a similar study. No other effects were found. There are several reasons that would be sufficient to explain our inability to demonstrate an effect of isoproterenol on the flow of aqueous humor, aside from the possibility that the drug might have no effect on flow. First, the drug may not have reached the ciliary processes in a concentration high enough to produce an effect. This situation may have existed despite our efforts to use a high concentration, to enhance corneal penetration with the use of a topical anesthetic, to instill double doses, and to repeat the doses at three hourly intervals to sustain a concentration in the eye. Isoproterenol, like other catecholamines, is highly polar and penetrates lipid membranes poorly. Also, the concentration in critical regions of living and adrenergically innervated tissue can be reduced by uptake mechanisms.

Second, the effect may have been too transient to be detected by the technique, or the flow measurement may have been carried out too soon or too late to detect it. The method of flow measurement that was used is affected by flow that occurs near the end of the procedure, in the last 3 hr, and is not affected by flow that occurs in the early hours after application of fluorescein. A previous study has demonstrated that maximum lowering of intraocular pressure occurs 6 hr after a single dose. If flow were affected similarly, the timing of our flow study was appropriate to detect it.

Third, the effect of isoproterenol on flow could have been too small to have been detected by the method employed. In this regard, we have calculated the statistical power of the experiment to detect a drug effect of varying magnitudes utilizing the method of Cochran. Using a value of α of 0.05, a value of β of 0.2, and a value of the coefficient of variation of the method of 16%, it can be calculated that our sample size of 20 pairs of eyes would have had an 80% chance of detecting a 14% change in flow caused by a drug or a 95% chance of detecting a 19% change in flow. Thus, it is likely that an effect on flow of less than 15% could have gone undetected, but this would be of little clinical significance.

The study shows that no clinically important alteration of the flow of aqueous humor occurs after acute topical administration of this drug in the normal eye. The cause of the fall of intraocular pressure remains unexplained by this study and could have been due to a factor that was not measured, such as uveoscleral flow.

**Key words:** isoproterenol, aqueous humor formation, fluorophotometry, human eye, intraocular pressure, beta adrenergic agonist

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The Effect of Vanadate on Aqueous Humor Dynamics in Cynomolgus Monkeys

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Topical administration of 1% vanadate in a formulation designed to enhance penetration lowered intraocular pressure in monkeys' eyes. The decrease in intraocular pressure was associated with significant decreases in aqueous humor flow. Tonographic outflow facility was unaltered by topical vanadate. Invest Ophthalmol Vis Sci 25:359-361, 1984

Vanadate, given either as sodium metavanadate (NaVO₃) or sodium orthovanadate (Na₃VO₄), lowers intraocular pressure equally in rabbits. The fall in intraocular pressure is not associated with significant changes in outflow facility or episcleral venous pressure. A reduction in the rate of aqueous humor secretion, resulting from the inhibition of ciliary epithelium (Na⁺K⁺)ATPase by vanadate, could explain these findings. On the basis of the results of tonographic studies, the calculated aqueous humor flow decreases approximately 30% in rabbits 2 hours after topical administration of 1% vanadate. Direct measurements of the effect of vanadate on aqueous flow have not been reported.

The topical application of 0.5% Na₃VO₄ was also reported to lower intraocular pressure in rhesus monkeys. In initial trials, we found inconsistent effects of NaVO₃ at the maximum soluble concentration of 1% on the intraocular pressure of cynomolgus monkeys and poor penetration of NaVO₃ into rabbit eyes.

We now report the effects of NaVO₃ prepared with agents to enhance penetration, dimethylsulfoxide (DMSO) and Tween 80 on intraocular pressure, outflow facility measured by tonography, and aqueous humor flow directly measured by fluorophotometry in cynomolgus monkeys.

Materials and Methods. Eight adult cynomolgus monkeys, 4–5 kg, were studied. The monkeys were kept in primate chairs throughout each experiment. A drop of a local anesthetic (0.5% proparacaine hydrochloride) was applied to the eye before all measurements. The intraocular pressure was measured in awake animals, and the outflow facility and aqueous humor flow were measured in animals anesthetized with ketamine hydrochloride, 5–10 mg/kg given intramuscularly.

Vanadate, as sodium metavanadate (NaVO₃) (E. Merck, Darmstadt, Germany), was prepared in distilled water with 10% DMSO and 5% Tween 80 just prior to topical ocular delivery. Solutions were adjusted to a pH between 7.0 and 8.0 with 1 N hydrochloric acid. For all experiments, two 50-μl drops of 1% vanadate, 3–5 min apart, were applied to either the right or left eye, chosen at random. An equal volume of the same diluent containing DMSO and Tween 80 was administered to the fellow control eye. Baseline intraocular pressure was measured with a calibrated pneumotonometer twice prior to administration of the drops. Repeat intraocular pressure measurements were made at 30, 60, 120, 240, and 360 min after administration of 1% vanadate.

Tonography was performed with an Alcon EDT-103 tonography unit. Baseline outflow facility was determined from 9 AM to 10 AM. The tonography results were obtained at 2 hours after administration of 1% vanadate.