A Chemically Induced Experimental Ocular Hypertension for Evaluating Drug Effects in Albino Rabbits* (33942)

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The search for an experimental model of increased intraocular pressure (IOP) in animals to simulate clinical glaucoma has revealed many chemical and mechanical means for producing ocular hypertension. Some of the procedures described previously include: replacement of aqueous humor with methylcellulose (1), subconjunctival injection of phenol (2), placement of polyethylene tubing in the angle of the anterior chamber (3), injection of alpha-chymotrypsin into the posterior chamber (4) and "water loading" animals *via* gastric intubation (5). None of these methods fulfilled all of the criteria for an optimal test system. Some did not elevate IOP in the usual laboratory animal, albino rabbits; some involved procedures which were complicated and time-consuming and some required puncture of the cornea during the procedure. The continued search for a nonsurgical method of short duration for experimentally raising IOP seemed desirable.

Clinically, glaucoma has been reported to be frequently associated with acute inflammatory processes in the anterior chamber (6, 7). Formalin has been used experimentally for production of "inflamation" and "edema" in the foot pad of rats (8) and is known to be irritating to the eye (9). Preliminary experiments in which a solution of formalin was applied topically to rabbit eyes, cannulated for direct IOP recording, demonstrated a sharp rise in IOP following this treatment (Langston, J. B., unpublished data). An onset of less than 5 min and duration of approximately 1 hr was observed following topical application of a 2.5% solution of formalin. Based on these data, the following simple nonsurgical experimental model for production of ocular hypertension in animals was devised. Drugs known to be effective in clinical glaucoma have been evaluated using this test system.

Methods and Materials. Young adult albino rabbits of either sex weighing 1.8–2.5 kg were used. Animals were anesthetized with pentobarbital sodium (30 mg/kg) *via* the marginal ear vein and placed on their left side. The right eye, therefore, was horizontal and exposed for treatment. Only one eye of each animal was used.

Two drops (0.1 ml) of a 2.5% solution of formalin were instilled into the conjuctival sac. Five min later, excess fluid was carefully blotted from the eye and IOP was recorded. Approximately 1 animal in 30 did not respond to formalin application with ocular hypertension and was discarded.

Measurement of intraocular pressure. The IOP was measured using a Posner-Inglima applanation tonometer (Institute for Glaucoma Research, Inc.) with a 5 g weight. The IOP (mm Hg) was read directly from the Posner calibration chart (1964) (10–12).

During the initial time–response study, IOP measurements were taken 15, 30, and 45 min following application of the 2.5% formalin solution. In all studies of drug effects on the experimentally induced ocular hypertension the "pretreatment" or initial IOP was recorded 5 min after instillation of the formalin solution at the time when excess fluid was blotted. Animals with an IOP of less than 27 mm Hg were rejected. Drugs were then administered and IOP was measured at 20 and 30 min from this time.

Drug administration. All test drugs were prepared as equimolar concentrations in physiological saline solution and administered as 2 drops (0.1 ml) applied topically to the test eye. Epinephrine hydrochloride 1.0% was included as a reference standard in each test to insure equivalence of individual tests throughout the study. Chemical assays for

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TABLE I. Effects of Drugs on Ocular Hypertension Induced by Topical Application of Formalin.

Test material	Drug conc (%)	n	IOP (mm Hg) 0 time	20 min	30 min	Av	t test $p < 0.05$
None, normal pressure decay		15	30.8	27.9	26.4	27.4	—
Epinephrine hydrochloride (reference standard)	1.0	20	32.8	17.1	14.7	16.4	Sig.
bitartrate	1.0	4	32.0	16.0	15.0	15.5	Sig.
Isoproterenol bitartrate	2.0	4	33.0	17.5	15.5	16.5	Sig.
Carbachol chloride	3.0	4	33.5	20.0	18.0	19.0	Sig.
Pilocarpine hydrochloride	4.0	4	31.3	18.5	15.8	17.1	Sig.
Physostigmine salicylate	2.0	4	29.3	18.5	15.7	17.5	Sig.
Phenoxybenzamine hydrochloride	2.0	4	30.7	24.5	20.3	22.8	Not sig.
Propranolol hydrochloride	2.0	4	32.8	26.3	28.3	27.3	Not sig.
Atropine sulfate	1.0	4	30.0	23.0	21.5	22.3	Not sig.
	4.0	4	28.7	20.0	21.0	20.5	Sig.
Hexamethonium chloride	2.0	4	32.5	22.3	20.3	21.5	Sig.

epinephrine content were done periodically to confirm constancy of the reference standard throughout the study.

Analysis of data. Twenty and 30 min IOP values for each animal were averaged. These averages were then used to determine numbers plotted on the concentration–response curves. Regression lines of best fit were calculated by the method least squares (13). Statistical comparisons were made between the mean IOP values recorded following application of the test drug and the mean IOP values for the normal decay curve following formalin application. The Student's t test was used to make this determination. A p value of less than 0.05 was regarded as significant.

Results. Topical application of 2.5% formalin solution to the eye of an anesthetized rabbit resulted in edema of the palpebral conjunctiva and nictitating membrane, miosis, and an increase in IOP which persisted for 45–60 min. The miosis induced by application of formalin was reversable by topical application of adrenergic drugs such as epinephrine hydrocholoride 1.0% (reference standard). Blanching and in some instances a reduction in palpebral swelling were also grossly apparent in animals receiving this material.

Biomicroscopic examination (Zeiss, model 100/16) using fluorescein staining during the period of miosis revealed little difference in the appearance of the formalin-treated cornea compared to the untreated cornea.

Intraocular pressure (IOP) values recorded following topical administration of selected concentrations of various pharmacologically representative drugs are tabulated in Table I.

Epinephrine bitartrate 1.0%, isoproterenol bitartrate 2.0%, carbachol chloride 3.0%, pilocarpine hydrochloride 4.0%, physostigmine salicylate 2.0%, atropine sulfate 4.0%, and hexamethonium chloride 2.0% were effective in lowering IOP significantly in these studies. Phenoxybenzamine hydrochloride 2.0%, propranolol hydrochloride 2.0%, and atropine sulfate 1.0% did not significantly alter IOP.

Concentration–response curves for representative compounds from four pharmacologic classes are illustrated in Fig. 1. This figure depicts the response of the test model to topical application of epinephrine bitartrate (A), isoproterenol bitartrate (B), carbachol chloride (C), and physostigmine (eserine) salicylate (D). Approximately equivalent response regression lines were obtained with epinephrine bitartrate at concentrations of 0.063, 0.25, and 1.0%, with isoproterenol bitartrate at concentrations of 0.5, 1.0, and 2.0% and with carbachol chloride

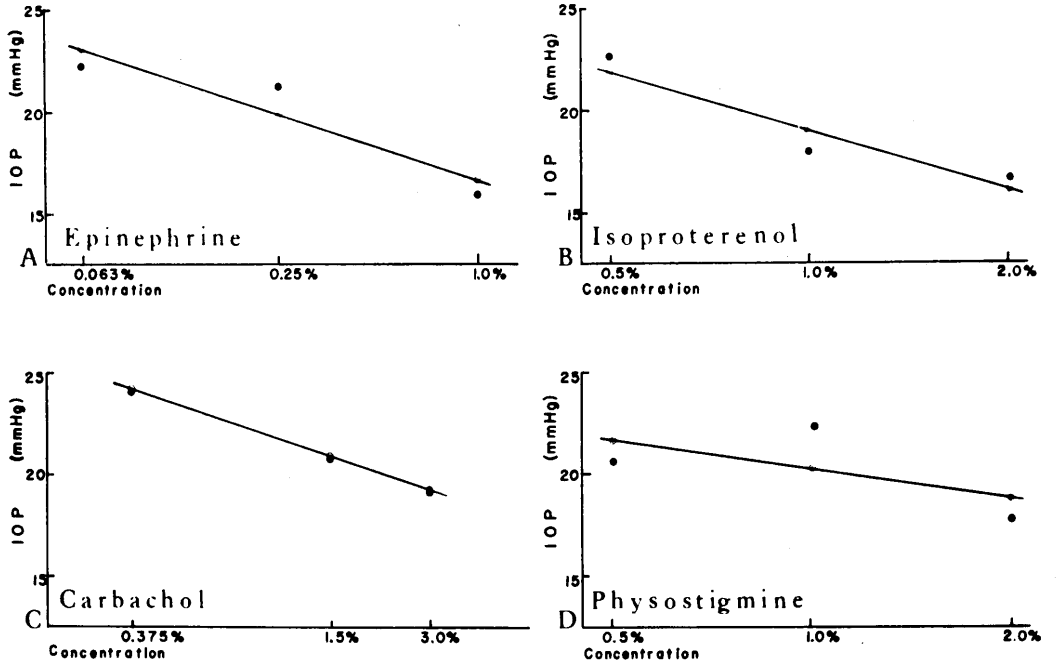

FIG. 1. Intraocular pressure responses following topical administration of selected compounds to eyes with formalin-induced ocular hypertension: (A), epinephrine bitartrate concentration–response curve; calculated best fitting regression line; (●) = actual recorded values; $n = 4$ at each concentration tested. (B) isoproterenol bitartrate concentration–response curve; calculated best fitting regression line; legend same as above. (C) Carbachol chloride concentration–response curve; calculated best fitting regression line; legend same as above. (D), Physostigimine (eserine) salicylate concentration–response curve; calculated best fitting regression line; legend same as above.

at concentrations of 0.375, 1.5, and 3.0%. Although physostigmine (escrine) salicylate did produce a concentration-dependent lowering of IOP at concentrations of 0.5, 1.0, and 2.0% the slope of the curve was shallow and the deviation of the observed values from the calculated best fitting regression line was large. Pilocarpine hydrochloride, at a concentration of 4.0% significantly lowered IOP but at concentrations of 2.0% or lower a consistent concentration dependent response was not elicited in these experiments.

Discussion. The use of inflammation or irritation as an approach to the production of experimental "glaucoma" in rabbits was grounded initially in the association clinically of glaucoma with acute inflammatory conditions in the anterior chamber (6). A number of chemical irritants are known to cause a transient rise in intraocular pressure when applied topically (7) and the known ocular toxicity of formalin (9) made this material a logical experimental choice. The duration of edema and miosis observed following topical application of formalin paralleled the elevation of IOP and suggested involvement of inflammation or irritation in the ocular hypertensive mechanism similar to that reported by Thomas for other compounds (7).

Initial tests using direct cannulation manometric techniques for IOP measurement following topical application of formalin (Langston, J. B., unpublished data) indicated that the resultant ocular hypertension reached peak response between 5 and 10 min after application of formalin, and pressure levels gradually returned to normal over a 60-min period. Time response studies using the Posner-Inglima applanometer confirmed that IOP elevation following a time course similar

to that recorded by direct cannulation could be adequately evaluated using applanation techniques.

In order to more clearly characterize the applicability of the proposed test model to the selection of potential antiglaucomic agents, representative members of each major pharmacological class of materials known to be topically effective in lowering IOP clinically were tested. Additionally, representative materials with other pharmacologic actions were evaluated to gain a greater insight into the mechanism involved in the production of ocular hypertension by this method.

Reports of increased IOP, miosis, local vasodilation, and aqueous flare following chemical or mechanical irritation of the cornea or stimulation of the trigeminal nerve to the eye have been summarized by Sears (14). Evidence was presented by this worker to implicate a neuroreflex mechanism in these responses. Further, since miosis and pressure changes were completely blocked by retrobulbar anesthesia, the suggestion was made that at least a portion of the reflex pathway must be extrabulbar. Additional evidence was obtained during the present study which further indicates that the neuro-pathway involved is multineuronal in nature. Hexamethonium, a ganglionic blocking agent, and high concentrations of atropine (presumably at a dose which will produce ganglionic blockade, 4.0%) significantly lowered IOP (Table I) although to a lesser degree than did the adrenergic and cholinergic agents tested. These effects are interpreted as due to interruption of the reflex neuro-pathway involved in the production of ocular hypertension and as a confirmation of the reflex nature of "irritation-induced" ocular hypertension. A concentration of atropine (1.0%) which produced mydriasis in normal rabbit eyes, did not lower IOP significantly. This suggests that muscarinic cholinergic blockade is not involved in the ocular hypotensive response described for atropine 4.0% and hexamethonium.

Phenoxybenzamine, an α-adrenergic blocking agent and propranolol, a β-adrenergic blocking agent did not reduce IOP. This suggests that an adrenergic mechanism is not involved in this model of ocular hypertension.

Propranolol has been reported to have local anesthetic properties in humans (15). Experiments in these laboratories demonstrate it to be an effective topical anesthetic in rabbit eyes with a minimal effective concentration (MEC) of 0.11 $\pm$ 0.02% (Leaders, F. E., unpublished data). These data indicate that corneal anesthesia does not inhibit "irritation-induced" ocular hypertension and suggest that the site at which the hypertensive response is initiated is not at the corneal surface.

The effectiveness of atropine 4.0% in lowering IOP is not entirely consistent with the report of Thomas (7) in which this concentration of atropine did not prevent miosis induced by irritation (injection of air into the anterior chamber). Although the test systems are not equivalent, the difference in response points to a consideration inherent to all of the models of "irritation-induced" ocular hypertension. Irritation of the cornea and adnexa may alter drug penetration to an unknown extent. This variation undoubtedly also occurs clinically, dependent upon the glaucoma involved, and hence does not invalidate the present test model. The possibility of altered drug absorption must be recognized, however, and compounds found to have activity in this model should undergo further testing in a system in which the eye remains "normal."

Adrenergic stimulants of the catecholamine type demonstrated the most marked efficacy in lowering IOP in this test model. This was true of agents with combined α and β-adrenergic effects (epinephrine) and those with only β-adrenergic activity (isoproterenol). These findings are consistent with the findings of Thomas (7) that the adrenergic agents are more effective in preventing "irritation-induced" ocular hypertension than are the cholinergic agents. The present work, in addition, extends this effectiveness to include β-adrenergic as well as the α-adrenergic materials and demonstrates that in the model described "irritation-induced" ocular hypertension can be reversed as well as prevented (7).

Cholinergic stimulants, both direct acting (carbachol, pilocarpine) and inhibitors of cholinesterase (physostigmine) were effective ocular hypotensives but were not as effective overall as were agents through an adrenergic mechanism. Although a concentration related response was observed following topical administration of physostigmine (eserine), the regression slope was quite shallow and hence differed from that observed with carbachol administration. Concentrations of pilocarpine below 4.0% did not produce a consistent concentration related response in this test model.

Summary. A test model is described for production of experimental ocular hypertension in albino rabbits. The procedure employs applanation tonometry for intraocular pressure (IOP) measurement; is simple in design and requires little time to perform. Eight pharmacologic classes of drugs were evaluated using this procedure. Good correlation is described between drugs topically effective in clinical treatment of glaucoma and those lowering IOP in this test system. The described method was most sensitive for the adrenergic stimulants of both α and β variety. Physostigmine (cholinesterase inhibitor) effectively lowered IOP as did the direct-acting cholinergic stimulant carbachol. A concentration–response relationship with pilocarpine, however, was not observed. Ganglionic blocking agents effectively lowered IOP and confirm the involvement of a neuroreflex mechanism involved in production of "irritation-induced" hypertension. Adrenergic blocking agents, muscarinic cholinergic blocking agents, and local anesthetics were ineffective ocular hypotensive agents in this test model.

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