The Influence of Reserpine and Propranolol upon the IOP Response to Vanadate in the Rabbit

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The authors examined the influence of intravenously administered sodium ortho-vanadate upon the intraocular pressure (IOP) of the albino rabbit. Vanadate was administered by intravenous injection and the IOP was measured byplanation tonometry. Vanadate (2 mg/kg) caused a marked reduction of IOP which lasted for several hours. Pretreatment with systemic reserpine 24 hr prior to vanadate administration markedly diminished ocular hypertensive response to vanadate. Similarly, systemic treatment with propranolol prevented the IOP-lowering effect of vanadate. In addition, propranolol administered during the course of the vanadate-induced hypertensive response caused the IOP to return to a level close to the control value. The IOP-lowering effect of vanadate appeared to be unrelated to cardiovascular changes; vanadate was observed to have no significant influence upon the blood pressure of anesthetized animals even though the IOP was markedly reduced. On the basis of these experiments, the authors suggest that adrenergic mechanisms contribute to the IOP-lowering effect of vanadate. Invest Ophthalmol Vis Sci 26:1442-1445, 1985

Sodium ortho-vanadate, applied topically to the eye, has been shown to lower intraocular pressure both in rabbits and nonhuman primates.1,2 The vanadate ion is a potent inhibitor of Na,K-ATPase,3 and it has been suggested that it could inhibit the activity of the sodium pump in the ciliary body4 and thereby compromise aqueous humor formation. A reduced aqueous humor flow rate following vanadate treatment has, in fact, been demonstrated experimentally by Podos et al.2 However, direct evidence is lacking to support the concept that transport enzyme inhibition does actually underlie the reduced aqueous flow rate following vanadate application.

A typical pharmacologic response to vanadate in vivo is an alteration of vascular tone, suggestive of an influence upon sympathetic nervous activity (see Erdmann et al5 for review). This aspect of vanadate action led us to examine the possibility of adrenergic involvement in the IOP response to vanadate. In order to avoid the recognized uncertainty of penetration of topically applied vanadate,6 we determined the intraocular pressure response to systemically administered vanadate in the albino rabbit. The response to vanadate was measured in control groups of animals and also in animals pretreated with reserpine or propranolol.

Materials and Methods. Chemicals: Sodium ortho-vanadate was purchased from Fisher Chemical Company (Fair Lawn, NJ). All other drugs were obtained from Sigma Chemical Company (St. Louis, MO). The sodium ortho-vanadate was dissolved in sterile isotonic saline and the pH was adjusted to 7.4 with 0.1 M hydrochloric acid. All the other compounds were dissolved in sterile isotonic saline.

Measurement of IOP: The IOPs of conscious albino rabbits were measured using an Alcon Digilab applation tonometer (Alcon; Fort Worth, TX). Prior to measurement of the IOP, the animals were gently restrained by wrapping in a cloth towel, and the cornea was anesthetized with 0.5% proparacain. Measurements of IOP were undertaken several times during a 24-hr period before each experiment to ensure that the animals became accustomed to handling. The IOP was measured in each rabbit prior to and after the administration of each drug.

Administration of drugs: All compounds were administered by intravenous injection into the marginal ear vein. Reserpine was administered (1.0 mg/kg) 24 hr before the intravenous injection of sodium ortho-vanadate. This dose of reserpine has been used previously in rabbits.6 Propranolol (1.0 mg/kg) was administered to a separate group of animals, 2 hr prior to the vanadate. The reserpine-induced depletion of catecholamines is slow in onset, and at this dose, is essentially complete within 24 hr. The blockade of beta receptors by propranolol is, on the other hand, a rapid phenomenon, the actual rate being dependent upon the bioavailability of the drug following intravenous injection. The pretreatment dosage regimens for reserpine and propranolol were therefore chosen to optimize the pharmacologic activity of these drugs.

Anesthesia and measurement of blood pressure: To determine whether the IOP effect of sodium orthovanadate could be attributed to a systemic hypotensive response, we monitored the arterial blood pressure in...
the presence of this compound. The animals were anesthetized with urethane (1.7 mg/kg intravenously) and the femoral artery was cannulated and then connected to a manometrically calibrated pressure transducer (Bell and Howell; Pasadena, CA). The blood pressure was monitored for 1 hr prior to and several hours after the intravenous injection of sodium ortho-vanadate.

It should be noted that all the experiments in this study conformed to the ARVO Resolution on the Use of Animals in Research.

**Results.** Systemic administration of 2 mg/kg sodium ortho-vanadate resulted in a prompt reduction of intraocular pressure (Fig. 1). The change in intraocular pressure was maximal after 90 min; at this time, the IOP was $13.5 \pm 0.4$ mmHg, compared to a control value (determined prior to treatment) of $17.4 \pm 0.2$ mmHg (mean ± SEM, n = 4).

In a separate series of experiments, rabbits were treated with 1 mg/kg reserpine in order to deplete catecholamines. We determined that reserpine treatment had no significant influence upon the IOP; the control IOP was $19.8 \pm 0.2$ mmHg, while that measured 4 hr post-treatment was $19.7 \pm 0.1$ mmHg and at 24 hr was $19.1 \pm 0.4$ mmHg (mean ± SEM, n = 4). After a 24-hr time period, the reserpine pretreated animals were injected with vanadate (2 mg/kg intravenously) and the IOP response measured. It was observed that pretreatment with reserpine markedly diminished the IOP fall normally induced by vanadate (Fig. 2).

We examined whether the vanadate-induced intraocular pressure decrease could be influenced by propranolol. It has been established previously that propranolol does not alter the IOP in the rabbit. In these experiments, the IOP measured 60 min after propranolol administration (1.0 mg/kg intravenously) was 18.6 ± 0.6 mmHg (mean ± SEM, n = 4) compared to a control (pretreatment) IOP of $19.0 \pm 0.3$ mmHg. Propranolol treated animals were given vanadate (2 mg/kg intravenously) after 60 min. It was observed that propranolol almost completely abrogated the IOP-lowering effect of vanadate (Fig. 3). Using a separate group of animals, we demonstrated that the IOP reduction caused by vanadate could be rapidly reversed when propranolol was administered at the time of the maximal response to vanadate (Fig. 4).

To establish whether the change of IOP induced by vanadate might be related to a reduction of systemic blood pressure, we performed separate experiments to measure the blood pressure by manometric techniques in anesthetized animals. It was observed that vanadate in doses as high as 2.0 mg/kg did not cause a reduction

![Fig. 1](image1.png)

**Fig. 1.** The effect of sodium ortho-vanadate on the intraocular pressure (IOP) of the albino rabbit. Vanadate (2 mg/kg) was administered intravenously in conscious rabbits and the IOP was measured by applanation tonometry. Each value is the mean ± SEM for six rabbits. The dotted line represents the mean control IOP.

![Fig. 2](image2.png)

**Fig. 2.** The effect of reserpine upon the intraocular pressure response to sodium ortho-vanadate. Rabbits were pretreated with reserpine (1 mg/kg) 24 hr prior to the intravenous injection of 2 mg/kg vanadate. Each value is the mean ± SEM for six rabbits. The dotted line represents the mean control IOP.

![Fig. 3](image3.png)

**Fig. 3.** The effect of propranolol upon the intraocular pressure response to sodium ortho-vanadate. Propranolol (1 mg/kg) was administered by intravenous injection 60 min prior to vanadate. Each value is the mean ± SEM for six rabbits. The dotted line represents the mean control IOP.
of blood pressure, although the heart rate altered (Fig.
5). Simultaneous measurement of IOP by applanation
tonometry in these same animals revealed a typical
vanadate-induced ocular hypotensive response.

Discussion. In this study, we have demonstrated that
intravenous administration of sodium ortho-vanadate
in the albino rabbit lowers the IOP for several hours.
This IOP-lowering effect of vanadate could be inhibited
by reserpine and, also, propranolol. Furthermore, the
IOP reduction following vanadate treatment appeared
to be unrelated to changes of blood pressure.

It has been shown that vanadate also lowers the IOP
following topical administration to monkeys and rab-
bits.\textsuperscript{1,2} Investigators have speculated that beca-
use vanadate has effects upon ATPase function, its IOP-lower-
ing effect might be related to inhibition of active
transport by the iris-ciliary body.\textsuperscript{4} However, Mittag et
al\textsuperscript{9} have recently presented a convincing argument
against this hypothesis; they determined that the
concentration of vanadate accumulating in ocular tissues
following topical administration was not sufficient to
cause a significant effect upon iris–ciliary body Na,K–
ATPase. The present study provides direct new evi-
dence to support the concept that mechanisms other
than Na,K–ATPase inhibition might contribute to the
IOP-lowering effect of vanadate.

The finding that both reserpine and propranolol
block the IOP-lowering effect of vanadate raises the
possibility that the action of vanadate upon the IOP is
connected with an influence on sympathetic neuronal
activity. Reserpine is a powerful catacholamine de-
pleting agent, and pretreatment with reserpine has pre-
viously been shown to cause effective sympathetic de-
nervation.\textsuperscript{7} In our study, propranolol inhibited and re-
versed the IOP-lowering effect of vanadate. Since
propranolol specifically blocks post-synaptic beta re-
cipients, this finding suggests a major contribution of
an adrenergic component to the vanadate effect.

Although we have demonstrated that vanadate does
not diminish systemic blood pressure, it is conceivable
that vanadate could exert a significant influence upon
localized blood flow. In fact, Morgan et al\textsuperscript{10} have pro-
vided experimental evidence to suggest that topical
application of vanadate alters blood flow in the rabbit
eye. It remains to be determined whether a vanadate-
induced alteration of ocular blood flow might contrib-
ute to the observed vanadate-induced reduction of IOP
in the rabbit.

Key words: vanadate, intraocular pressure, rabbit, propran-
olol, reserpine

Acknowledgments. The authors thank Elaine Butler for ex-
cellent technical assistance and Professor C. A. Paterson for
stimulating discussions during the course of this study.

From the Department of Ophthalmology, University of Colorado
Health Sciences Center, Denver, Colorado. Supported in part by
USPHS Grant No. EY-00506 from the National Eye Institute, and
by an unrestricted grant from Research to Prevent Blindness, Inc.
Submitted for publication: February 1, 1985. Reprint requests: Nich-
olas A. Delamere, Box B205, UCHSC, 4200 East Ninth Avenue,
Denver, CO 80262.

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Stereochronometry: Quantitative Measurement of Optic Disc Cup Changes
Takenori Takamoro and Bernard Schwartz

The authors have developed an objective, quantitative method for detecting optic disc cup changes that occur with time. Called stereochronometry, this new technique uses a stereoplotter to measure changes recorded in the combination of two single photographs taken of the same optic disc at different times. The reproducibility and sensitivity of stereochronometry were evaluated using a model eye. Stereochronometric measurements were significantly correlated with calibrated changes of depth and width of the cup of the model eye. Standard deviations for five measurements of cup changes without camera shift range from —26 μm to 18 μm, and errors (deviations of the mean of measurements from calibrated cup changes) ranged from —300 μm to +300 μm. Standard deviations and errors in the measurement of cup depth and width were significantly increased when the camera was shifted by 2.5 mm between photographs to simulate possible changes in photographic conditions. Invest Ophthalmol Vis Sci 26:1445–1449, 1985

Changes in the optic disc cup are an early sign of glaucoma; a quantitative screening method to detect such changes would prove a valuable clinical tool. Schirmer1 devised a procedure, which Goldmann and Lotmar2 called stereochronoscopy, that employs two single photographs of the optic disc taken at different times (a stereochronoscopic pair). This photographic pair, when viewed stereoscopically after a rotation of the photographs to eliminate stereoeffects caused by cup depth, shows cup changes as stereoeffects.3–4

Stereochronoscopy allows image shifts caused by optic disc cup changes to be detected subjectively, that is, according to the stereo acuity of the observer. Using a stereoplotter, we made objective measurements of the amount of image shift caused by optic disc cup changes. We call this quantitative method for determining optic disc changes stereochronometry.

We evaluated the reproducibility and sensitivity of stereochronometry by measuring changes in a model eye that could simulate alterations in optic disc cup depth and width that occur in time in glaucoma and ocular hypertension.

Materials and Methods. Our procedure comprised the following steps: (1) adjustment of a model eye (Fig. 1) to a different cup width and depth for each series of photographs; (2) photography of the model eye with a Donaldson stereoscopic fundus camera2 to produce simultaneous stereophotographs; (3) calibration of cup depth and width in the model eye; (4) assembly of a stereochronoscopic pair from a series of photographs so that the pair represented a depth change or a width change; (5) stereochronometric measurements of stereochronoscopic pairs using a Kern PG2 stereoplotter (Kern; Aarau, Switzerland); (6) correlations between stereochronometric measurements and cup changes in the model eye; and (7) computations of reproducibility of stereochronometric measurements.

The model eye (Fig. 1) was constructed to simulate changes in the optic cup. We fixed the position of the camera relative to the model eye so that all photographs taken from the same side, left or right, were taken along the same camera optical axis and were, therefore, geometrically identical.

To simulate changes in cup depth we varied the position of the micrometer head over a range of 600 μm, from —300 μm to +300 μm, measured relative to the fixed position of the ring. Two pairs of stereophotographs were taken at each of seven positions along this range, with intervals of 100 μm between each position.

We assembled stereochronoscopic pairs with no camera shift using only the left photograph of a stereopair so that the stereochronoscopic pair had identical geometry. To simulate changes that might occur in photographic conditions (eg, camera angle or distance,